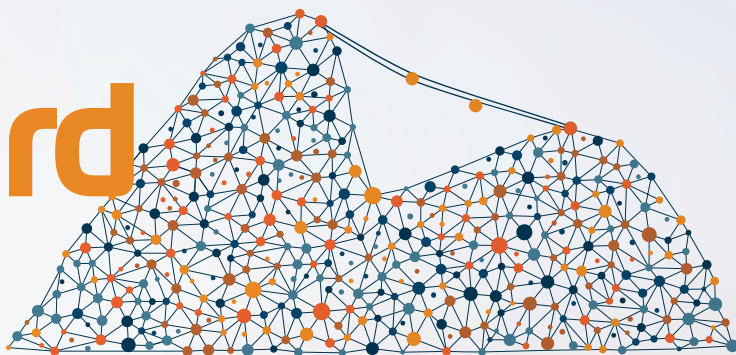


Proceedings

3rd



INTERNATIONAL MEETING **IN** **ONCOLOGY RESEARCH**

II Single Cell LATAM Symposium: Deepening Connections, Driving Discovery

Satellite Symposium for the 60th Anniversary of the IARC

Simpósio do INCT em Genômica do Câncer e Medicina de Precisão para o SUS

Celebration of 20 Years of the Graduate Program in Oncology (PPGO)

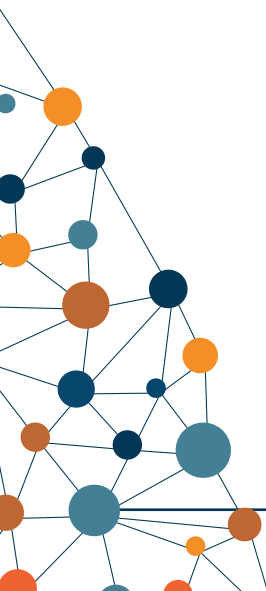
XIX Jornada de Iniciação Científica e XIV Jornada de Pós-Graduação

Prodigy Rio de Janeiro, December 1 to 4, 2025



MINISTÉRIO DA
SAÚDE





Dear Friends and Colleagues,

On behalf of the *Instituto Nacional de Câncer (INCA)*, we are pleased and honored to welcome you to the *III International Meeting in Oncology Research in Celebration of 20 Years of the Graduate Program in Oncology (PPGO)*. This meeting will also include the *II Single Cell LATAM Symposium: Deepening Connections, Driving Discovery* and the *Simpósio do INCT em Genômica do Câncer e Medicina de Precisão para o SUS*. As a Satellite Symposium, we are also celebrating of the *60th Anniversary of the International Agency for Research on Cancer (IARC)*. We are delighted to have among us several worldwide leaders in oncology and related areas, who were kind enough to spare some of their time to present us their most recent data and personal views.

The Graduate Program in Oncology together with the Oncology Research Training Program of the INCA, has held annually for 19 years the *Jornada de Pós-graduação e Iniciação Científica*, which has a goal to integrate the different areas of activity of our Institute, including research, education, prevention and control, and clinical care. This event has constituted a permanent institutional forum for scientific debate, bringing together the clinical staff and researchers, as well as students, around guest speakers, and the success of this meeting reflects our participation in the international scenario with regard to scientific contribution in cancer research.

This year (2025) PPGO will complete the 20th anniversary of its foundation and as part of this celebration, this year the *Jornada de Pós-graduação e Iniciação Científica* will be held in the format of an international meeting. The scientific program will follow excellences of topics and guest speakers, which is already a tradition in INCA meetings, valuing expertise as well as offering opportunities to young scientists. The format of the meeting includes four keynote conferences, ten symposia and two poster sessions, with a goal of assembling a group of specialists from both clinical and basic science that will discuss different aspects on oncology research, including epidemiology, tumor genetics and genomics, cancer immunology and immunotherapy, cancer metabolism and microenvironment, signaling and tumor biology, biomarkers and clinical research, among other topics.

Also, during this meeting will take place the *Workshop Planning a Resilient Cancer Control in Brazil*. To respond to the urgent need to strengthen health system resilience globally in particular in low- and middle-income countries, a workshop will be organised as a partnership between the IARC, INCA and PAHO/WHO focused on resilience and cancer control in crisis. This event will bring together Brazilian leaders and international experts in the field of cancer care and health systems to discuss preliminary findings from the IARC's Initiative for Resilience in Cancer Control (IRCC), provides an essential platform for presenting and validating the preliminary findings from the IRCC Brazil study.

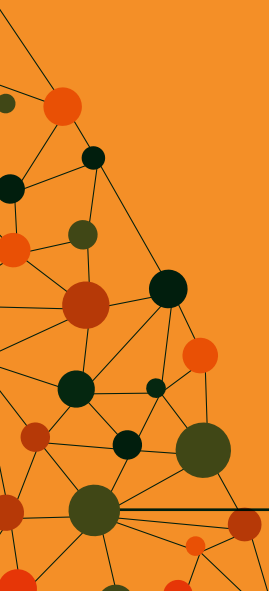
Cancer in Brazil is the second cause of death and is already considered a major public health problem for the Brazilian population. As our population is aging, it is estimated that cancer will be the leading cause of death in Brazil in the next 20 years. These observations demonstrate INCA's great challenge for a future that is not far off, since the Institute is responsible for elaborating the policy of cancer care for our country. Thus, we will take this opportunity to gather great experts in the field of cancer in order to propose actions that can contribute to the reduction of the incidence and mortality of this disease in Brazil, reaffirming our commitment to the quality of life of the population. In this event, the multiple aspects related to cancer will be addressed. For 85 years, INCA is part of the history of public health in Brazil, standing out as a reference institution for the medical community and health professionals and for society in general in oncology.

We are assured that we will have an excellent meeting in 2025! Welcome to the *III International Meeting in Oncology Research!*

The Scientific Committee.



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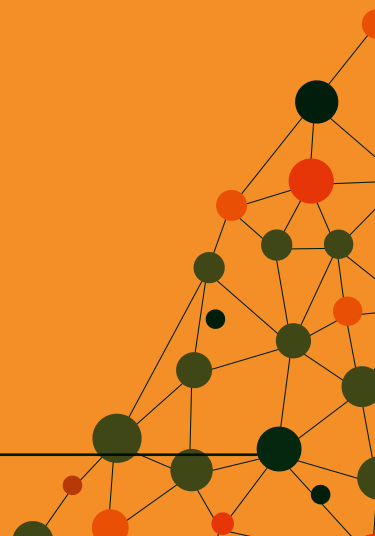
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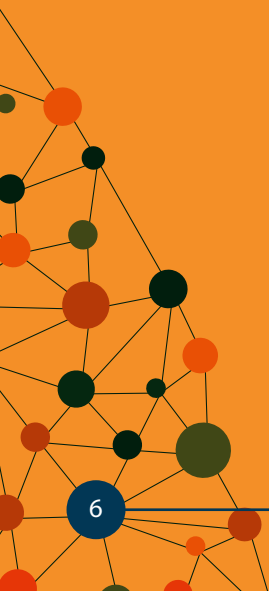
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Committees



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- Mariana Emerenciano
- Patricia Possik

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ORGANIZATION

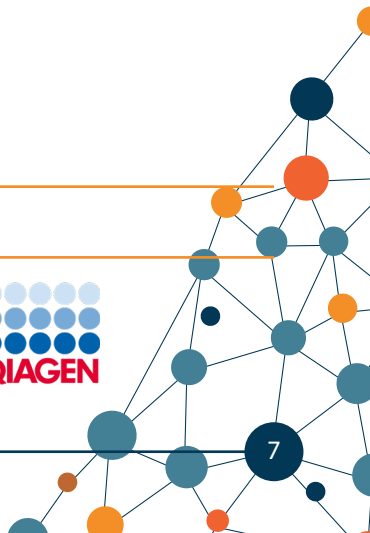
- Coordenação de Pesquisa e Inovação
- Coordenação de Pós-Graduação *Stricto Sensu* em Oncologia
- Coordenação Institucional de Iniciação Científica
- Programa de Formação em Pesquisa Oncológica

SUPPORT

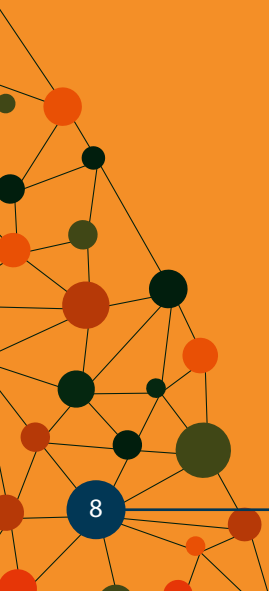


FUNDAÇÃO DO CÂNCER

illumina®



Speakers





Andrea Biondi

Tettamanti Center, Italy



Andreia Melo

INCA, Brazil



Arn Migowski

INCA, Brazil



Bruno Mattuck

Virginia Commonwealth University, USA



Bryan Strauss

USP, Brazil



Carlos Menck

USP, Brazil



Cristiana Libardi

UFMG, Brazil



Cristina Bonorino

UFCSPA, Brazil



David Adams

Wellcome Sanger Institute, UK



Dirce Carraro

Hospital AcCamargo, Brazil



Eliane Piaggio

Institut Curie, France



Fernando Wehrmeister

UFPEL, Brazil



Giuliana Mognol

UCSD, USA



Gulnar Azevedo e Silva

UERJ, Brazil



Helder Nakaya

Hospital Israelita Albert Einstein, Brazil



Isabelle Soerjomataram

IARC, France



Jessé Lopes da Silva

INCA, Brazil



Kelly Grace Magalhães

UNB, Brazil



Kenneth Gollob

Hospital Israelita Albert Einstein, Brazil



Luisa Villa

USP, Brazil



Mariana Boroni

INCA, Brazil



Mariana Emerenciano

INCA, Brazil



Nathalia Meirelles

INCA, Brazil



Patricia Neves

FIOCRUZ, Brazil



Patricia Possik

INCA, Brazil



Patrícia Prolla

UFRGS, Brazil



Paul Brennan

IARC, France



Pedro Galante

Hospital Sírio-Libanês, Brazil



Rodrigo Nalio

IDOR, Brazil



Roger Chammas

USP, Brazil



Sandra Avila

UNICAMP, Brazil



Sophia George

University of Miami Miller School of Medicine, USA



Taissa Vila

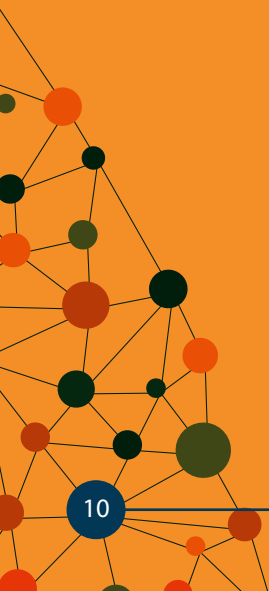
The Lancet Regional Health Americas



Tatiana Toporcov

USP, Brazil

General Information



VENUE

Prodigy Santos Dumont, Rio de Janeiro, RJ, Brazil

BADGE

Mandatory use for admission in the rooms and common areas of the venue.

SMOKING

Smoking is strictly forbidden in the Convention center, Foyers and Toilets.

CELL PHONES

Delegates are kindly requested to switch off mobile phones in the rooms where scientific sessions are being held.

PHOTOGRAPHY

Delegates are prohibited from taking photographs of the presentations during the conferences.

CERTIFICATES OF ATTENDANCE

Will be available online.

SIMULTANEOUS TRANSLATION

English is the official language of the event, there will not be simultaneous translation in the meeting.

POSTERS

The authors are responsible for the attachment and removal of their poster in the specified areas. The poster must be attached for presentation from 9am to 6:30pm and removed at the end of the day.

POSTER SESSIONS

Poster sessions will be held in the Corcovado 1 and 2.

SESSION 1: Tuesday, Dec 2, 16:30 - 18:30h

- Basic and Experimental Research **Posters #2–189**
- Translational Research and Diagnostic Applications **Posters #4–72**

SESSION 2: Wednesday, Dec 3, 16:30 - 18:30h

- Translational Research and Diagnostic Applications **Posters #73–188**
- Clinical Research and Technological Development **Posters #5–184**
- Epidemiological Research **Posters #10–183**

MEDIA DESK

The presentation material of the speakers must be delivered prior to 2 (two) hours, seeking for a good assistance and proper organization in the AV system.

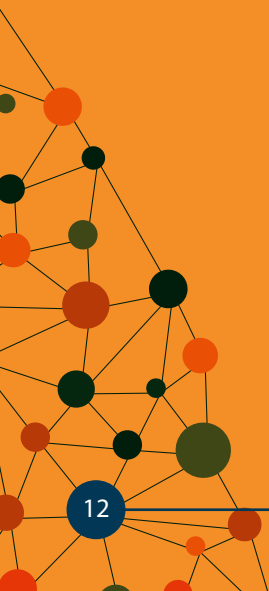
TIMETABLE

The Organizing Committee invites all participants of the Scientific Activities to strictly follow the schedule of their presentations.

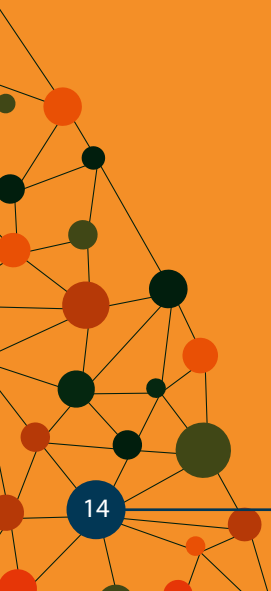
LUNCH

The hotel restaurant is located by the pool area at Orla 21 Rooftop.

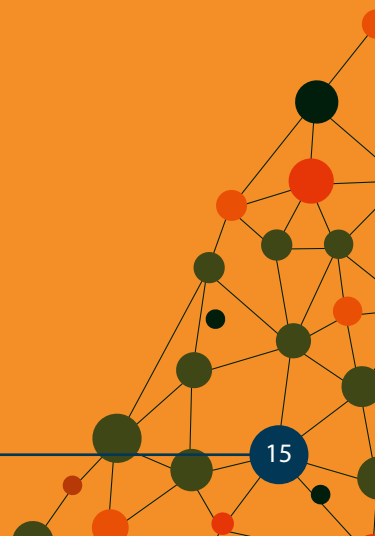
Program at a Glance



	MONDAY December 1	TUESDAY December 2	WEDNESDAY December 3	THURSDAY December 4
09:00 11:00		SYMPOSIUM Cancer Surveillance and Risk Factors	SYMPOSIUM Hereditary Cancer and Genetic Counselling	SYMPOSIUM Precision Oncology and Emerging Technologies
		SYMPOSIUM Cancer Genetics	SYMPOSIUM Charting the Complexity of Cancer One Cell at a Time	SYMPOSIUM Tumor Virology and Clinical Research
11:00 11:30		COFFEE BREAK	COFFEE BREAK	COFFEE BREAK
11:30 12:30		CONFERENCE	CONFERENCE	CLOSING CONFERENCE
12:30 14:00		LUNCH	LUNCH	CLOSING REMARKS AND AWARDS
14:00 16:00	REGISTRATION	SYMPOSIUM Research Integrity and the Responsible Use of Artificial Intelligence	SYMPOSIUM Disparities in Cancer Care and Research	WORKSHOP INCA/IARC/ WHO/PAHO Planning a Resilient Cancer Control in Brazil (participation under invitation)
		SYMPOSIUM Tumor Immunology and Immunotherapy	SYMPOSIUM Tumor Metabolism and Microenvironment	
16:00 16:30	OPEN CEREMONY	SATELLITE CONFERENCE	SATELLITE CONFERENCE	
16:30 17:30		POSTER EXHIBITION	POSTER EXHIBITION	
17:30 18:30	OPENING CONFERENCE			
18:30 22:00	COCKTAIL			



Scientific Program



DECEMBER 1, MONDAY

14:00 - 16:00 | REGISTRATION

16:00 - 17:30 | OPENING CEREMONY – Room Corcovado
Celebration of 20 Years of the Graduate Program in Oncology (PPGO)

17:30 - 18:30 | OPENING CONFERENCE – Room Corcovado
Chair: João Viola

Isabelle Soerjomataram - IARC, France

Resilience under pressure: The future of national health system and cancer control

18:30 - 22:00 | COCKTAIL

DECEMBER 2, TUESDAY

SYMPOSIUM – Room Corcovado 5

Cancer Surveillance and Risk Factors

Chair: Marianna Cancela

09:00 - 11:00

09:00 - 09:30 | *Arn Migowski - INCA, Brazil*

New Brazilian guidelines for a nationwide population-based cervical cancer screening program

09:30 - 10:00 | *Tatiana Toporcov - USP, Brazil*

Equity on cancer research and control

10:00 - 10:30 | *Gulnar Azevedo e Silva - UERJ, Brazil*

The challenges of epidemiology for cancer control

10:30 - 10:45 | *Isabela de Pinho Pestana - INCA, Brazil*

Epidemiological profile of patients with metastatic non-small cell lung cancer using Erlotinib and Gefitinib

10:45 - 11:00 | *Guilherme Augusto Barcello Costa - INCA, Brazil*

Missing staging in esophageal, gastric, and colorectal cancers in Brazil from 2010 to 2019: Analysis of data from hospital-based cancer registries

SYMPOSIUM – Room Corcovado 3 and 4

Cancer Genetics

Chair: Miguel Moreira

09:00 - 11:00

- 09:00 - 09:30 | *Nathalia Meirelles - INCA, Brazil*
TP53 as a hub for the functional deregulation mediated by HMGA and TDG in esophageal carcinogenesis
- 09:30 - 10:00 | *Mariana Emerenciano - INCA, Brazil*
Pediatric acute leukemia genetics: Standing by Brazilian children
- 10:00 - 10:30 | *Pedro Galante - Hospital Sírio-Libanês, Brazil*
The opportunities and findings in the dark side of the cancer transcriptomics
- 10:30 - 10:45 | *Raquel Monteiro de Barros Lemos - INCA, Brazil*
Investigation of IL-7R dysregulation in T-cell acute lymphoblastic leukemia
- 10:45 - 11:00 | *Vinícius Pereira de Oliveira - INCA, Brazil*
The effect of cyclin E1 overexpression on chromosome segregation and ultrafine DNA bridges formation in mammary epithelial cells

11:00 - 11:30 | COFFEE BREAK

11:30 - 12:30 | CONFERENCE – Room Corcovado

Chair: Luis Felipe Ribeiro Pinto

Paul Brennan - IARC, France

What's causing the increasing incidence of early onset colorectal cancer?

12:30 - 14:00 | LUNCH

SYMPOSIUM – Room Corcovado 5

Research Integrity and the Responsible Use of Artificial Intelligence

Chair: Alessandra Siqueira

14:00 - 16:00

- 14:00 - 14:30 | *Sandra Avila - UNICAMP, Brazil*
The day I discovered I was collaborating on a eugenics skin cancer project
- 14:30 - 15:00 | *Helder Nakaya - Hospital Israelita Albert Einstein, Brazil*
How good intentions and misaligned incentives lead to unethical AI
- 15:00 - 15:30 | *Taissa Vila – The Lancet Regional Health Americas*
Responsible innovation: Research integrity in the era of artificial intelligence
- 15:30 - 15:45 | *Karla Santos da Costa Rosa - INCA, Brazil*
NutriPal (Nutricional screening algorithm): Association with nutritional status, quality of Life, and survival, and development of personalized recommendations
- 15:45 - 16:00 | *Sebastian Alejandro Granja Bustos - INCA, Brazil*
Analysis of patients with rectal adenocarcinoma undergoing robotic resection after neoadjuvant treatment at INCA

14:00 - 16:00

SYMPOSIUM – Room Corcovado 3 and 4

Tumor Immunology and Immunotherapy

Chair: *Robson Monteiro*

- 14:00 - 14:30 | *Cristina Bonorino – UFCSPA, Brazil*
The natural repertoire as a source of novel anti-checkpoint antibodies
- 14:30 - 15:00 | *Giuliana Mognol – UCSD, USA*
Reversal of T cell exclusion in head and neck cancer by PI3K gamma inhibition
- 15:00 - 15:30 | *Andrea Biondi – Tettamanti Center, Italy*
Non-viral CAR T cells platform for hematological disorders
- 15:30 - 15:45 | *Rebecca Martins Cadimo do Nascimento – INCA, Brazil*
Evaluation and characterization of tumor-infiltrating lymphocytes (TIL) Isolated from acral melanoma
- 15:45 - 16:00 | *Barbara Oliveira-Vieira – INCA, Brazil*
Role of transcriptional co-factor IRF2BP2 in activation and differentiation of CD8 T cells

16:00 - 16:30

ILLUMINA SATELLITE CONFERENCE - Room Corcovado 5

Illumina Applications in Precision Oncology

16:30 - 18:30

POSTER EXHIBITION - Room Corcovado 1 and 2

DECEMBER 3, WEDNESDAY

09:00 - 11:00

SYMPOSIUM – Room Corcovado 5

Hereditary Cancer and Genetic Counselling

Chair: *Edenir Palmero*

- 09:00 - 09:30 | *Dirce Carraro – Hospital AcCamargo, Brazil*
Decoding triple-negative breast cancer: From hereditary risk to HRD mechanisms and liquid biopsy insights
- 09:30 - 10:00 | *Patrícia Prolla – UFRGS, Brazil*
Challenges of cancer genetic counseling in clinical practice
- 10:00 - 10:30 | *Carlos Menck – USP, Brazil*
Skin tumor mutation signatures in xeroderma pigmentosum patients
- 10:30 - 10:45 | *Anna Beatriz Ribeiro Elias – INCA, Brazil*
An unexpected role of PALB2 links homologous recombination and centrosome biology
- 10:45 - 11:00 | *Bianca Catarina Azeredo Cabral – INCA, Brazil*
Identification and characterization of patients with hereditary breast cancer in Brazil

SYMPOSIUM – Room Corcovado 3 and 4

Charting the Complexity of Cancer One Cell at a Time

Chair: Mariana Boroni

09:00 - 11:00

- 09:00 - 09:30 | *Rodrigo Nalio – IDOR, Brazil*
Macrophage profiling in colorectal cancer niches: a time-space perspective
- 09:30 - 10:00 | *Bruno Matuck – Virginia Commonwealth University, USA*
Charting spatial biology: Integrating proteotranscriptomics and AI in cancer
- 10:00 - 10:30 | *Sophia George - University of Miami Miller School of Medicine, USA*
Single-cell profiling of the breast and reproductive tract in women of African and Caribbean descent
- 10:30 - 10:45 | *Caroline Carvalho de Sá - INCA, Brazil*
Characterization of mutational signatures in tumor tissue from cervical cancer infected with different HPV types
- 10:45 - 11:00 | *Suzana Benedito - INCA, Brazil*
The cooperation between CALRdel52 and TP53 mutations increases the number of immature hematopoietic progenitors and enhances thrombopoietin-independent megakaryopoiesis

11:00 - 11:30 | COFFEE BREAK

11:30 - 12:30 | CONFERENCE - Room Corcovado

Chair: Patricia Possik

David Adams - Wellcome Sanger Institute, UK

Rewriting cancer's script: Insights from saturation genome editing in hereditary cancers

12:30 - 14:00 | LUNCH

SYMPOSIUM – Room Corcovado 5

Disparities in Cancer Care and Research

Chair: Sheila Coelho

14:00 - 16:00

- 14:00 - 14:30 | *Patricia Possik – INCA, Brazil*
Modelling acral melanoma in admixed Brazilians uncovers genomic drivers and targetable pathways
- 14:30 - 15:00 | *Jessé Lopes da Silva – INCA, Brazil*
When inequity shapes prognosis: National evidence of racial disparities in cancer care in Brazil
- 15:00 - 15:30 | *Fernando Wehrmeister – UFPEL, Brazil*
Occurrence and inequalities in cancer risk factors from a life course perspective: Evidence from the Pelotas cohorts
- 15:30 - 15:45 | *Tiago Eduardo dos Santos - INCA, Brazil*
Therapeutic pathway of patients with laryngeal cancer
- 15:45 - 16:00 | *Monique de Souza Almeida Lopes - INCA, Brazil*
Bacteriome analysis in esophageal squamous cell carcinoma

SYMPOSIUM – Room Corcovado 3 and 4

Tumor Metabolism and Microenvironment

Chair: *Patricia Bozza*

14:00 - 16:00

14:00 - 14:30 | *Mariana Boroni – INCA, Brazil*

Tumor microenvironment niches and chemotherapy resistance in ovarian cancer

14:30 - 15:00 | *Kelly Magalhães – UNB, Brazil*

From fat to tumor: How adipose tissue fuels cancer growth

15:00 - 15:30 | *Roger Chammás – USP, Brazil*

Targeting the mechanisms of tumor cell tolerance to therapy and metastases with nucleic-acid-based strategies

15:30 - 15:45 | *Michelle de Souza Ferreira Saraiva – INCA, Brazil*

MGAT5 affects the expression of genes related to tumor progression in colon adenocarcinoma

15:45 - 16:00 | *João Victor Roza Cruz – INCA, Brazil*

PIK3R3 activates PI3K/AKT signaling contributing to drive esophageal squamous cell carcinoma progression representing a promising therapeutic target

16:00 - 16:30

NEW ENGLAND BIOLABS SATELLITE CONFERENCE - Room Corcovado 5

Enabling single cell workflows with New England Biolabs

QIAGEN SATELLITE CONFERENCE - Room Corcovado 3 and 4

Advanced applications of molecular biology for investigating tumor pathways and mechanisms of resistance

16:30 - 18:30

POSTER EXHIBITION - Room Corcovado 1 and 2

DECEMBER 4, THURSDAY

SYMPOSIUM – Room Corcovado 5

Precision Oncology and Emerging Technologies

Chair: *Martin Bonamino*

09:00 - 11:00

09:00 - 09:30 | *Bryan Strauss – USP, Brazil*

Tumor on a chip as a model of cancer gene therapy

09:30 - 10:00 | *Patricia Neves – FIOCRUZ, Brazil*

Building Brazil's RNA platform: Unlocking new frontiers for cancer immunotherapy and precision medicine in Latin America

10:00 - 10:30 | *Kenneth Gollob – Hospital Israelita Albert Einstein, Brazil*

Predicting benefit versus harm: Divergent immune trajectories defined at baseline in NSCLC immunotherapy

10:30 - 10:45 | *Luiza Abdo – INCA, Brazil*

Advancing CAR-T Cell Engineering: Hybrid Transposons as Cost-Effective non-Viral Alternatives

10:45 - 11:00 | *Ana Rubia Alcantara Pelloso – INCA, Brazil*

Production of amplicon viral vectors for gene therapy: A novel purification system based site-specific recombination and magnetic bead separation

SYMPOSIUM – Room Corcovado 3 and 4

Tumor Virology and Emerging Technologies

Chair: *Marcelo Soares*

09:00 - 11:00

- 09:00 - 09:30 | *Andreia Melo – INCA, Brazil*
Immunotherapy in gynecologic cancers: Global advances, local challenges
- 09:30 - 10:00 | *Cristiana Libardi – UFMG, Brazil*
Exploring penile cancer through WES: Does HPV change the story?
- 10:00 - 10:30 | *Luisa Villa – USP, Brazil*
Elimination of Cervical Cancer: Where do we stand and what to expect
- 10:30 - 10:45 | *Juliana Domett Siqueira - INCA, Brazil*
Characterization of the cervical cancer microbiome
- 10:45 - 11:00 | *Eduardo Paulino - INCA, Brazil*
Endometrial cancer recurrence in p53 abnormal tumors without myometrial invasion

11:00 - 11:30 | COFFEE BREAK

11:30 - 12:30 | CLOSING CONFERENCE

Chair: *Leonardo Teixeira*

Eliane Piagio – Institut Curie, France

Immunoprofiling of Tregs: Implications for tumor immunity and cancer therapy

12:30 - 13:00 | CLOSING CEREMONY – Room Corcovado

13:00 - 14:00 | LUNCH

WORKSHOP – Room Copacabana

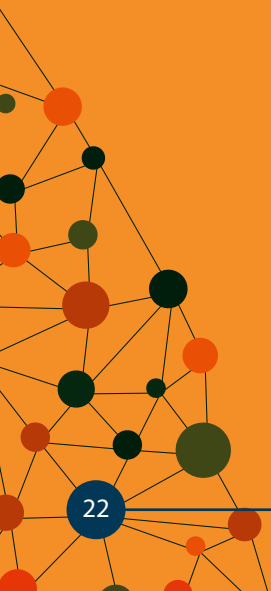
(participation under invitation)

Planning a Resilient Cancer Control in Brazil

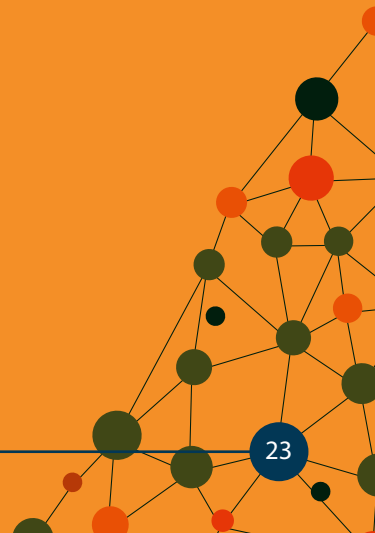
Chair: *Isabelle Soerjomataram and João Viola*

14:00 - 18:00

- 14:00 - 14:20 | **Opening Remarks** (*INCA, PAHO/WHO, IARC*)
- 14:20 - 14:50 | **Presentation of Preliminary Findings** (*Case study: Brazil*)
- 14:50 - 15:20 | **Work group: Stakeholder responses and reflections**
- 15:20 - 15:40 | COFFEE BREAK**
- 15:40 - 16:40 | **Interactive Breakout Groups by domain: Governance, Service Delivery, Workforce, Resources**
- 16:40 - 17:30 | **Plenary Discussion: Synthesis of group insights**
- 17:30 - 17:50 | **Policy Dialogue: WHO Policy Note – feasibility and next steps**
- 17:50 - 18:00 | **Closing Remarks & Future Collaboration Opportunities**



Scientific Papers



BASIC AND EXPERIMENTAL RESEARCH (BE)

PAPER CODE: 3

TWIST1 IS A KEY REGULATOR OF IL17RA AND IL17D IN BREAST CANCER

SILVA, C.B.D.A.1; DA CONCEIÇÃO, J.O.1; FERREIRA, G.M.1; GOMES, R.B.1; PIRES, B.R.B.2; ABDELHAY, E.S.F.W.1. 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. UERJ, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Breast cancer (BC) is a heterogeneous disease composed of multiple subtypes with distinct molecular characteristics and clinical outcomes. Metastasis, regulated by transcription factors involved in embryonic development, such as TWIST1, is one of the main causes of BC mortality. TWIST1 acts as a regulator of gene expression by binding to consensus sequences known as E-boxes (CANNTG) within the promoter regions of target genes. Previously, our group demonstrated the overexpression of TWIST1 levels in the HER2+ BC subtype, influencing the IL-17 signaling pathway, which plays a critical role in the tumor microenvironment.

OBJECTIVES: We sought to examine the role of TWIST1 as a regulator of IL-17 signaling pathway genes in BC.

Method: We analyzed correlations between TWIST1 and IL-17 family members (assessing RNA, protein, and phospho-protein levels) using TCGA-BRCA and CPTAC-BRCA datasets. Positively correlated IL-17 genes had promoter sequences retrieved for TWIST1 binding-site prediction using bioinformatics tools. Furthermore, we modulated TWIST1 (overexpressing it in MCF7 and knocking it down in SKBR3) and measured the expression of the proposed regulatory targets by RT-qPCR.

RESULTS: Our bioinformatics analyses revealed a positive and statistically significant correlation of TWIST1 with IL17RA ($R=0.82$, $p=2.027e-3$) and with IL17D ($R=0.51$; $p<0.001$). Promoter region analyses identified two TWIST1 binding sites in IL17D (+296 bp and +321

bp; $MSS=0.99$ and 0.93 , respectively) and one site in IL17RA (-226 bp; $MSS = 0.86$), which were evolutionarily conserved in Metazoa. In accordance, RT-qPCR results demonstrated a statistically significant increase in IL17RA in the MCF7-pTWIST1 model ($p=0.035$), and a decrease in the SKBR3 Sh-TWIST1 model ($p=0.01$).

CONCLUSION: Our findings demonstrated that IL17D and IL17RA levels positively correlate with TWIST1 through bioinformatics analyses using BC databases. Furthermore, IL-17 family genes with predicted TWIST1 binding sites were supported by the modulation of IL17RA expression following the silencing or overexpression of TWIST1 in BC cellular models. As a perspective, ChIP assays will be conducted to confirm the direct binding of TWIST1 to the predicted promoter regions.

Keywords: Breast Cancer; TWIST1; IL17.

PAPER CODE: 8

Study of the role of SLPI protein in the cell cycle regulation in AML Leukemic Stem Cells

BRUM, M.C.M.; AZEVEDO, P.L.; GOMES, R.B.. INSTITUTO NACIONAL DO CÂNCER (INCA), RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Acute Myeloid Leukemia (AML) is a hematological malignancy characterized by differentiation and apoptosis block, leading accumulation of immature precursors in the bone marrow (BM). AML arise from the transformation of Hematopoietic Stem Cells (HSCs) into Leukemic Stem Cells (LSCs), influenced by BM microenvironment changes. Mesenchymal stromal cells (MSCs), key to HSC maintenance, may play a role in this process. To explore MSC-HSC signaling in AML, our group performed a proteomic analysis and showed that the secretome from co-culture assays with MSCs from AML patients (AML-MSCs) and healthy HSC, comparing to healthy controls presented increased levels of SLPI protein, which, besides its protease inhibitor activity, has been reported to regulate cell cycle-related genes.

OBJECTIVES: To evaluate whether changes in SLPI expression in AML-MSCs can influence the gene expression of cell cycle-related genes in HSCs.

METHOD: Silencing assays will be performed using the transient silencing method. For this purpose, the SLPI gene siRNA duplex (Thermo Fisher Scientific) will be transfected into AML-MSCs cells expressing high levels of SLPI. Scramble siRNA will be used as the transfection control. Through quantitative real-time PCR (qRT-PCR) we will evaluate the efficiency of SLPI silencing, as well as analyze the expression of cell cycle genes regulated by this protein.

RESULTS: AML-MSCs were subjected to SLPI silencing at 24, 48, 72, and 96 hours. Thus, we obtained two groups: SLPI-silenced (AML-MSC-siSLPI) and non-silenced (AML-MSC-SLPI). Through qRT-PCR, we will confirm the optimization of SLPI inhibition at the silencing times already performed. Once the optimal SLPI knockdown time is established, we will proceed with the remaining assays, such as Western blotting to confirm SLPI protein silencing.

CONCLUSION: Through coculture assays with MSC-AML cells silenced or not by SLPI and HSC cells from healthy donors, we will evaluate whether SLPI can alter the gene expression of cell cycle genes in HSCs. Transcriptomic analyses and investigation of interaction networks and biological functions linked to the genes found to be differentially expressed in HSCs after the coculture assays will also be performed.

Keywords: Acute myeloid leukemia; Mesenchymal stromal cells; SLPI.

PAPER CODE: 9

THE IMPACT OF THE AML MICROENVIRONMENT ON THE ACTIVATION OF THE PI3K/AKT PATHWAY IN HEMATOPOIETIC STEM CELLS

BASTOS, L.J.R.D.S.. INSTITUTO NACIONAL DO CÂNCER, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: The bone marrow microenvironment includes hematopoietic and non-hematopoietic

cells, such as mesenchymal stromal cells (hMSCs), which regulate hematopoiesis by controlling the balance between self-renewal and differentiation of hematopoietic stem cells (HSCs). In acute myeloid leukemia (AML), studies have shown that hMSCs from patients (hMSCs-AML) exhibit molecular alterations and our group has demonstrated that hMSCs-AML modulate gene expression in HSCs in coculture. In this study, we identified 133 differentially expressed genes, including AKT3 and other genes from PI3K/AKT pathway, frequently activated in leukemic stem cells (LSCs), suggesting their involvement in AML alterations.

OBJECTIVES: To evaluate the proteins secreted by hMSCs-AML that may be involved in the induction of the PI3K/AKT pathway in HSCs.

METHOD: For this, we used the Human Cytokine Array C5 ELISA (RayBiotech, USA), which allows the simultaneous detection of 80 human cytokines and growth factors. This assay was performed using the supernatant from the hMSC-AML and hMSCs from healthy donors culture at passage 3 after 72 hours of culture.

RESULTS: The analysis revealed increased expression of the secreted proteins HGF, VEGF, IGFBP4, OPG, and TIMP1 in AML-hMSCs compared to hMSCs from healthy donors. These factors may act as mediators of the activation of the PI3K/AKT pathway in hematopoietic stem cells (HSCs).

CONCLUSION: Initial data indicate a possible role for these proteins in modulating the PI3K/AKT pathway in the AML microenvironment from hMSC-AML. As a next step, functional assays will be performed in cocultures with healthy HSCs to confirm the influence of these proteins on the activation of this signaling pathway.

Keywords: Acute myeloid leukemia (AML); Secretome; PI3K/AKT signaling pathway.

PAPER CODE: 12

INVESTIGATION OF THE REGULATORY ROLE OF IRX3 IN GASTRIC CANCER

DA CONCEIÇÃO, J.O.; ROHAN, P.H.N.; DOS SANTOS, E.C.; ABDELHAY, E.S.F.W.. INCA, RIO DE JANEIRO - RJ - BRASIL.

Introduction: The IRX3 gene, a member of the Iroquois homeobox (IRX) family, encodes a transcription factor that regulates various developmental processes and cancer-related pathways. In gastric cancer (GC), our previous findings showed that IRX3 expression was reduced. However, its function in GC remains unknown.

OBJECTIVES: This study aimed to elucidate the role of transcription factor IRX3 in GC.

METHOD: We analyzed transcriptomic data from GEO and TCGA-STAD (2,214 samples). Three cohorts were defined: TCGA-STAD (n=412), Affymetrix (n=577), and Illumina (n=1,225). The IRX3 regulatory network was inferred using ARACNe, CLR, and GENIE3, integrating results across methods and cohorts. Candidate targets were validated by identifying the Iroquois Binding Site (IBS), ACAnTGT, within 3 kb upstream promoter regions. We evaluated IRX3 expression in GC cell lines by RT-qPCR.

RESULTS: The common interactions were selected resulting in 707 putative IRX3-gene interactions. Next, we ranked the regulatory interactions by the strength within each network, defined as the evidence level of interaction. From these, we selected the top 50 targets for each three cohorts. Subsequently, we found 17 common genes between the cohorts that were considered the most potential IRX3 targets. Of these, 14 had consensus IBS in the promoter region. Between these, the most probable targets were HOMER2, SCGB2A1, CCKBR, APLP1, ORM1, ENPP5, FOLR1, CXCL14, MAP7D2, CAPN9, SH3GL2 and also important gastric related genes such as LIPE, ATP4A, PSCA. The regulatory relationships found were positive, and we subsequently verified that the expression level of these targets is reduced in the tumor compared to the adjacent tissue, which is also the case of IRX3. We also identified GC cell lines with distinct IRX3 expression profiles by RT-qPCR, establishing a model for subsequent studies.

CONCLUSION: This study gathered evidence of putative targets of IRX3 in GC and established a model using cell lines with distinct IRX3 expression profiles.

Keywords: Gastric Cancer; IRX3; Regulatory Network.

PAPER CODE: 13

Comparison between allogeneic 19BBz CAR-T cells generated with CRISPR and Sleeping Beauty transposons

CORREIA, E.M.; ABDO, L.; ARAGÃO, E.; STEIMBACH, J.V.; ALVES, P.D.; NEUMANN, A.; NETO, L.; DE VASCONCELOS, B.M.; BONAMINO, M.H.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: CAR-T cell, despite being a promising technique that redirects T cell response to a specific tumor target through a chimeric antigen receptor (CAR), has some limitations. These include manufacturing and use restricted to a single patient, low-quality starting material, manufacturing delays, and high costs. To enable these cells to be used in more than one patient, genes encoding the TCR can be knocked out (KO), and the CAR transgene can be delivered via a transposon, such as Sleeping Beauty (SB), or donor DNA for site-specific recombination in the TCR alpha chain (KI). SB can improve CAR-T production by being cheaper and less labor-intensive than viral assembly and delivery. Donor DNA can also enhance the cells generated through knock-in (KI) of the transgene into specific genetic sites.

OBJECTIVES: Therefore, the present study aims to generate and compare allogeneic CAR-T 19BBz cells using CRISPR and SB approaches against the NALM6 tumor cell line.

METHOD: PBMCs were isolated and purified with CD3 columns, then electroporated using a 4D nucleofector with CRISPR RNPs, SB, or donor DNA to produce experimental groups: Mock (only electroporated cells), RNP (KO for TCR), RNP+SB (KO for TCR and CAR via SB), SB (only SB plasmids), and KI. TCR knockout cells were evaluated by flow cytometry.

RESULTS: Previously, we conducted an in vivo experiment pairing the CAR-T cell dose between the KI and SB groups, with the RNP+SB group receiving half the dose. We observed an improved survival curve for the KI compared to the others, and similar survival curves between the SB and RNP+SB groups. This indicates better tumor growth control with KI. Another in vivo trial paired the dose between the SB and RNP+SB groups, with the KI group receiving half the dose. We saw a slight increase in survival for RNP+SB compared to others, and similar survival between KI and SB groups. The KI at half the dose achieved the same tumor control effect as the full dose of SB. In our third in vivo experiment, we paired the dose among KI, SB, and RNP+SB groups and observed no difference in survival among all groups treated with CAR-T.

Conclusion: In conclusion, all allogeneic CAR-T cells appear to provide equal or higher tumor control and may also offer the benefits associated with allogeneic CAR-T therapy.

Keywords: CAR-T; CRISPR; Allogeneic.

PAPER CODE: 14

Development of Cellular Immunotherapies for Gastric Carcinomas

STEIMBACH, J.V.1; CORREIA, E.M.1; KHAYAT, A.S.2; DE ASSUMPTÃO, P.P.2; BONAMINO, M.H.1. 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. UFPA, BELÉM - PA - BRASIL.

INTRODUCTION: Gastric cancer is the fifth most common and deadly malignancy worldwide. Curative treatment relies on gastrectomy combined with chemotherapy and monoclonal antibodies. Since the 2000s, cellular therapies such as in vitro-expanded tumor-infiltrating lymphocytes (TIL) and anti-Claudin 18.2 CAR-T cells have been tested in advanced cases, but with limited success. Like most solid tumors, CAR-T cells in gastric cancer show low persistence and expansion

OBJECTIVES: This project aims to identify therapeutic targets in patients and develop cell therapy products from healthy donors' lymphocytes or patient-derived TIL to elicit strong, durable antitumor responses

METHOD: Patient transcriptomics was evaluated to explore targetable immune interactions. CRISPR/Cas9 gene-editing was used to disrupt PDCD1, HAVCR2, CTLA4, and LAG3 expression in anti-Claudin 18.2 CAR-T cells and TIL.

RESULTS: Preliminary RNA-seq analysis of 120 Brazilian patients revealed differential expression of tumor microenvironment genes. In primary tumors, CTLA4 and HAVCR2 were significantly upregulated compared to adjacent tissues. In peritoneal metastases, expression of CD274, LGAL9, FGL1 (ligands for PD-1, TIM-3, and LAG-3, respectively) and HAVCR2 was increased. Additionally, CLDN18.2 was predominant over isoform 1, highlighting its potential as an immunotherapy target. Notably, anti-Claudin 18.2 CAR-T cells were generated using CRISPR/Cas9 to knock-in the CAR construct at the CTLA4 loci, ensuring gene disruption in CAR+ cells. CAR-T cells with CTLA4 knock-in exhibited potent antitumor activity against a gastric cancer cell line overexpressing CLDN18.2, confirming the functional efficacy of this strategy. In addition, TIL were isolated and expanded from ascitic fluid of a gastric cancer patient, yielding 75 million cells with an effector and minimally exhausted phenotype at the end of expansion.

CONCLUSION: This project aims to enhance cell resistance and expansion in the suppressive tumor microenvironment, advancing cellular therapy for gastric cancer, with the goal of improving patient outcomes.

Keywords: Cell Therapy; Gastric Cancer; Inhibitory Receptors.

PAPER CODE: 16

EVALUATION OF RAS INHIBITION AS A POTENTIAL THERAPEUTIC STRATEGY IN ACRAL MELANOMA

MAGALHÃES, M.V.D.C.1; ALVES, T.C.1; BARROS, P.S.D.R.1; ANNAMALAI, G.2; APLIN, A.2; ADAMS, D.3; POSSIK, P.A.1. 1. BRAZILIAN NATIONAL CANCER INSTITUTE (INCA), RIO DE JANEIRO - RJ - BRASIL; 2. THOMAS JEFFERSON UNIVERSITY, PHILADELPHIA - ESTADOS UNIDOS DA AMERICA; 3. WELLCOME SANGER INSTITUTE, CAMBRIDGE - REINO UNIDO.

INTRODUCTION: Acral melanoma (AM) is a subtype of melanoma that develops on the palms, soles and subungual areas. This is the one of deadliest subtypes of melanoma and there are no therapeutic strategies designed specifically to treat AM patients. In this context, our group has generated a platform of tumor tissue samples, patient-derived xenografts (PDX) and cell lines derived from AM patients. In our cohort, missense mutations in Ras family genes account for 27% of the cases. These RAS-mutant tumors are targettable by a RMC-7977, a Ras inhibitor that has been showing promising effects in lung, colorectal and pancreatic cancer cell lines, in vitro and in vivo. In this way, it is important to search for new targets that can increase the cytotoxicity with Ras inhibition.

OBJECTIVES: Thus, the aim of this project is to evaluate the therapeutic potential of RMC-7977 and search for combinatorial targets that can enhance the effects of Ras inhibition in AM.

METHOD: Cell viability was evaluated in AM007 (NRASQ61P) and AM016 (KRASQ61H) cell lines using dose-response assays after 72h of RMC-7977 treatment. ERK and AKT phosphorylation levels were analysed in AM007 cells after 1h treatment by Western blotting. To characterize the expression profile, three RAS-mutant AM cell lines were treated with RMC-7977 or DMSO for 8 hours and submitted to RNA sequencing. Differential gene expression and pathway enrichment analyses were performed comparing both conditions.

RESULTS: Both AM007 and AM016 cell lines showed sensitivity to RMC-7977, with IC20 values of 37.91 nM

and 15.53 nM, respectively. An IC50 of 93.59 nM was estimated for AM007 whereas AM016 cells did not reach the IC50 concentration, indicating less sensitivity. A dose-dependent reduction in ERK phosphorylation was observed in AM007 cells treated with RMC-7977 for 1 hour. No effect in AKT phosphorylation was observed. Differential expression analyses revealed downregulation of MAP Kinase pathway genes and upregulation of epithelial to mesenchymal transition (EMT)-related genes.

CONCLUSION: In conclusion, RMC-7977 reduced viability of RAS-mutant AM cell lines in addition to reducing ERK phosphorylation levels without changes on AKT phosphorylation. Further exploration of the RNA sequencing data combined with functional assays will help to characterize the response patterns of RAS-mutant AM cells to RMC-7977.

Keywords: Acral melanoma; Ras inhibition; RMC-7977.

PAPER CODE: 17

Uncovering the Impact of SAA1 in Leukemic Transformation

THOMPSON, M.F.; AZEVEDO, P.L.; GOMES, R.B.. INSTITUTO NACIONAL DO CANCER (INCA), RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Acute myeloid leukemia (AML) is a heterogeneous hematological neoplasm characterized by the proliferation of myeloid cells, reduced apoptosis, and a block in cellular differentiation, originating from the transformation of hematopoietic stem cells (HSCs) into leukemic stem cells. The bone marrow microenvironment (BMM) is crucial for the maintenance of HSCs and alterations in this microenvironment may influence this transformation. Through proteomic analysis of bone marrow (BM) plasma, we identified an increased expression of SAA1 protein in the plasma of AML patients, suggesting it as a potential biomarker for AML. SAA1 is a secreted protein involved in inflammatory processes and may be associated with the dysregulation of signaling pathways linked to leukemic transformation.

OBJECTIVES: The aim of this study was to evaluate the impact of SAA1 on leukemic transformation.

METHOD: To confirm the increased expression of SAA1, we performed an ELISA assay using BM plasma from 22 AML patients and compared it with BM plasma from 16 healthy donors (HD). To identify which cell types express SAA1, we used the WEBGestalt tool. To assess the gene expression of SAA1 in Mesenchymal Stromal Cells (MSCs), we performed qPCR. Finally, to analyze pathways potentially related to SAA1, we used the Reactome Pathway database.

RESULTS: Using individual BM plasma samples from AML patients and HD in the ELISA assay, we confirmed the increased expression of SAA1 in AML patients. Since elevated levels of SAA1 were found in plasma, we decided to investigate which cell types present in the BM could be related to SAA1 expression. For this, we conducted an analysis using WEBGestalt. In this analysis, the cell types associated with MO are differentiated cells of hematopoietic origin. This result indicated that the increased expression of SAA1 is not related to differentiated hematopoietic cells. Thus, we decided to evaluate other cell types present in the BM that could express SAA1. As our group works with MSCs, we assessed by qPCR whether these cells express SAA1. Our results show that MSCs do not express SAA1. In parallel, we evaluated possible pathways activated by SAA1 using the Reactome Pathway database. Interestingly, we found that the NF- κ B pathway can be activated by SAA1.

CONCLUSION: So far, we have observed that SAA1 expression is elevated in the BM plasma of AML patients, but we still do not know which cell type may be associated with this increased expression. To continue, we will evaluate the expression of SAA1 in HSCs from both AML patients and HD. Furthermore, we will assess whether components of the NF- κ B pathway are altered in HSCs.

Keywords: SAA1; bone marrow microenvironment (BMM); acute myeloid leukemia (AML).

PAPER CODE: 18

IDENTIFICATION AND CHARACTERIZATION OF THE NUCLEAR LOCALIZATION SIGNAL (NLS) OF THE TUMOR SUPPRESSOR PALB2

DE SOUZA, G.F. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Cancer is the second leading cause of death worldwide, and genomic instability is one of its hallmarks. A major contributor is dysfunction of DNA repair pathways, such as homologous recombination (HR), which repairs DNA double-strand breaks during the S and G2 phases. PALB2 (Partner and Localizer of BRCA2) is a tumor suppressor that links BRCA1 and BRCA2, forming a complex essential for HR. Pathogenic PALB2 variants disrupt this complex and are linked to tumorigenesis. Mutations in its WD40 domain may expose the nuclear export signal (NES), driving cytoplasmic localization. Although PALB2 is mainly nuclear, no nuclear localization signal (NLS) has been defined. This study investigates the putative NLS of PALB2.

OBJECTIVES: To identify and characterize the nuclear localization signal (NLS) of PALB2 through in silico prediction, molecular cloning, and subcellular localization analysis in a human cell model.

METHOD: Three bioinformatic tools (NLStradamus, cNLS Mapper, NoD) were used to predict NLS motifs. Constructs encoding full-length or truncated PALB2 fused to EGFP were generated. Eight constructs (N1, N2, N3, C1, C2, C3, Δ NLS, L35P- Δ NLS) were transfected into U-2 OS cells. Subcellular localization of wild-type and mutant PALB2 proteins was analyzed by fluorescence microscopy and by immunoblotting of nuclear and cytoplasmic fractions.

RESULTS: Bioinformatic analysis identified a conserved sequence (146RRKKQQR153) as a candidate NLS in the N-terminal region of PALB2. Constructs containing this region (FL-WT, N1, N2, N3) localized predominantly to the nucleus, while those lacking it (C1, C2, C3) were cytoplasmic. Deletion of the putative NLS or the L35P mutation combined with NLS deletion shifted

localization toward the cytoplasm, though not fully. These findings support the in silico prediction of a functional NLS in PALB2's N-terminal region. Ongoing immunoblotting of nuclear and cytoplasmic fractions will further validate microscopy data and clarify the link between localization and NLS integrity.

CONCLUSION: We identified a functional nuclear localization signal in PALB2 (146RRKKQQR153). Constructs containing this motif localized to the nucleus, whereas those lacking it were cytoplasmic. Characterizing sequences that control PALB2 localization is crucial to understanding how this tumor suppressor preserves genome stability, as mislocalization may disrupt BRCA1–PALB2–BRCA2 complex formation, impair homologous recombination, and promote tumorigenesis.

Keywords: PALB2; Tumor suppressor; Nuclear Localization Signal.

PAPER CODE: 31

MGAT5 AFFECTS THE EXPRESSION OF GENES RELATED TO TUMOR PROGRESSION IN COLON ADENOCARCINOMA

SARAIVA, M.D.S.F.1; TOUÇA, N.2; BINATO, R.1; FREITAS, J.2. 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. STATE UNIVERSITY OF RIO DE JANEIRO (UERJ), RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: The acquisition of a malignant cellular phenotype may be associated with alterations in the glycan profile. In colorectal tumors, the expression of N-acetylglucosaminyltransferase 5 (MGAT5) is increased, leading to the production of branched N-glycans (tri- or tetra-antennary). Given the importance of these glycans in tumor progression — including adhesion loss, migration, signaling activation, and metastasis — this study aims to investigate the consequences of Mgat5 inhibition (via CRISPR-Cas9) on the global gene expression profile of murine colon adenocarcinoma cells (MC38).

OBJECTIVES: The objective is to identify pro-tumoral molecular mechanisms dependent on branched N-glycans.

METHOD: TCGA data were analyzed using bioinformatics platforms (GEPIA, UALCAN, cBioPortal, and UCSC XENA). Biological processes were identified with Metacore software. Expression and protein levels were assessed by qRT-PCR and western blotting. Functional assays included viability (trypan blue), proliferation and clonogenic potential (crystal violet), migration/invasion (transwell), and cell cycle analysis.

RESULTS: In silico analyses showed elevated MGAT5 protein levels across multiple cancers, with high expression linked to poorer disease-free survival. In colorectal cancer, MGAT5 varied among CMS subtypes, being highest in CMS4, the most aggressive group. In MC38 cells, Mgat5 knockout altered HIF1A targets, including Cdkn1a (p21), confirmed by expression and protein data. MGAT5 and CDKN1A were negatively correlated in colon tumors, with CDKN1A higher in normal tissue. Clinically, high CDKN1A correlated with better survival, and patients with low MGAT5/high CDKN1A had improved outcomes. Functionally, Mgat5 KO cells showed reduced proliferation and colony formation, likely due to Cdkn1a modulation. Since branched N-glycans stabilize receptor tyrosine kinases and AKT signaling drives cytoplasmic p21, reduced pathway activity may favor nuclear p21 retention. Supporting this, AKT and p21 phosphorylation decreased in KO cells, which accumulated in G1, reinforcing the tumor suppressor role of p21.

CONCLUSION: Finally, potential transcription factors involved in MGAT5-mediated regulation of p21 are being explored. In summary, this study provides new insights into the role of MGAT5 and branched N-glycans in tumorigenesis and their impact on the regulation of tumor suppressor pathways.

Keywords: colorectal cancer; MGAT5; p21.

PAPER CODE: 32**THE ROLE OF IROQUOIS FAMILY GENES IN GASTRIC CANCER CARCINOGENESIS**

DOS SANTOS, E.C.; DA CONCEIÇÃO, J.O.; ROHAN, P.H.N.; ABDELHAY, E.S.F.W.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Gastric cancer (GC), the fifth leading cause of cancer-related deaths, accounted for 660,175 deaths and 968,784 new cases in 2022. Our previous data indicated that Iroquois genes, transcription factors, exhibit reduced expression in GC. These genes regulate processes such as cell differentiation and development, being associated with pathways like wingless, hedgehog, TGF- β , and PI3K/Akt, which are altered in cancer. Their functions in cancer are context-dependent, potentially acting as oncogenes or suppressors. Notwithstanding, information in GC remains limited.

OBJECTIVES: This study aims to evaluate the role of Iroquois (Irx) family genes in gastric carcinogenesis.

METHOD: In silico analysis used R (v4.3.2) with TCGA/ GEO data, processed via DESeq2 (v1.44.0), affy (v1.82.0), ComBat sva (3.52.0). Networks were built with ARACNE, CLR, GENIE3, visualized in Cytoscape (v3.10.3), with correlations via corrpilot and enrichment via free software. GC cell lines and normal tissue were cultured. Gene expression was quantified by RT-qPCR. Lentiviral plasmids (abm) were used for Iroquois overexpression/ inhibition models.

RESULTS: A database were built from 2214 public GC samples (TCGA-STAD: 412 RNA-seq; Affymetrix GEO: 577 from 4 studies; Illumina GEO: 1225 from 8 studies). Reduced IRX3 and IRX5 expression were confirmed in GC samples. Regulatory networks reconstructed identified 73 targets for IRX3 and 46 for IRX5, with shared targets, suggesting regulatory interaction. Correlation between IRX3 and IRX5 was confirmed. Enrichment analysis revealed pathways like EMT, KRAS, and angiogenesis, indicating that low IRX3 and IRX5 expression may impact key processes in gastric carcinogenesis. Six gastric cell lines (AGS, NCI-N87, AGP01, HGC-27, KATOIII, MNP01) were cultured to evaluate Irx gene expression.

RT-qPCR showed variable IRX3 expression (increased in AGP01, HGC-27; decreased in NCI-N87; undetectable in AGS, KATOIII) and reduced IRX5 in all tumor lines. Lentiviral transduction of siRNA-IRX5 in MNP01 and AGP01 confirmed by GFP expression.

CONCLUSION: In silico analysis identified downregulated IRX3 and IRX5 and differentially expressed genes, in GC across 2214 samples from diverse populations, confirming generalizability. Reduced IRX3/IRX5 expression may impact key carcinogenesis pathways. Cell line studies showed variable IRX3 and reduced IRX5 expression. Ongoing silencing experiments will further elucidate their regulatory roles.

Keywords: Gastric Cancer; Iroquois; Regulatory Network.

PAPER CODE: 33**ANTITUMOR POTENTIAL OF MEVALONATE PATHWAY INHIBITORS THROUGH MODULATION OF N-GLYCOME IN COLORECTAL CANCER CELLS**

MYNSEN, G.F.D.L.. INSTITUTO NACIONAL DE CÂNCER (INCA), RIO DE JANEIRO - RJ - BRASIL.

Introduction: During the progression of solid tumors, including colorectal cancer (CRC), alterations in the N-glycosylation modulate protein functionality and, in certain cases, contribute to the acquisition of malignant phenotype. CRC cells are known to exhibit not only an overall increase in N-glycan levels, but also elevated levels of specific glycan structures. Such structural modifications in N-glycans at the tumor cell surface play a critical role in regulating cellular mechanisms associated with malignancy, including proliferation, epithelial-mesenchymal transition (EMT), migration, and invasion. To date, no therapeutic strategies aimed at remodeling the glycome have been translated into clinical practice. Consequently, drug repurposing has emerged as a promising approach.

OBJECTIVES: This study evaluated the antitumor effects of statins on the N-glycome of CRC cells by inhibiting the mevalonate pathway and disrupting dolichol biosynthesis involved in N-glycosylation.

METHOD: Two colorectal cancer cell lines (SW480 and SW620) were treated with fluvastatin or simvastatin to inhibit the mevalonate pathway. Cell viability (MTT), clonogenicity, migration/invasion (transwell), and N-glycome changes (lectin-based flow cytometry) were evaluated. In silico analyses (GEPIA2, UALCAN, cBioPortal), qRT-PCR, Western blot, and immunofluorescence were used to assess gene/protein expression and cytoskeletal or adhesion alterations.

RESULTS: Our results demonstrated that SW480 cells were more sensitive to statin treatment than SW620 cells, as reflected by their lower IC50 values. Among the statins tested, simvastatin exhibited greater potency in reducing cell viability compared with fluvastatin in both cell lines. Additionally, statin treatment significantly reduced clonogenic capacity and migration potential. Unexpectedly, inhibition of the mevalonate pathway by either simvastatin or fluvastatin did not result in substantial alterations in total N-glycan content or in glycan structures typically associated with tumor progression.

CONCLUSION: These findings suggest that the antitumor effects of statins in CRC extend beyond glycosylation remodeling, warranting further investigation into the underlying molecular mechanisms and their therapeutic potential.

Keywords: colorectal cancer; N-glycome; statins.

PAPER CODE: 35

Impact of irradiation on the remodeling of the tumor extracellular matrix and its effects on the cell polarity in CCR

COSTA, R.M.B.. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: The organization of cell polarity complexes is supported by the cell adhesion to the extracellular matrix (ECM). Modifications in this polarity

complex are considered hallmarks of cancer, causing epithelial-mesenchymal transition and invasion. The ECM remodeling provides a unique molecular and biomechanical niche that regulate tumor behavior through the activation of a variety of intracellular signaling pathways triggered by the cell-ECM contact. However, the knowledge of the mechanisms involved in cell-ECM interaction is scarce on literature, particularly when tumors are subjected to radiotherapy treatment, a very common type of treatment in colorectal cancer (CRC). Thus, developing research in this area is relevant to understand CCR progression and recurrence

OBJECTIVES: Elucidate the impact of irradiation on the remodeling of the tumor ECM and its effects on CCR cells, analyzing alterations in cell polarity and the development of epithelial-mesenchymal transition

METHOD: SW-480 cells were irradiated with a single dose of 5Gy, incubated for 24h and replated. After 48h, cells ECM (ECMIR) was obtained and immunofluorescence of collagen and fibronectin (FN) were performed. Nonirradiated SW-480 cells were seeded on top of ECMs, cell lysed obtained and Western blot for protein analysis was performed. In parallel, cell migration was assessed by using the Wound Healing. Survival curves (univariate Kaplan-Meier) and gene expression were generated using TCGA

RESULTS: We observed an increase in FN fluorescence but no in collagen 1 in ECMIR compared to the control. We observed an increase in the protein levels of SLUG, N-cadherin, α -SMA, α -DLG and pSCRIB when SW-480 nonirradiated cells were grown the ECMIR compared to the control group. We did not observe differences in the expression of vimentin and E-cadherin between groups. TCGA data showed that increased expression of DLG1 is associated with poor survival in CRC patients. Cell polarity proteins were closely related to cell junctions and TCGA data show a positive correlation between DLG1 with occluding and ZO-2. In addition, we observed a decrease in the protein expression levels of occludin and ZO-2 in SW-480 cells, which were seeded on ECMIR. Supporting these findings, TCGA data showed that the decrease of ZO-2 is associated with poorer survival in CRC patients. Finally, cells seeded on the ECM IR migrate more compared to the control

CONCLUSION: Our results suggest that irradiation causes tumor ECM remodeling and the contact with non-irradiated CRC cells influences the organization of the cell polarity complex and cell junctions, triggering EMT and cell migration. Bioinformatics analyses and immunoblotting suggests that ECM remodeling may be related to changes in cell polarity and intercellular junction proteins and that this may be associated with worse patient survival. More experiments are underway to confirm these results

Keywords: Extracellular matrix; Irradiation; Cell polarity.

PAPER CODE: 36

The Wnt/ β -catenin pathway as a central player in the development of epithelial-mesenchymal transition and stem-cell features as chemoresistance mechanisms in colorectal cancer

DE PINA, D.F.T.. INSTITUTO NACIONAL DO CANCER, PRAIA - CABO VERDE.

INTRODUCTION: Células tumorais com alta plasticidade, como as que passam pela transição epitélio-mesenchimal (TEM), são capazes de desenvolver características de células-tronco cancerígenas (CSCs) por meio da interação com células estromais associadas ao tumor, o que aumenta a resistência à quimioterapia, levando à recorrência tumoral e à formação de metástases. A ativação persistente da via Wnt/ β -catenina tem sido hipotetizada em células de câncer colorretal (CCR) resistentes à quimioterapia. No entanto, a associação entre essa via e o desenvolvimento de células-tronco na EMT induzida por resistência terapêutica permanece obscura.

OBJECTIVES: O objetivo deste estudo é avaliar se a via Wnt/ β -catenina medeia o surgimento de CSCs de células em EMT durante a aquisição de quimiorresistência.

METHOD: Neste estudo, utilizamos células HCT-116 resistentes ao tratamento com 5-fluorouracil (HCT116-5FUR), obtidas e caracterizadas em nosso laboratório. As células foram descongeladas e a manutenção do

fenótipo de resistência foi devidamente confirmada pelo tratamento das células com concentrações crescentes de 5-FU (1-100 μ M), e a viabilidade celular foi analisada por MTT. Foram analisados parâmetros morfométricos (área, perímetro e circularidade). A localização subcelular de proteínas envolvidas na via EMT e Wnt/ β -catenina

RESULTS: O ensaio de viabilidade celular mostrou que o HCT116-5FUR manteve o fenótipo resistente após o descongelamento, em comparação com as células controle. A análise morfométrica dos esferoides revelou que, ao longo do período de cultura de 7 dias, a linhagem celular parental apresentou uma organização estrutural compacta, esférica e uniforme, enquanto a linhagem resistente apresentou morfologia irregular com múltiplos esferoides, menor compactação e presença de projeções de membrana. A análise de imunofluorescência por microscopia confocal mostrou que as proteínas claudina-3 e β -catenina estavam localizadas na membrana celular das células controle.

CONCLUSION: Nossos resultados indicam que o modelo de resistência ao 5-FU obtido anteriormente manteve o fenótipo de resistência e pode servir como um modelo experimental adequado para experimentos futuros. Além disso, o modelo 3D, aqui desenvolvido, abre caminhos para a continuidade do nosso projeto.

Keywords: colorretal cancer; model 3D; resistance.

PAPER CODE: 37

ANTITUMOR RESPONSE EVALUATION OF ANTI-GD2 AND ANTI-HER2 CAR-T CELL THERAPY IN ACRAL MELANOMA

DA COSTA, M.R.1; BONAMINO, M.H.2; POSSIK, P.A.2. 1. NATIONAL CANCER INSTITUTE (INCA), RIO DE JANEIRO - RJ - BRASIL; 2. NATIONAL CANCER INSTITUTE, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Acral melanoma (AM) is the most aggressive subtype among cutaneous melanomas, which is characterized by malignant melanocytes in body extremities such as palms, soles and subungual tissue. Among some treatment options in the

metastatic landscape, checkpoint inhibitors (anti-CTLA4 or anti-PD1/PDL1) remain the most effective. However, despite improved survival and disease regression in some cases compared to other treatments, response rates remain relatively lower compared to other melanoma subtypes. In this context, new therapeutic approaches are constantly being explored, such as CAR-T cell therapy. Among promising targets, HER2 and GD2 are highlighted for their high expression in some tumors, their biological functions and the existence of preliminary CAR-T studies in melanoma.

OBJECTIVES: Following this premise, the main objective of this project is to evaluate the antitumor potential of anti-GD2 and anti-HER2 CAR-T cells in acral melanoma models.

METHOD: Six AM cell lines were analyzed by flow cytometry for GD2 and HER2 levels. Five of those were transduced with lentivirus carrying a sequence encoding the GFP protein followed by sorting of GFP+ cells. Peripheral blood mononuclear cells (PBMC) from healthy donors will be recovered and modified for CAR-T by plasmidial transfection with the Sleeping Beauty transposon system. The effect of CAR-T cells will be analysed by co-culturing them with GFP+ cells using time-lapse fluorescence microscopy.

RESULTS: All cell lines showed expression of HER2 and GD2, although the frequency of GD2+ cells varied across cell lines, with lower levels in AM015a, AM015b and AM022a cells. In addition, the measurement of Mean Fluorescence Intensity (MFI) per cell showed that the mean levels of GD2 were higher in AM007, AM016 and AM021b cells compared to other cell lines, whereas HER2 showed higher MFI measures in AM007, AM021b and AM022a. In parallel, GFP+ AM cell lines were successfully established and stored for subsequent experiments.

CONCLUSION: Our preliminary results showed that HER2 and GD2 are present in all AM cell lines analyzed, although at different levels. AM007 and AM021b were selected for co-culture experiments due to their higher frequency of HER2 and GD2 positive cells with high levels of these biomolecules.

Keywords: Melanoma; CAR-T; GD2.

PAPER CODE: 39

MULTI-OMICS MODEL FOR PREDICTING THERAPY RESISTANCE IN HIGH-GRADE SEROUS OVARIAN CANCER

TEIXEIRA, C.E.1; TESSAROLLO, N.1; BAPTISTA, G.1; PAREDES, M.E.1; SERAIN, A.1; CHIAVEGATTO, A.2; BORONI, M.1. 1. INSTITUTO NACIONAL DE CÂNCER, RJ - RJ - BRASIL; 2. UNIVERSIDADE DE SÃO PAULO, SP - SP - BRASIL.

INTRODUCTION: Ovarian cancer (OC) is the deadliest gynecologic malignancy, with poor survival in advanced stages. High-grade serous ovarian cancer (HGSOC), the most common subtype, is mainly treated with surgery and platinum–paclitaxel chemotherapy, but outcomes remain limited.

OBJECTIVES: We aim to predict treatment response in HGSOC using clinical and multi-omics data (DNA-Seq and RNA-seq) from The Cancer Genome Atlas (TCGA) and Brazilian National Cancer Institute (INCA) cohorts.

METHOD: Patients were stratified as platinum-resistant ($PFI \leq 6$ m) or platinum-sensitive ($PFI > 6$ m), further classified into partially (6–12 m), sensitive (12–24 m), and highly sensitive (> 24 m). Differential gene expression and mutational profiles were analyzed. Data were split into training (80%) and test (20%). Feature selection used RFE, Cox, and Boruta. Machine learning models were trained with cross-validation. Performance was evaluated by AUC and sensitivity, and SHAP identified key biomarkers.

RESULTS: Following feature selection and mutational profiling, 212 and 40 features related to gene expression and mutations, respectively were identified as relevant. The deep multi-modal fusion network incorporating a gated algorithm demonstrated strong predictive performance (AUC = 0.900; sensitivity = 0.860). Explainable AI analysis using SHAP highlighted L3MBTL1 as a key predictor, a gene known to suppress p53 activity through chromatin regulation. Additional important predictors included CXCR4 and HINT4, both associated with cell proliferation pathways, as well as mutations in TP53 and KIAA1109. Ongoing functional analyses aim to elucidate the clinical relevance of these predictors in ovarian cancer.

CONCLUSION: Developed through the integration of TCGA and Brazil's INCA cohort data, this multi-omics model is robust and generalizable, showing strong potential for clinical translation within the public health system to enable precise patient stratification and support personalized treatment strategies for ovarian cancer.

Keywords: machine learning; ovarian cancer; multiomics.

PAPER CODE: 40

Investigation of dietary influence on lipid droplets in colon cancer development in vivo

BRANCO, J.M.C.R.; OLIVEIRA-VIEIRA, B.; VIOLA, J.P.B..
INSTITUTO NACIONAL DE CANCER (INCA), RIO DE JANEIRO
- RJ - BRASIL.

INTRODUCTION: Colon cancer is one of the most prevalent malignancies worldwide and is strongly associated with obesity and chronic inflammation, both of which are influenced by lifestyle factors, particularly dietary habits. Technological advances in food processing and availability have increased the consumption of hypercaloric diets, leading to excessive caloric intake and lipid accumulation within cells. This accumulation interferes with metabolic pathways linked to poor tumor prognosis. In cancer cells, altered lipid metabolism involves the modulation of key metabolic regulators and may represent potential therapeutic targets for anti-cancer strategies.

OBJECTIVES: This study aimed to investigate the influence of different dietary patterns on the profile and frequency of lipid droplets (LDs) during tumor progression using a murine model.

METHOD: C57BL/6J mice were fed for 12 weeks with one of three diets: Chow (control), high-fat high-sucrose (HFHS), or a fast-food mimicking diet (FFMD). After the dietary intervention, colorectal carcinogenesis was induced using azoxymethane (AOM) followed by three cycles of dextran sulfate sodium (DSS).

RESULTS: After 12 weeks, mice on high-calorie diets (HFHS and FFMD) consumed fewer calories than the Chow group but still gained more weight and visceral fat. These diets caused hepatic steatosis and insulin resistance. The AOM/DSS protocol induced tumors in all groups, but mice on high-calorie diets developed larger tumors and had higher mortality, especially the FFMD group. Although the overall frequencies of B cells, T cells, and CD4⁺/CD8⁺ T cell subsets in mesenteric lymph nodes remained unchanged, the diets altered activation profiles of CD4⁺ and CD8⁺ T cells. These changes differed by sex and involved increased expression of exhaustion markers PD-1, Tim-3, and Lag-3 on CD8⁺ T cells.

CONCLUSION: Together, these findings suggest that diet composition and sex exert distinct effects on immune activation and exhaustion during colorectal tumorigenesis, highlighting the interplay between metabolic and immune pathways in cancer progression.

Keywords: Lipid Droplets; Diet-induced carcinogenesis; Tumor progression.

PAPER CODE: 43

AN UNEXPECTED ROLE OF PALB2 LINKS HOMOLOGOUS RECOMBINATION AND CENTROSOME BIOLOGY

ELIAS, A.B.R.1; GOMES, T.T.1; NEPOMUCENO, T.C.2;
CANAVARRO, F.S.3; SUAREZ-KURTZ, G.1; MONTEIRO, A.N.2;
CARVALHO, M.A.3. 1. INCA, RIO DE JANEIRO - RJ - BRASIL;
2. MOFFIT, TAMPA - ESTADOS UNIDOS DA AMERICA; 3.
IFRJ, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Mutations in PALB2 are associated with predisposition to breast and pancreatic cancers. It encodes a homonymous protein responsible for maintaining genomic integrity by playing a crucial role in the DNA damage response. PALB2 interacts with BRCA1 (through the coiled-coil domain (CC) of both proteins), and BRCA2 (through the WD40 domain of PALB2), working as a linker between these two proteins in the homologous recombination (HR) pathway,

enabling the recruitment of RAD51 recombinase to DNA-damaged sites. Despite the growing interest in PALB2 in recent years, its molecular function beyond the HR pathway is poorly understood.

OBJECTIVES: Identifying new PALB2 interactors may help clarify the broader biological roles of this protein.

METHOD: Using a yeast two-hybrid screen, we identified ROCK2, a centrosome duplication regulator, as a putative new interaction partner of PALB2. We used human cells protein extracts in protein co-immunoprecipitation and GST-pulldown assays to validate and characterize this interaction. We also used confocal microscopy to investigate the presence of PALB2 in centrosome structure using g-tubulin protein as a specific centrosome marker. DNA damages were induced by IR treatment in different experiments.

RESULTS: We confirmed PALB2-ROCK2 interaction in human cells and revealed that this interaction occurs through PALB2 N-terminal region, where the CC is situated. We observed that PALB2 mutants that compromise the CC, and consequently the BRCA1 interaction, exhibited enhanced binding to ROCK2, suggesting a ROCK2 and BRCA1 competition for PALB2 binding. We also induced DNA damage and observed a decrease in the PALB2-ROCK2 interaction. Since PALB2 phosphorylation is important for BRCA1 binding, we also treated protein extracts with phosphatase and observed increased PALB2-ROCK2 interaction, corroborating our hypothesis. Furthermore, we identify PALB2 colocalization with g-tubulin in human cells BRCA1-proficient and BRCA1-deficient, indicating a BRCA1-independent phenomenon. Moreover, we provided evidence that PALB2 is less present at the centrosome after DNA damage induction when compared with non-treated cells, which is in consonance with our previous hypothesis.

CONCLUSION: Collectively, our results identified ROCK2 as a new interaction partner of PALB2 and suggest a competition with BRCA1 for PALB2 interaction in the DNA damage response. Additionally, we revealed a previously unrecognized localization of PALB2 at the centrosome, uncovering a novel role of PALB2 beyond

the HR pathway. These results provide insight into a better understanding of the PALB2 functions in cellular biology and cancer development.

Keywords: DNA damage; Centrosome; PALB2.

PAPER CODE: 44

Identification and Characterization of HPV45 Transcriptional Profiles in Cervical Cancer

GONÇALVES, L.F.A.; SCHERER, N.D.M.; BORONI, M.; FELIX, S.P.; FURTADO, C.; MOREIRA, M.Â.M.. INSTITUTO NACIONAL DO CÂNCER, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Human Papillomavirus (HPV) is a small, circular, and double-stranded DNA virus whose persistent infection is primarily associated with cervical cancer (CC). HPV produces polycistronic mRNA that undergoes alternative splicing. Although non-obligatory, the viral genome can integrate into the human genome and may lead to chimeric (human+viral) transcripts. HPV transcripts are pivotal for infection and carcinogenesis, playing roles in cellular gene expression regulation, development of lesions and tumor progression. Of the fifteen high-risk HPV (HR-HPV) genotypes, HPV45 is the third most prevalent in cervical cancer, however, its transcripts remain poorly characterized.

OBJECTIVES: This study aims to characterize and quantify HPV45 transcripts/isoforms (viral and chimeric) from CC, to build HPV45 transcript map and compare genes/biological processes associated with HPV45/16/18.

METHOD: Total RNA was extracted from 25 HPV45+ CC samples. Libraries were prepared with rRNA depletion approach, and sequenced on NovaSeq X Plus (Illumina) platform. Transcripts were de novo assembled with Trinity (v2.15.1) and aligned to the human genome, HR-HPV genomes and HPV6/11 using BLAT (v2.5.1). Reads aligned to the HPV45 genome were characterized through PaVE BLAST and Human BLAT tools. Transcript quantification, read alignment (back-mapping), and enrichment analyses will be further performed.

RESULTS: The processing of 4 samples failed at different steps of the workflow. From the 21 samples analyzed, a total of 138 HPV45-related transcripts were identified, of which 118 also mapped to the human genome. These chimeric transcripts aligned to the human genome in chromosome 1 (ENSG00000308843 and ENSG00000305041), 2 (ENSG00000286223), 3 (TP63, NSUN3, TMCC1 and intergenic region), 4 (SLC10A6 and intergenic region), 6 (LINC01600), 10 (MANCR), 11 (MAP6 and FAM168A), 13 (ELF1 and intergenic region), 14 (RAD51B), 17 (NTN1), 18 (intergenic region), 20 (STK4) and X (CHRD1 and STS). Most transcripts mapped to intronic regions (n=67), followed by exonic (n=15) and combined regions (n=11). Regarding HPV45, 14 transcripts aligned to a single HPV gene: E1 (n=2), E2 (n=1), L1 (n=5), L2 (n=5) and LCR (n=1). The other distinct HPV45 transcripts aligned to multiple viral genes: E6+E7 region in 108 transcripts, LCR in 104, L1+L2 in 32, L1 in 20, L2 in 7, E5α in 16, E1+E2 in 20, E1 in 33 and E2 in 20.

CONCLUSION: HPV45 is a genotype with limited information on its transcripts. Here, we report a large diversity of HPV45 assemblies and a high frequency of viral genome integration resulting in chimeric transcripts. Transcript sequences are currently being analyzed to elucidate splicing and integration events. Future analyses will evaluate transcript/isoform abundance, assess transcript assembly support through back-mapping, and compare HPV45, HPV16, and HPV18 profiles using enrichment approaches.

Keywords: RNA-seq; Transcription map; Cervical cancer .

PAPER CODE: 46

THE ROLE OF THE IRF2BP2 PROTEIN IN CELL CYCLE CONTROL AND TRANSFORMATION

PASTOR, T.D.P.; DE TOLEDO, N.E.; BORONI, M.; VIOLA, J.P.B.
INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Interferon regulatory factor 2 binding protein 2 (IRF2BP2) is a member of IRF2BP family of proteins, which were initially identified as

transcriptional corepressors that were dependent on IRF2. IRF2 is known for controlling the expression of much interferon (IFN)-responsive genes, which in turn act in the regulation of the cell cycle and/or apoptosis. Although the actual involvement of IRF2BP2 in these genes' transcription has not yet been evaluated and its physiological role is still poorly understood, recent data suggest the participation of IRF2BP2 in cell cycle control, cell death and angiogenesis. These findings indicate that an imbalance in IRF2BP2 function could be related to the pathophysiology of cancer.

OBJECTIVES: The main objective of this study is to investigate the role of IRF2BP2 in cell cycle control and transformation, and thus to determine the relevance of this protein in tumorigenesis.

METHOD: NIH3T3 were transduced with retroviral vector containing the Irf2bp2 gene under the control of a tetracycline-responsive promoter. Cell proliferation, death and transformation were evaluated by crystal violet labeling, clonogenic assays, PI, BrdU and annexin-V-labeled flow cytometry, focus formation, growth in semi-solid medium and tumor formation. In addition, transcriptome analysis was carried out using RNA extracted from transduced NIH3T3 cells and gene expression profiles were analyzed.

RESULTS: Overexpression of Irf2bp2 decreased cell proliferation due to a delay in S phase entry. Additionally, overexpression of Irf2bp2 in cells transformed with H-ras-V12 diminished the formation of culture foci, semi-solid colonies and mouse tumors. Different cellular pathways important in the carcinogenesis process were enriched in cells overexpressing IRF2BP2, such as the cell cycle pathway, hypoxia, angiogenesis, and pathways related to immune response.

CONCLUSION: These data showed that Irf2bp2 may have a tumor suppressive role, inducing lower proliferative potential through repressing the expression of cell cycle genes in NIH3T3 cells.

Keywords: IRF2BP2; cell cycle; gene expression regulation.

PAPER CODE: 51

FROM FIBROBLASTS TO CANCER-ASSOCIATED ADIPOCYTES: A PRECLINICAL MODEL OF ADIPOCYTE-COLORECTAL CANCER CROSSTALK TO EXPLORE TUMOR PROGRESSION

DA SILVA, S.C.; MALTA, A.C.D.S.A.; DÍAZ, J.A.M.; MURAD, L.B.. BRAZILIAN NATIONAL CANCER INSTITUTE - INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Colorectal cancer (CRC) is among the most prevalent malignancies worldwide, and obesity is a major risk factor that accelerates its progression. Within the tumor microenvironment (TME), adipocytes undergo functional reprogramming into cancer-associated adipocytes (CAAs), which promote proliferation, invasion, chemoresistance, and stemness through mechanisms such as lipid transfer, adipokine secretion, and exosome-mediated signaling. However, the lack of standardized in vitro models limits the mechanistic understanding of the adipocyte-CRC crosstalk. Establishing reproducible experimental protocols to induce CAAs is essential to explore how adipocyte-derived factors drive CRC aggressiveness, as well as to identify potential therapeutic targets within the obesity-cancer axis.

OBJECTIVES: To standardize a reproducible protocol for generating CAAs through indirect interaction with colorectal cancer cell lines.

METHOD: The protocol included three stages: (1) differentiation of NIH3T3/GFP fibroblasts into adipocytes using a cocktail of dexamethasone, IBMX, insulin, and rosiglitazone; (2) collection of conditioned medium from CRC lines (HT29, HCT116, SW480); and (3) induction of CAAs via indirect co-culture with tumor-conditioned medium. Experimental parameters were standardized, and adipocyte reprogramming was evaluated by morphological and morphometric analysis, Oil Red O staining and cell quantification.

RESULTS: Differentiation of NIH3T3/GFP fibroblasts resulted in marked lipid accumulation and the acquisition of typical adipocyte morphology, as confirmed by Oil Red O staining under microscopy. Conditioned medium

from CRC cell lines were collected 48 hours after culture, centrifuged, filtered, and stored under standardized conditions to ensure reproducibility and stability. Adipocytes exposed to CRC-conditioned medium for 48 hours exhibited distinct morphological alterations and enhanced Oil Red O staining compared to control cells. These findings demonstrate effective reprogramming of adipocytes into CAAs. The standardized protocol established here provides a robust and reproducible framework to investigate how CRC-derived factors influence adipocyte phenotypes and contribute to the development of a tumor-supportive microenvironment.

CONCLUSION: This protocol established a reproducible in vitro model for the generation of CAAs by exposing adipocytes to conditioned medium from colorectal cancer cells. It provided a reliable platform for investigating the bidirectional interactions between adipocytes and CRC cells. Subsequent studies by our group will explore the functional changes in CRC cell lines after exposure to CAAs-conditioned medium, as well as the associated transcriptomic and signaling pathway changes.

Keywords: Colorectal cancer; Cancer-associated adipocytes; Conditioned medium.

PAPER CODE: 55

Modeling the TP53R175H mutation in iPSCs derived from myeloproliferative neoplasms with CALRdel52

SILVA, M.L.D.S.1; BENEDITO, S.1; BERNARDO, P.S.2; RODRIGUES, M.D.L.2; PADILHA, T.F.1; ARKOUN, B.3; OYARZUN, C.M.3; PLO, I.3; MONTE-MÓR, B.1. 1. MOLECULAR BIOLOGY LABORATORY, DIVISION OF SPECIALIZED LABORATORIES (DILABESP), NATIONAL CANCER INSTITUTE (INCA), RIO DE JANEIRO - RJ - BRASIL; 2. CELLULAR AND MOLECULAR HEMATO-ONCOLOGY LABORATORY, NATIONAL CANCER INSTITUTE (INCA), RIO DE JANEIRO - RJ - BRASIL; 3. INSTITUT NATIONAL DE LA SANTÉ ET DE LA RECHERCHE MÉDICALE, UNITÉ MIXTE DE RECHERCHE 1287, GUSTAVE ROUSSY, UNIVERSITÉ PARIS-CITÉ, PARIS - FRANÇA.

INTRODUCTION: Myeloproliferative neoplasms (MPNs) are clonal disorders of hematopoietic stem cells driven by JAK2V617F, CALR indels, or MPL mutations. The common mechanism of these mutations is the constitutive activation of the JAK/STAT pathway, leading to excessive myeloid proliferation. MPNs may progress to secondary acute myeloid leukemia (sAML), with poor prognosis. TP53 mutations are associated with higher transformation rates and reduced survival; about one third of sAML cases show biallelic TP53 inactivation. While TP53 mutations typically impair its tumor suppressor role, some may confer oncogenic gain-of-function, such as increased proliferation and invasion. Among hotspot mutations, R175H is the most frequent in cancer, but its contribution to the leukemic transformation of MPNs remains unclear.

OBJECTIVES: This study aims to analyze the impact of the TP53R175H mutation on the transcriptional landscape of MPN model cells.

METHOD: Our group used CRISPR-Cas9 to generate TP53R175H knock-in (KI) in a CALRdel52 iPSC model from an MPN patient. In this study, we analyzed on and off-target editions by DNA sequencing and the expression of mutated alleles was verified by cDNA sequencing. KI iPSCs and isogenic controls (TP53WT and p53 knockout (KO) iPSCs) were treated or not with Nutlin-3, an MDM2 antagonist. p53 protein expression was assessed by Western blot, and expression of p53 target genes was quantified by qPCR.

RESULTS: iPSC MPN models carrying CALRdel52 and the TP53R175H mutations were generated. KI clones in homozygous (TP53R175H/R175H, 2 clones) or compound heterozygous (TP53R175H/frameshift, 2 clones) states were selected. In this study, Sanger sequencing confirmed the specific KI, with no off-target editing in the six regions analyzed. TP53R175H allele expression was detected by cDNA sequencing, while frameshift mutations were not expressed. Following Nutlin-3 treatment, CALRdel52 TP53WT iPSCs expressed p53, whereas the p53 KO cell line showed no protein expression. TP53R175H-carrying lines exhibited p53 expression in steady state and upon Nutlin-3 treatment. Accordingly, CALRdel52 TP53WT iPSCs displayed significant induction of p53 target gene expression

upon Nutlin-3 treatment: CDKN1A (59.2-fold), BBC3 (13.8-fold), PMAIP1 (3.8-fold) and BAX (3.3-fold). In contrast, this transcriptional upregulation was severely impaired in TP53R175H-expressing lines, as in the p53 KO cell line.

CONCLUSION: These results demonstrate that our CALRdel52 TP53R175H KI iPSC models express p53 mutant protein, lacking its canonical transcriptional activity. As a perspective, we will use these models to investigate the impact of TP53R175H on the transcriptional landscape through RNAseq, thereby assessing potential gain-of-function (GOF) effects in CALRdel52 hematopoietic cells. Therefore, this study may help clarify the molecular mechanisms underlying leukemic transformation in MPNs.

Keywords: Myeloproliferative neoplasms (MPN); CALR; TP53.

PAPER CODE: 58

The secretome derived from irradiated colorectal cancer cells promotes an inflammatory phenotype in cancer-associated fibroblasts via STAT3 activation.

COELHO, B.M.D.L.1; MEDINA, Y.K.C.1; SANT'ANNA, A.L.2; PEREIRA, D.D.A.1; MURAD, L.B.1; COSTA, R.M.B.1; DÍAZ, J.A.M.1. 1. BRAZILIAN NATIONAL CANCER INSTITUTE, RIO DE JANEIRO - RJ - BRASIL; 2. FEDERAL UNIVERSITY OF RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Growing evidence indicates that the sustained inflammatory state driven by cytokines and growth factors secreted by tumor cells submitted to irradiation, provides crucial cues that modulate the behavior of cells within the tumor microenvironment (TME), promoting the acquisition of malignant traits. Irradiation and stimuli from TME, induces the activation and differentiation of CAFs subtype as well as alters the extracellular matrix (ECM) organization, triggering cellular responses related with tumor cell progression. However, the mechanisms/signaling by which colorectal cancer (CRC) cells influences CAFs phenotype definition and how this alters ECM remodeling, contributing to tumor progression, remain poorly understood.

OBJECTIVES: Investigate the influence of the secretome derived from irradiated CRC cells on CAFs subtype definition, alterations in the ECM organization, and how these modifications contribute to CRC progression.

METHOD: In silico analyses investigated the relationship between CAFs subtypes, ECM regulation and CRC progression. In vitro, we used the conditioned medium (CMi) of irradiated cells to stimulate fibroblasts (FGH). CAFs subtypes was assessed by protein levels of specific marker and subcellular localization by Western blotting and immunofluorescence, respectively. ECM was obtained following protocol established in our laboratory and their organization by immunofluorescence to collagen and fibronectin.

RESULTS: In silico analyses showed that patients overall survival didn't differ among myCAFs and vCAFs subtypes but were associated with poorer disease-free survival. Functional analyses revealed differences among myCAFs, iCAFs, and apCAFs, especially in ECM remodeling. myCAFs showed higher expression of adhesion, cytoskeletal, and ECM proteins, whereas iCAFs were enriched in immune-associated proteins. Gene set enrichment analysis indicated activation of epithelial mesenchymal transition, Hedgehog, and Wnt/ β -catenin pathways in myCAFs, and TNF α /NF- κ B, MYC, and DNA repair in iCAFs. myCAFs upregulated ECM structural genes while iCAFs showed ECM degradation genes. In vitro, fibroblasts treated with CMi showed an inflammatory phenotype characterized by fusiform morphology, nuclear localization and increased pSTAT3 levels. Immunofluorescence to collagen I and fibronectin revealed altered ECM organization.

CONCLUSION: Our data suggest that CMi induces an inflammatory phenotype in cancer-associated fibroblasts through pSTAT3 activation, accompanied by a spindle-shaped morphology and alterations in the ECM organization. Additional experiments are underway to confirm this hypothesis.

Keywords: Colorectal Cancer; Cancer associated fibroblasts; Extracellular Matrix.

PAPER CODE: 62

Regulation of PALB2 expression and protein stability in response to DNA damage

CHRISPIM, D.S.1; ELIAS, A.B.R.1; GOMES, T.T.1; SUAREZ-KURTZ, G.1; MONTEIRO, A.N.2; CARVALHO, M.A.3. 1. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL; 2. H. LEE MOFFITT CANCER CENTER & RESEARCH INSTITUTE, TAMPA, FLORIDA - ESTADOS UNIDOS DA AMERICA; 3. INSTITUTO FEDERAL DE EDUCAÇÃO, CIÊNCIA E TECNOLOGIA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Cancer remains one of the leading causes of death and a major global health concern. Genomic instability, a hallmark of cancer, arises from defects in DNA repair pathways. Among them, homologous recombination (HR) is the most accurate mechanism for repairing double-strand breaks. The tumor suppressor PALB2 acts as a molecular bridge between BRCA1 and BRCA2, facilitating RAD51 recruitment to damage sites. Although its function in HR is well established, little is known about the transcriptional control of PALB2 expression and the stability of its protein product during the DNA damage response, leaving important gaps in understanding its regulation in genome maintenance and tumorigenesis.

OBJECTIVES: This study aims to investigate the mechanisms regulating PALB2 gene expression and the protein stability in response to DNA damage through molecular and cellular approaches.

METHOD: Putative PALB2 transcriptional regulators will be identified using yeast one-hybrid screening and confirmed through chromatin immunoprecipitation and RT-qPCR. Expression and protein levels will be analyzed in synchronized and irradiated MCF7 cells. mRNA stability will be assessed with actinomycin D, and protein degradation with cycloheximide and MG-132. Mutants affecting nuclear localization will be used to assess the relationship between subcellular localization and stability.

RESULTS: Data indicate that PALB2 protein levels fluctuate throughout the cell cycle, peaking during late S and G2/M phases. Upon DNA damage, MCF7

cells exhibited G1 arrest and delayed cycle progression, accompanied by altered PALB2 expression dynamics. Immunoblotting revealed increased nuclear PALB2 accumulation after irradiation, suggesting a role in active HR-mediated repair. Variants disrupting the WD40 domain or nuclear localization exhibited cytoplasmic accumulation and reduced protein stability, supporting the hypothesis that proper subcellular distribution is critical for PALB2 stability and function. These findings indicate coordinated regulation of PALB2 expression and protein degradation, possibly governed by both transcriptional and post-translational mechanisms in response to DNA damage.

CONCLUSION: The data suggest that PALB2 expression and protein stability are dynamically regulated during cell cycle and after DNA damage. Nuclear localization appears essential for its stability and function in homologous recombination. Understanding these regulatory mechanisms may reveal new aspects of PALB2 biology and its involvement in tumorigenesis, offering insights for therapeutic targeting in cancers associated with defective DNA repair.

Keywords: PALB2; DNA damage response; cancer.

PAPER CODE: 68

EVALUATION OF THE RELATIONSHIP BETWEEN INTRATUMORAL COLLAGEN AND LYMPHOCYTIC INFILTRATE IN ACRAL LENTIGINOUS MELANOMA

RANGEL, B.D.S.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Acral melanoma, a subtype of cutaneous melanoma, affects the palms, nail bed, and soles, which are regions composed of glabrous skin. It is not associated with ultraviolet radiation exposure, and the etiological factors related to its development remain unclear. However, some evidence suggests that the structure of glabrous skin may influence tumor development, indicating a potential role of the extracellular matrix (ECM) composition. It is known that the amount of collagen, the main ECM component present in the tumor microenvironment, can hinder the

presence of tumor-infiltrating lymphocytes (TILs), either by forming a physical barrier that limits T cell migration or by activating signaling pathways that reduce T cell infiltration, proliferation, and cytotoxic activity within the tumor.

OBJECTIVES: Therefore, this study aims to investigate the association between intratumoral collagen abundance and the profile of intratumoral CD4+ and CD8+ T lymphocytes in primary acral melanoma (AM).

METHOD: To quantify TILs, sequential serial tissue sections were stained with anti-CD3, anti-CD4, and anti-CD8 using HRP green, BioSB®. Five fields with the highest density of intratumoral CD3+ lymphocytes were selected. Corresponding areas stained with anti-CD4 and anti-CD8 were analyzed to determine the absolute number of CD4+ and CD8+ lymphocytes using QuPath 0.5.1. For collagen quantification, sections were stained with Picrosirius Red (PSR) and are currently being analyzed using CaseViewer.

RESULTS: To date, sixty-six primary AM samples from patients diagnosed at INCA whose paraffin blocks were available were analyzed, have been stained with PSR and also labeled for CD3, CD4, and CD8. Forty of those have been quantified so far, all of which were positive for CD3, including both CD8+ and CD4+ T cells, with no difference in their abundance ($p = 0.66$).

CONCLUSION: All AM samples included in this study have detectable abundance of lymphocytic cells, with similar proportions of CD4+ and CD8+ cells. Quantification of the remaining 26 samples will be important to determine the lymphocytic profile of the complete cohorts and to investigate how this profile relates to collagen abundance and clinical outcome of patients.

Keywords: collagen; tumor-infiltrating lymphocytes; tumor microenvironment.

PAPER CODE: 74

Association between the Hailstorm Phenomenon and Gene Expression in Acral Melanoma

ALVES, T.C.1; FACCILO FILHO, A.C.2; SQUIAVINATO, A.C.M.S.2; HERRERA, M.D.C.V.2; BASURTO-LOZADA, P.3; RAMIREZ, J.R.C.W.3; ESPINOZA, C.D.R.3; ADAMS, D.2; POSSIK, P.A.1. 1. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL; 2. WELLCOME SANGER INSTITUTE, HINXTON - INGLATERRA; 3. LIIGH UNAM, MEXICO CITY - MEXICO.

INTRODUCTION: Acral melanoma (AM) is a rare and aggressive subtype of melanoma that arises on the palms, soles, and nail beds. It accounts for a higher proportion of melanoma cases in non-European and admixed populations and shows notable geographic variation. AM is frequently diagnosed at advanced stages, is associated with poor prognosis, and has limited therapeutic options due to low response rates to current treatments. Unlike UV-driven cutaneous melanomas, AM shows low mutation burden but frequent complex chromosomal alterations, including “hailstorms”, regions characterized by high copy number transition (CNT) density, significant amplifications, and large segment sizes. These genomic events may play a key role in tumor development, presenting significant challenges for treatment strategies.

OBJECTIVES: This study investigates the molecular landscape of AM through integrative analysis of whole-exome and RNA sequencing, with a focus on hailstorms and their relationship with gene expression.

METHOD: Whole-exome and RNA sequencing data from AM samples were processed using Trimmomatic, bwa-mem v0.7.17 (WES alignment), and STAR (RNA-seq). CNV was estimated using Sequenza, and hailstorm events were identified based on increased CNT density, amplified segments relative to ploidy, and a minimum 5 Mb width between CNTs. Differential gene expression was analyzed with DESeq2, comparing samples with and without hailstorms in chromosomal arms.

RESULTS: Whole-exome and transcriptome sequencing of 29 samples from a Brazilian cohort of AM, collected at INCA from 25 patients, revealed low mutation burden but predominance of copy number alterations, including hailstorms most commonly on chromosomes 11q (38.5%), 5p (34.6%), and 22q (26.9%). Gene expression analysis showed 212 differentially expressed genes (198 up, 14 down) in samples with 11q hailstorms. Among 410 genes in this hailstorm, 38 were upregulated, including PAK1 and GAB2. On 5p, 15 genes were differentially expressed (9 up, 6 down), but none of those are located within the hailstorm region. Overall gene expression analysis for chromosome 22q showed that 152 genes (130 up and 22 down) were differentially expressed in hailstorm samples. Of these, 23 out of 135 genes within the hailstorm regions were upregulated, including EP300, LZTR1, and CRKL.

CONCLUSION: The results suggest that genes expressed within hailstorms on chromosomes 11q, 5p, and 22q are associated with amplifications and rearrangements of genes previously linked to acral melanoma. The consistent upregulation of genes previously described as melanoma drivers indicates that these regions are crucial for tumor biology and provide new insights into the genomic structure characterizing acral melanoma.

Keywords: hailstorms; acral melanoma; DEGs.

PAPER CODE: 78

Potential oncogenic roles of CDC5L and NPM1 in T-cell acute lymphoblastic leukemia

DE OLIVEIRA, R.S.1; DA SILVA, L.L.F.1; CARELS, N.2; CARNEIRO, F.R.G.1. 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive and heterogeneous hematological malignancy. Treatment regimens are prolonged and toxic, underscoring the need for

oncogenes and molecular targets to reduce adverse effects and therapy limitations. We previously analyzed public RNA-seq data from two T-ALL cell lines and the human interactome to find upregulated and highly-connected genes. Identified candidates included oncogenes, chaperones, and regulatory proteins, with NPM1 and CDC5L emerging as promising targets. NPM1 is vital for ribosome biogenesis, centrosome duplication, and histone assembly. CDC5L regulates pre-mRNA splicing and the cell cycle. Their overexpression, network centrality, and roles in carcinogenesis pathways strongly suggest their oncogenic potential in T-ALL.

OBJECTIVES: This study aims to investigate the oncogenic potential of NPM1 and CDC5L genes in T-ALL cell lines through cell proliferation, viability, apoptosis, and cell cycle progression assays.

METHOD: We evaluated NPM1 and CDC5L overexpression via RT-qPCR using SyBr Green and by Western blot. We then performed shRNA lentiviral transduction to knockdown NPM1 and CDC5L in T-ALL cell lines, followed by phenotypic analysis. Functional assays included cell proliferation and viability, assessed by trypan blue exclusion test and MTT. Apoptosis was evaluated by activated caspase 3 labeling following by flow cytometer analysis. Cells were analyzed by a 96-hour time course.

RESULTS: Overexpression of both NPM1 and CDC5L was confirmed in Jurkat and Molt-4 cells via RT-qPCR and Western blot, corroborating our previous in silico analysis. Gene knockdown was highly efficient for the two targets using two specific shRNA sequences per gene in each cell line. Thus, all functional assays were performed in the two T-ALL lines. Knockdown cells showed a significant reduction in cell proliferation and viability. Furthermore, caspase 3 labeling assays indicated increased apoptosis in silenced cells, demonstrating the crucial role of these genes in cell survival.

CONCLUSION: These findings strongly suggest that NPM1 and CDC5L are crucial for cell division and survival

in T-ALL cell lines. The data support the efficacy of our bioinformatics-driven approach for identifying possible therapeutic targets and oncogenes in this aggressive disease. Ultimately, the results provide a foundation for future translational research.

Keywords: Leukemia; T-ALL; Oncogenes.

PAPER CODE: 81

IN VITRO VALIDATION OF CDC37 AS A POTENTIAL THERAPEUTIC TARGET FOR T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

LOPES, A.D.S.C.1; DE OLIVEIRA, R.S.1; DA SILVA, L.L.F.2; VIOLA, J.P.B.1; CARNEIRO, F.R.G.3. 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. UFRJ, RIO DE JANEIRO - RJ - BRASIL; 3. FIOCRUZ/INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: T-cell acute lymphoblastic leukemia (T-ALL) is characterized by the accumulation of immature T cells. The multiple and frequent therapies used to treat this neoplasia can result in significant toxicity, potentially leading to the emergence of other diseases. Identifying novel therapeutic targets and drugs to reduce treatment-related side effects is crucial. For this purpose, we used in silico analysis on the T-ALL cell lines Jurkat and Molt-4 to find overexpressed genes, along with the evaluation of the human interactome to identify highly connected proteins. Among the identified targets, we selected the CDC37 gene for this study. This gene acts to form complexes with the chaperone HSP90, providing stability for the folding of a spectrum of protein kinases.

OBJECTIVES: This work aims to validate in vitro CDC37 as a potential therapeutic target for T-ALL through proliferation, viability, apoptosis and cell cycle assays.

METHOD: To validate this potential target, we first performed qRT-PCR and western blot analysis to evaluate the overexpression in T-ALL cell lines. Then, we performed knockdown experiments by lentiviral

transduction of plasmids expressing specific short hairpin (shRNA) molecules for this gene and assessed the knockdown efficiency by western blotting. Furthermore, we conducted a phenotypic evaluation in the Jurkat cell line by the trypan blue exclusion test for cell viability and proliferation.

RESULTS: The qRT-PCR and western blot confirmed that CDC37 is overexpressed in T-ALL cell lines compared to peripheral blood mononuclear cell (PBMC) control samples. The knockdown was efficient in both tested cell lines, with a high decrease in protein levels. Furthermore, the phenotypic analysis demonstrated a reduction in cell number over time and a decrease in cell viability in the knockdown for CDC37.

CONCLUSION: The results obtained are preliminary but suggest that CDC37 may have crucial functions in cell division and survival in the Jurkat cell line. However, many other assays still need to be performed to validate the CDC37 gene as a therapeutic target for T-ALL.

Keywords: Leukemia; T-ALL; CDC37.

PAPER CODE: 85

Evaluation of Cyclin E1 effect in centromere integrity and function in breast epithelial cells

BOECHAT, S.G.; FAGUNDES, R.; TEIXEIRA, L.K.. BRAZILIAN NATIONAL CANCER INSTITUTE (INCA), RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Cell cycle is a fundamental process for cells to successfully replicate and segregate their genome. Cyclin E1, together with CDK2, regulates the initiation and progression of DNA replication. During DNA replication, sister chromatids are held together through the centromere region. Centromeres are essential for the proper alignment and segregation of chromosomes during mitosis. High levels of Cyclin E1 induce replication stress, leading to genomic instability, and are associated with a worse prognosis in several types of tumors, including breast cancer. Our group has identified that high levels of Cyclin E1 in breast epithelial cells induce premature separation of sister chromatids.

However, the mechanism by which high levels of Cyclin E1 lead to this phenomenon has not been elucidated yet

OBJECTIVES: The main objective of this project is to evaluate the effect of high levels of Cyclin E1 on the integrity and function of centromeres in immortalized human mammary epithelial cells (HME1).

METHOD: High levels of Cyclin E1 were induced by adenoviral transduction of HME1 cells with virus encoding the human Cyclin E1 cDNA (Adv-Cyclin E1) or control adenovirus (Adv-Control). Cell cycle progression, protein levels of targets of interest and chromosome integrity were analyzed over a 7-day period post-transduction. Chromosome segregation and centromere dynamics will be evaluated in a HME1 cell line expressing H2BGFP, TubulinBFP2, and CENP-AmCherry through live cell imaging.

RESULTS: Adv-Cyclin E1 cells show higher levels of Cyclin E1 when compared to Adv-Control cells. Adv-Cyclin E1 cells show alterations in cell cycle progression, such as a decrease in G1-phase population, an increase in S-phase population and a decrease in BrdU incorporation. Adv-Cyclin E1 cells show several chromosomal alterations, such as endoreduplication, hyperfragmentation and, especially, premature separation of sister chromatids. Regarding centromeres, Adv-Cyclin E1 cells do not show alterations in the transcriptional levels of α -satellite regions, but show alterations in CENP-A deposition in the nucleus. To evaluate chromosome segregation and centromere dynamics during mitosis, HME1 cells expressing Histone H2BGFP, TubulinBFP2, and CENP-AmCherry, which allow for the visualization of chromosomes, mitotic spindle, and centromeres, respectively, are being analyzed through live cell imaging.

CONCLUSION: Our results indicate that high levels of Cyclin E1 interfere with cell cycle progression and lead to premature separation of sister chromatids in mitosis. High levels of Cyclin E1 induce alterations in CENP-A deposition, which may interfere with the maintenance of sister chromatids cohesion during mitosis.

Keywords: Breast cancer; Cyclin E; Chromosome instability.

PAPER CODE: 88**CHARACTERIZATION OF HPV DIVERSITY IN ANAL CANAL AND PENILE TUMORS AND THEIR PRECURSORS LESIONS**

DE MOURA, L.O.M.1; ARAÚJO, R.O.D.C.1; VALADÃO, M.1; SILVA, J.A.D.D.C.E.2; LEAL, F.E.1; SOARES, M.A.1; SIQUEIRA, J.D.1; GOES, L.R.3. 1. INSTITUTO NACIONAL DE CâNCER, RIO DE JANEIRO - RJ - BRASIL; 2. HOSPITAL UNIVERSITÁRIO ANTÔNIO PEDRO, NITERÓI - RJ - BRASIL; 3. INSTITUTO NACIONAL DE CâNCER; UNIVERSIDADE FEDERAL DO ESTADO DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Human papillomavirus (HPV) is a double-stranded circular DNA non-enveloped virus that has a tropism for epithelial cells. These viruses belong to the Papillomaviridae family and can be classified according to their oncogenic potential as low or high risk, both of which are associated with cancer development. Anal canal and penile cancers, although uncommon, have increasing incidence and mortality rates worldwide. In 2022, there were an estimated 54,306 cases and 22,035 deaths from anal cancer, and 37,700 cases and 13,738 deaths from penile cancer. HPV is present in 88% of anal cancer cases and 50% of penile cancer cases. However, studies on the prevalence and viral diversity in these tumors are still limited and are essential for the prevention strategies and management of infection.

OBJECTIVES: This study aims to assess the prevalence and diversity of HPV in biopsy samples of anal canal lesions, anal cancer, and penile cancer from patients followed at the National Cancer Institute (INCA).

METHOD: Tissue samples from penile cancer, anal canal cancer, and non-tumoral anal canal tissue were stored in the Biobank of the National Tumor Bank (BNT) at the National Cancer Institute. Total DNA was extracted and samples were subsequently subjected to HPV genotyping using the reverse hybridization method on the HybriSpot 12 platform (Mobius) with the multi HPV flow chip kit (XGEN), which contains probes for 35 types of HPV, including both high and low-risk types.

RESULTS: So far, 3 penile cancer samples, 27 anal cancer samples, and 33 non-tumoral anal canal samples have been processed. Of the 63 samples analyzed, 62 tested positive for HPV, with 3 from penile cancer, 26 from anal canal cancer, and 33 from precursor lesions of the anal canal. A total of 32 different HPV types were detected, with HPV 16 being the most prevalent. HPV 16 was present in all penile cancer samples, 24 samples (92.3%) of anal canal cancer, and 24 samples (78.8%) of anal canal precursor lesions. Multiple HPV co-infections were observed in 2 samples of penile cancer, 20 samples of anal canal cancer, and 25 samples of anal canal precursor lesions.

CONCLUSION: These findings indicate a high prevalence and diversity of HPV infections in anal canal and penile tumors, as well as in their precursor lesions, with a particular focus on the frequency of HPV 16. There was also a high frequency of co-infection with multiple genotypes, highlighting the importance of genotypic screening and molecular surveillance as key strategies for the monitoring, prevention, and clinical management of these HPV-associated tumors.

Keywords: HPV; Anal Canal Cancer; Penile Cancer .

PAPER CODE: 90**Study of the relationship between p53 and mir-210 in cancer and its impact on the response to radiotherapy in vitro and in silico**

RODRIGUES, M.D.L.1; VASCONCELOS, F.2; HANCIO, T.3; MAIA, R.C.1; BERNARDO, P.S.1. 1. LABORATÓRIO DE HEMATO-ONCOLOGIA CELULAR E MOLECULAR, PROGRAMA DE HEMATO-ONCOLOGIA MOLECULAR, INSTITUTO NACIONAL DE CâNCER, RIO DE JANEIRO - RJ - BRASIL; 2. DIVISÃO DE LABORATÓRIOS ESPECIALIZADOS, INSTITUTO NACIONAL DE CâNCER, RIO DE JANEIRO - RJ - BRASIL; 3. LABORATÓRIO NACIONAL DE BIOCÊNCIA, CENTRO NACIONAL DE PESQUISA EM ENERGIA E MATERIAIS (CNPEM), CAMPINAS - SP - BRASIL.

INTRODUCTION: Cancer remains a major global health problem due to its high incidence and mortality. Many studies aim to identify the molecular basis of treatment resistance to improve diagnosis, therapy,

and patient survival. p53, the “guardian of the genome”, activates repair and cell death pathways in response to DNA damage or cellular stress. TP53 is mutated in over 50% of tumors, promoting tumorigenesis. MicroRNA, small non-coding RNAs that inhibit mRNA translation, modulate gene expression. The interaction between p53 and miR-210 has been indirectly observed in cancer-associated processes such as the Warburg effect, epithelial-mesenchymal transition, and therapy resistance. Our group previously showed that TP53 mutation may contribute to radiotherapy sensitivity mediated by miR-210, in vitro.

OBJECTIVES: The aim of this work is to evaluate the involvement of TP53 mutational status of in ionizing radiation (IR) response and in expression levels of miR-210 in cancer, in vitro and in silico

METHOD: miR-210 expression was investigated in silico across tumor types, and its correlation with the p53 pathway was assessed using miRTarBase and TRUST platforms. T98G (TP53R273K) and TE-1 (TP53M272V, temperature-sensitive) cell lines were treated with APR-246 to reactivate p53, and exposed to IR, for cytotoxicity evaluation by MTT and trypan blue assays, cell cycle assessment by flow cytometry, and p53 pathway activation via CDKN1A and BAX expression by RT-qPCR.

RESULTS: In silico, miR-210 was upregulated in tumor versus non-tumor tissues and in TP53 mutant versus wild-type samples in breast, prostate, and lung cancers. In glioblastoma, miR-210 was overexpressed in TP53 mutant samples, while in esophageal cancer it was higher in tumor tissues. Correlation analysis identified four common targets — HPK2, E2F3, IGFBP3, and EPHA2 — associated with cell cycle regulation and apoptosis. In vitro, APR-246 concentrations of 8, 16, 32, and 64µM were tested for 24 and 48h; 64µM showed cytotoxic effects in both cell lines, while 8µM did not produce significant treatment differences. T98G cells were more resistant than TE-1. Based on these results, 24h treatment time and concentrations of 16 and 32µM were selected for both cell lines to confirm p53 reactivation and evaluate IR response. Preliminary data showed that 16 or 32µM of APR-246 associated with IR led to a greater reduction in cell viability in comparison to isolated treatments in TE-1 cell line.

CONCLUSION: In silico, miR-210 is overexpressed in multiple cancer types, without impact on staging or nodal metastasis. In vitro, APR-246 treatment decreases cell viability and alters the cell cycle, indicating increased cellular sensitivity. miR-210 is strongly associated with TP53 mutation in breast cancer and glioblastoma.

Keywords: Radiotherapy resistance; microRNAs; p53.

PAPER CODE: 93

EVALUATION AND CHARACTERIZATION OF TUMOR-INFILTRATING LYMPHOCYTES (TIL) ISOLATED FROM PATIENTS WITH ACRAL MELANOMA

DO NASCIMENTO, R.M.C.1; SILVA, L.R.B.2; DE OLIVEIRA, J.L.1; BONAMINO, M.H.1; POSSIK, P.A.1. 1. INSTITUTO NACIONAL DE CâNCER - INCA, RIO DE JANEIRO - RJ - BRASIL; 2. FUNDAÇÃO OSWALDO CRUZ (FIOCRUZ), RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Acral melanoma (AM) is a rare and aggressive subtype of melanoma that affects glabrous skin (palms, soles, and subungual regions). Its frequency across the melanoma subtypes is higher in Latin Americans compared to populations of European descent. Often diagnosed at advanced stages, AM is associated with poor prognosis and limited treatment options. Adoptive Cell Therapy (ACT) using ex vivo expanded tumor-infiltrating lymphocytes (TIL) was recently approved by the Food and Drug Administration (FDA), demonstrating efficacy in patients with advanced melanoma, including those refractory to immune checkpoint inhibitors (ICIs). However, its application in AM is underexplored due to scarce functional characterization of TIL and limited clinical data of AM patients' response to ACT.

OBJECTIVES: This study aims to isolate, expand, and characterize TILs from AM patients treated at the Brazilian National Cancer Institute (INCA) and to evaluate their therapeutic potential.

METHOD: Tumor samples from six patients were processed by enzymatic dissociation or direct fragment culture with high dose IL-2 (6,000 IU/mL). TIL cultures

were monitored for morphology and expansion and analyzed by flow cytometry. Tumor fragments were implanted into immunocompromised mice to generate autologous patient-derived xenografts (PDX) and isolate AM cells for in vitro culturing. Cell cultures were checked for melanocytic origin by SOX10 immunofluorescence.

RESULTS: TILs were successfully expanded from all AM samples. Those from four patients were characterized, showing that within the immune compartment (CD45+ cells), CD3+ T cells were predominant (median: 84.6%, $\pm$ SD 17.1). CD4+ and CD8+ cells averaged 46.1% ($\pm$ SD 27.6) and 47.3% ($\pm$ SD 27.1), respectively. Regulatory T cells were rare (median: 0.6%, $\pm$ SD 1.6). PD-1 expression was high in three samples (median: 93.2%, $\pm$ SD 32.6), while LAG-3 and TIM-3 were expressed at lower levels (LAG-3 median: 0.3%, $\pm$ SD 2.7; TIM-3 median: 6.5%, $\pm$ SD 5.2). PDX models were established from three patient samples, from which corresponding SOX10+ AM cell cultures were generated. Cells are currently being monitored for growth stability. One culture remained stable for ≥ 10 passages, while the others are still developing.

CONCLUSION: These results demonstrate the successful expansion of TILs and the generation of PDX models and cell cultures from AM samples, establishing a robust platform to rigorously evaluate their functional activity and advance personalized immunotherapy strategies for AM.

Keywords: acral melanoma; tumor-infiltrating lymphocytes (TILs); adoptive cell therapy (ACT).

PAPER CODE: 94

In vitro evaluation of TRIM28's role in T-Cell Acute Lymphoblastic Leukemia Tumorigenesis

VALENTIM, L.V.1; LOPES, A.D.S.C.2; DA SILVA, L.L.F.2; DE OLIVEIRA, R.S.2; CARNEIRO, F.R.G.2. 1. FIOCRUZ/INCA, RIO DE JANEIRO - RJ - BRASIL; 2. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive disease that accounts for approximately 15% of pediatric and 25% of adult cases

of acute lymphoblastic leukemia. It is an aggressive disease that requires long-term treatment and causes significant side effects. Previous in silico analyses performed by our group using RNAseq data from Jurkat and Molt-4 cell lines indicated that the TRIM28 gene is overexpressed in these cells. Analysis of the human interactome indicates that TRIM28 is highly connected, and its targeting may destabilize the protein network. Therefore, we speculate that this gene may play a role in T-ALL tumorigenesis. TRIM28 is a widely expressed multifunctional protein that regulates cancer pathways, promoting growth, immune evasion, and survival.

OBJECTIVES: This study aims to investigate the role of TRIM28 as a potential oncogene for T-ALL by evaluating its expression and function in Jurkat and Molt-4 cell lines.

METHOD: Jurkat and Molt-4 cells were grown in RPMI with 10% FBS. Gene expression analysis were performed by RT-qPCR (SYBR) to validate TRIM28 overexpression in T-ALL cell lines compared to PBMC samples from healthy individuals. ACTB and RPL13 were used as RT-qPCR reference genes. Cell extracts were prepared and quantified for western blotting to verify TRIM28 protein levels. TRIM28 shRNA lentiviral particles transduction was performed to knock down TRIM28 for future functional assays.

RESULTS: Significant TRIM28 mRNA overexpression was observed in Jurkat and Molt-4 cell lines compared to controls, using both reference genes (ACTIN and RPL13). A high amount of TRIM28 protein was also observed in these cell lines. These results confirm the in silico analysis. Subsequently, TRIM28 was knocked down by shRNA. Western blot analysis showed highly efficient silencing of TRIM28 in both cell lines and with the two shRNA sequences used. The silenced cells will support future functional assays.

CONCLUSION: The results confirm TRIM28 overexpression in T-ALL cell lines. Additional functional assays, such as cell proliferation and viability analysis, will be necessary to investigate TRIM28's role as a potential oncogene in T-ALL.

Keywords: T-cell Acute Lymphoblastic Leukemia (T-ALL); TRIM28; Oncogene.

PAPER CODE: 98

Identification and classification of germline variants potentially associated with the development of early-onset colorectal cancer

PEREIRA, A.S.1; CABRAL, B.C.A.1; DE MARSILLAC, S.M.1; PEREIRA, J.P.1; DAMASCENA, A.C.O.1; MONTEIRO, M.L.P.F.1; TORRESAN, C.1; FURTADO, C.1; ARAÚJO, R.1; DE MELO, A.C.1; DE PAULA, D.J.G.1; CARRARO, D.M.2; UNO, M.3; BARROS, L.R.C.3; CHAMMAS, R.3; DOS SANTOS, A.K.C.R.4; TORRES, L.C.5; DE ALBUQUERQUE, C.G.P.6; ANTONIAZZI, A.P.7; ASHTON-PROLLA, P.8; DOS SANTOS, A.C.E.1; MOREIRA, M.Â.M.1; PALMERO, E.I.1. 1. INSTITUTO NACIONAL DE CÂNCER (INCA), RIO DE JANEIRO - RJ - BRASIL; 2. A.C. CAMARGO CANCER CENTER, SÃO PAULO - SP - BRASIL; 3. INSTITUTO DE CÂNCER DO ESTADO DE SÃO PAULO, SÃO PAULO - SP - BRASIL; 4. UNIVERSIDADE FEDERAL DO PARÁ, BELÉM - PA - BRASIL; 5. HOSPITAL DE CÂNCER DE PERNAMBUCO, RECIFE - PE - BRASIL; 6. INSTITUTO DE CÂNCER DO CEARÁ, FORTALEZA - CE - BRASIL; 7. HOSPITAL DE CÂNCER DE BARRETOS, BARRETOS - SP - BRASIL; 8. HOSPITAL DAS CLÍNICAS DE PORTO ALEGRE, PORTO ALEGRE - RS - BRASIL.

INTRODUCTION: Colorectal cancer (CRC) is the third most diagnosed malignancy worldwide and the second leading cause of cancer-related death. The incidence and number of deaths from CRC have decreased over time; however, when we consider only the early-onset CRC (EO-CRC; defined by diagnosis before the age of 50), these rates have increased. Notable disparities—including molecular differences—have been observed in EO-CRC when compared to cases diagnosed in individuals over 50. We hypothesize that additional genes associated with low or moderate risk, in combination with specific genetic alterations, environmental and occupational exposures, or lifestyle factors, may contribute to the development of the disease. Therefore, a deeper understanding of the molecular basis of EO-CRC is needed.

OBJECTIVES: The study aims to identify germline variants potentially associated with the development of early-onset colorectal cancer, and the relevant biological pathways involved in carcinogenesis.

METHOD: Individuals diagnosed with CRC at or before 50 years, treated at 10 reference cancer centers in Brazil will be selected for germline exome sequencing. Variants will be classified according to ACMG criteria. Cases with variants of uncertain significance or splice site alterations will undergo RNA sequencing. Loss of heterozygosity will be assessed for pathogenic variants in genes not typically associated with CRC. Sociodemographic, clinicopathological, and family history data will be collected.

RESULTS: To date, 341 participants have been enrolled in the study. DNA has been extracted from all participants; exome sequencing has already been performed for 2 individuals, and 1 presented a pathogenic variant in MSH2. Additionally, 72 libraries have been prepared for sequencing. Data have been collected from 148 participants, with a mean age at diagnosis of 40 years (17–50; SD: 7.37), and most of whom are women (70.3%). The identified tumor sites were the colon (66.5%), rectum (31.9%), cecum or hepatic flexure (1.6%). Fifteen participants reported a second primary tumor, and two of them reported a third primary tumor. The tumor types were thyroid (4), CRC (3), endometrial (2), breast (2), prostate (1), and unspecified tumors (3). Approximately 91% of participants reported a family history of cancer (2 cases with consanguineous parents), and 11% reported a history of Lynch Syndrome or Polyposis. The most reported cancer types among relatives were CRC (62), breast (42), and prostate (33).

CONCLUSION: The majority of the participants were from Brazil's South and Southeast regions. Most reported a family history of cancer, and one of them presented a pathogenic variant in MSH2, proving its high risk for developing hereditary tumors. This way, their relatives will be able to receive risk-appropriate management. Furthermore, the study will include and evaluate 1,000 individuals over the next few months to contribute to the understanding of the development of EO-CRC in Brazil.

Keywords: Early-onset colorectal cancer; Hereditary Syndromes; whole exome sequencing.

PAPER CODE: 102

MAPPING SPATIAL NICHES IN OVARIAN CANCER RELATED TO CHEMOTHERAPY RESPONSE

SOARES, L.C.1; DE LANNA, C.A.2; TESSAROLLO, N.1; PINHO, P.V.1; SERAIN, A.F.1; DE PAULA, D.J.G.1; MORREUW, L.D.C.1; MACEDO, F.D.C.1; CHAVES, C.B.P.1; DE MELO, A.C.1; VIOLA, J.P.B.1; BORONI, M.1. 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. LNCC, PETRÓPOLIS - RJ - BRASIL.

INTRODUCTION: Ovarian cancer is the 7th most common malignancy in women worldwide and the 8th in Brazil. High-grade serous ovarian carcinoma (HGSOC), representing 70-80% of cases, is highly aggressive, with recurrence rates reaching 85% within two years after treatment. Most patients are diagnosed at advanced stages due to the absence of specific symptoms and the ovary's anatomical location, which facilitates metastasis. Spatial Transcriptomics enables in situ analysis of gene expression, advancing the study of tumor biology.

OBJECTIVES: This study aims to investigate the cellular and molecular heterogeneity of HGSOC, focusing on spatial gene expression patterns related to chemotherapy response and tumor resistance.

METHOD: Fresh-frozen tumor samples from four patients were sequenced with the Visium kit (10x Genomics) and classified as favorable (FR, n=2) or poor responders (PR, n=2). Data were quality-controlled with Seurat, integrated with scvi-tools, and clustered using the Leiden algorithm. Differential expression (Wilcoxon-Rank-Sum), pathway enrichment (KEGG database), deconvolution (CARD), regulon analysis (VIPER/DoRothEA) and survival analysis (Survminer) were conducted.

RESULTS: Ten clusters were identified across all samples, showing distinct distributions between groups. Cluster 10 was more abundant in PR tumors and enriched for FOXO signaling, with high expression of FOXO3 and its target SOD2, indicating oxidative stress adaptation. Cluster 2, found in the vicinity of cluster 10, also more prominent in PR, was enriched for HIF and glycolysis pathways, as well as hypoxia- and autophagy-related gene signatures, and displayed clear activation of HIF1A

regulon. Cluster 2 also contained the highest proportion of hypoxia-associated macrophages, and areas expressing the hypoxic marker LDHA coincided with these regions. Notably, cluster 2 displayed enrichment for autophagy scores, consistent with reports in HCC linking hypoxia-induced autophagy mediated by FOXO3 to therapy resistance. TCGA analysis revealed shorter PFI in samples with high FOXO3 expression, supporting this association.

CONCLUSION: Together, these findings suggest the formation of adaptive hypoxic niches composed of tumor and immune cells capable of surviving oxidative stress and therapeutic pressure, potentially serving as reservoirs for recurrence and disease progression. Further studies are needed to explore cell-cell communication and the role of these niches in tumor progression.

Keywords: ovarian cancer; tumor microenvironment; spatial transcriptomics.

PAPER CODE: 103

EVALUATION OF THE IMPACT OF HMGA1 AND HMGA2 OVEREXPRESSION ON NEOPLASTIC TRANSFORMATION OF ESOPHAGEAL EPITHELIAL CELLS

JORGE, F.L.1; BOECHAT, S.G.1; JÚNIOR, A.P.2; PINTO, L.F.R.1; TEIXEIRA, L.K.1; DA COSTA, N.O.M.1. 1. INSTITUTO NACIONAL DE CÂNCER (INCA), RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO (UFRJ), RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Esophageal cancer (EC) is a highly lethal disease often diagnosed at advanced stages, limiting treatment options. It is subdivided into two main histological subtypes: esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC). Understanding the molecular and cellular mechanisms involved in its genesis and progression is important for developing new therapies. HMGA (High Mobility Group A) proteins, which regulate gene expression and play crucial roles in various cellular processes, are overexpressed in several human tumors and correlated with poor prognosis. Our group has shown that HMGA2

is overexpressed in ESCC, while HMGA1 is induced in EAC, and this fact is associated with the progression of both tumors, highlighting their potential as therapeutic targets.

OBJECTIVES: In this study, we are investigating the role of HMGA1 and HMGA2 in the neoplastic transformation of esophageal epithelial cells to better understand their involvement in esophageal carcinogenesis.

METHOD: We have used retroviral transduction to generate esophagus epithelial cell lines (HET-1A and EPC-hTERT) overexpressing HMGA1 and HMGA2. mRNA and protein levels of HMGA genes and targets will be assessed by RT-qPCR and Western blotting at 1, 15, 30, and 60 days. Functional assays (viability, proliferation, cell cycle, migration, invasion, and colony formation) will be performed at the same time points to characterize the resulting phenotypes.

RESULTS: The first half of the project, which includes the molecular biology procedures, has been completed. We have successfully cloned the HMGA1 and HMGA2 genes individually into the pQCXIP retroviral vector. Retroviral production was performed in the Phoenix Amphi cell line, yielding sufficient viral titers to transduce normal esophagus cell lines. We have successfully established the cell lines. Currently, we are evaluating the mRNA and protein expression levels of HMGA1 and HMGA2, as well as their target genes involved in carcinogenesis.

CONCLUSION: In conclusion, we have successfully generated the target cell lines. We are currently evaluating HMGA expression at both the mRNA and protein levels, and our next step will be to investigate the cellular phenotypes. We anticipate that these data will provide valuable insights into the role of HMGA proteins in esophageal cell transformation.

Keywords: Esophageal cancer; HMGA; esophageal cellular transformation.

PAPER CODE: 104

Role of NFAT1 transcription factor in the generation and function of Memory-like CD8+ T lymphocytes in young and aged mice

BARBOZA, B.A.; OLIVEIRA-VIEIRA, B.; BRUZZI, H.F.; FERREIRA, L.V.L.; FREIRE, L.L.; POLIDO, V.P.; SEBBA, M.E.C.; SILVA, K.L.; VIOLA, J.P.B.. BRAZILIAN NATIONAL INSTITUTE OF CANCER, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Memory-like cells, also known as Memory-phenotype, are antigen-inexperienced memory T cells present within hosts that have not been exposed to foreign antigen(s), such as non-immunized/unprimed mice. It is described that this population increases with aging.

OBJECTIVES: Knowing that NFAT1 transcription factor has an important role in "classic" CD8 T lymphocytes, we questioned its part in the generation and function of Memory-like CD8 T cells during aging.

Method: Peripheral immune tissues (peripheral lymph nodes and spleen) from non-immunized mice were analyzed by Flow Cytometry.

RESULTS: We observed that young and aged mice lacking NFAT1 (NFAT1^{-/-}) presented a higher frequency of cells expressing high levels of CD44 compared with the control group (C57BL/6). As CD44 is a marker of activation, these results suggest an increase in activated/memory cells in the knockout mice (NFAT1^{-/-}). However, most of this population did not express CD49d and expressed high levels of CD62L and CD127, indicating that these are Memory-like cells. We also noted that this group of cells increased with age when we compared Middle-Aged (13-16 months) and Old (22-23 months) mice with the younger ones (2-3 months).

CONCLUSION: We still need additional experiments to have conclusions. So, for that, we are planning on analyzing additional markers, like NKG2D and T-bet, so we can better classify these cells. We also going to study the expression of some transcription factors on the Thymus, like PLZF and Eomes, to understand the generation of this group of cells. Furthermore, we intend to analyze functional properties in vitro, like IFN-

gamma and Granzyme B production, proliferation, and cytotoxic capacity.

Keywords: Memory-like; NFAT1; Aging.

PAPER CODE: 108

Exploring the functional and mechanistic aspects of PALB2 homodimerization

SKAF, L.B.1; GOMES, T.T.1; ELIAS, A.B.R.1; SUAREZ-KURTZ, G.1; MONTEIRO, A.N.2; CARVALHO, M.A.3. 1. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL; 2. H. LEE MOFFITT CANCER CENTER, RIO DE JANEIRO - RJ - BRASIL; 3. INSTITUTO FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

Introduction: Approximately 10% of all cancers have a hereditary origin, mainly due to mutations in genes involved in the DNA damage response. PALB2 is associated with hereditary predisposition to breast and pancreatic cancer and acts in homologous recombination (HR)-mediated DNA double-strand break repair. Recruitment of PALB2 to damage sites occurs through heterodimerization with BRCA1 via its coiled-coil (CC) motif, a process regulated by PALB2 phosphorylation. PALB2 homodimerization, also mediated by its CC, may negatively regulate HR by competing with BRCA1 heterodimerization. However, the functional relevance and regulatory mechanisms of PALB2 homodimerization remain unclear, and the impact of PALB2 variants on this process is poorly understood.

OBJECTIVES: To systematically evaluate the homodimerization capacity of PALB2 missense variants and explore the mechanisms that regulate this interaction.

METHOD: In silico analysis was performed using the DeepCoil algorithm for CC structure prediction. Protein-protein interactions were evaluated using (i) Bimolecular Fluorescence Complementation (BiFC) and (ii) co-immunoprecipitation (Co-IP) assays.

RESULTS: Eleven N-terminal missense variants, including seven within the CC motif, were analyzed. In silico prediction indicated CC structural disruption only for the p.L35P variant. Flow cytometry-based BiFC

showed that all variants, except p.L35P, retained wild-type (WT)-like homodimerization. Two independent Co-IP assay models revealed that p.K18R behaved similarly to WT, whereas p.L24A, p.Y28C, p.L32V, p.L35P, and p.R37H showed reduced homodimerization in both models. In contrast, p.P8L, p.K30N, p.H46Y, p.L169I, and p.S319Y exhibited assay-dependent effects. Ongoing analyses aim to elucidate mechanisms modulating the interplay between PALB2 homodimerization and BRCA1 heterodimerization, including responses to ionizing radiation and phosphorylation-dependent modulation. Previous data suggest that ATR and CDK-mediated phosphorylation events affecting PALB2-BRCA1 binding may also impact homodimerization dynamics.

CONCLUSION: Our findings provide novel insights into the functional consequences of PALB2 variants and improve understanding of the mechanisms controlling PALB2 homodimerization.

Keywords: PALB2; BRCA1; homologous recombination.

PAPER CODE: 110

THE RELATIONSHIP BETWEEN TREATMENT RESISTANCE AND TUMOR MICROENVIRONMENT HETEROGENEITY IN HIGH-GRADE SEROUS OVARIAN CANCER

GUIMARÃES, G.R.; TESSAROLLO, N.; SERAIN, A.F.; DE PAULA, D.J.G.; MORREUW, L.D.C.; CHAVES, C.B.P.; DE MELO, A.C.; VIOLA, J.P.B.; BORONI, M.. BRAZILIAN NATIONAL CANCER INSTITUTE, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Ovarian cancer encompasses a spectrum of heterogeneous diseases with distinct histotypes. Among them, the high-grade serous ovarian cancer (HGSOC) has the highest incidence and remains a clinical challenge, with a 5-year survival rate of only 34%. In Brazil, it leads to over 3,000 deaths annually, primarily due to late diagnosis and a lack of effective early detection strategies. The standard first line of treatment is an aggressive multimodal approach, combining cytoreductive surgery to resect and stage the tumor, followed by adjuvant platinum-

based chemotherapy. Non-responder patients face a limited drug landscape, especially if they do not present Homologous Recombination Deficiency.

OBJECTIVES: To characterize the cellular profile and tumor immune microenvironment architecture of HGSOC from chemotherapy-responding and non-responding patients through spatial and single-cell transcriptomics

METHOD: After quality control, we integrated 10 publicly available single-cell transcriptomes with a novel Brazilian cohort profiled by single-nuclei RNA-seq using scANVI. Integrated cells were dimensionally reduced using UMAP, clustered with the Leiden algorithm, and manually annotated by examining canonical markers and their association with differentially expressed genes across cell populations.

RESULTS: The atlas comprises 833,958 high-quality cells, including 146 samples from 101 patients, covering different anatomical sites from healthy donors, such as the fallopian tube and ovary, as well as samples from patients diagnosed with HGSOC, including primary tumor, omentum, peritoneum, and ascites. Preliminary analyses reveal heterogeneous immune and stromal populations, with transcriptionally distinct states across tumor sites and treatment responses. Among malignant cells, we identified a clonal population expanding in non-responder patients, characterized by upregulation of the Wnt signaling pathway. Differential expression and pathway enrichment analyses are being performed to identify molecular signatures associated with chemoresistance.

CONCLUSION: Our integrated single-nucleus transcriptomic approach is uncovering key features of the HGSOC microenvironment associated with treatment response. The inclusion of a novel Brazilian cohort expands the diversity and relevance of available datasets. Future directions include expanding the cohort and incorporating spatial transcriptomics (Xenium platform) to map spatial context and cell-cell interactions, aiming to inform more personalized and effective therapeutic strategies.

Keywords: single-cell RNA-seq; high-grade serous ovarian cancer; tumor microenvironment.

PAPER CODE: 111

single-cell resolution of Isoform signatures reveals functional diversity among tumor clones in HGSOC

DA FONSECA, G.F.C.; DE TOLEDO, N.E.; GUIMARÃES, G.R.; SILVA, A.C.P.E.; TESSAROLLO, N.; BAPTISTA, G.; BORONI, M.. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: The most deadly gynecologic cancer is still ovarian cancer, and high-grade serous ovarian carcinoma (HGSOC) is particularly difficult to treat because of its marked resistance to treatment. A fundamental RNA processing method called alternative splicing (AS) allows for the selective inclusion or exclusion of particular exons and introns, resulting in the production of several isoforms from a single gene. Tumor initiation and progression are greatly impacted by this mechanism, which is essential for many biological functions. It contributes to the hallmarks of cancer, including metastasis, immune evasion, and proliferation. Tumor-specific splice isoforms that contribute to these characteristics can be produced as a result of dysregulated alternative splicing.

OBJECTIVES: The present study seeks to characterize AS derived isoforms generated by distinct tumor cell types in HGSOC.

METHOD: To identify specific splicing errors and their related isoforms, we used publically available raw single-cell RNA-sequencing datasets from HGSOC patients (study id: phs002262.v3.p1). BAM alignments are obtained using CellRanger, while AS links are detected using STARsolo. The output goes through a full pipeline for quality control, scvi-tool integration, and Seurat cell-type identification. AS characteristics were linked to specific cellular populations using the MARVEL platform.

RESULTS: In total, we detected on average 18,013 protein-coding transcripts, 11,622 lncRNAs, and additional RNA categories expressed at low abundance. In our initial analysis, we compared AS events differentially regulated between epithelial cells and malignant clones, distinguished by copy number

variation inference. Differential splice junction (SJ) analysis revealed 4,088 dysregulated events involving 938 genes. Among them, PABPC1 exhibited specific isoforms antagonizing the nonsense-mediated decay (NMD) pathway. By comparing SJ expression between epithelial and malignant cells, we observed that these junctions were more expressed in clones, corresponding to isoforms associated with malignant populations. In clones, SJs were linked to five protein domains of PABPC1, whereas in normal epithelial cells they were restricted to a single domain. Expression validation using TCGA and GTEx RNA-seq confirmed deregulated PABPC1 isoforms, linked to tumor progression and poor outcomes in HGSC.

CONCLUSION: We discovered overexpressed isoforms identified in this pathway in tumor clones, compared them with normal cells, and evaluated the prognostic significance of these isoforms using data from the TCGA cohort. By analyzing these splicing patterns and their isoforms, our goal is to identify new biomarkers as therapeutic targets and broaden our understanding of tumor progression.

Keywords: scRNA-seq; ovarian cancer; isoforms.

PAPER CODE: 116

CHARACTERIZATION OF MUTATIONAL SIGNATURES IN TUMOR TISSUE FROM CERVICAL CANCER INFECTED WITH DIFFERENT HPV TYPES

DE SÁ, C.C.1; PAREDES, M.E.1; SANTOS, R.B.V.1; FELIX, S.P.1; BRANT, A.C.1; MOREIRA, M.Â.M.1; SOARES, M.A.1; BORONI, M.1; SIQUEIRA, J.D.1; GOES, L.R.2. 1. INSTITUTO NACIONAL DO CÂNCER (INCA), RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO NACIONAL DO CÂNCER (INCA); UNIVERSIDADE FEDERAL DO ESTADO DO RIO DE JANEIRO (UNIRIO), RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Chronic infection by HPV is associated with the development of virtually all cases of cervical cancer (CC). There are more than 450 HPV types characterized, with types 16 and 18 being the most common, responsible for 70% of CC cases. Viral

infections can induce mechanisms of innate immunity, such as APOBEC enzymes. This family of proteins has a cytidine deaminase domain, causing nucleotide mutations from cytosine to uracil in DNA/RNA, acting as a restriction factor for HPV. However, APOBEC editing can also induce mutations on the host genome, and mutational signatures characteristic of these enzymes have been found in cervical cancer samples. Our group have demonstrated that HPV18-positive cervical tumors appeared to have higher expression of APOBEC3B compared to other HPV types.

OBJECTIVES: Therefore, the current study aims to explore the mutational profiles in the genomic DNA of cervical cancer cells and compare these profiles according to the infecting HPV types.

METHOD: The whole genome (WGS) from 47 CC samples from patients from the Brazilian National Cancer Institute (INCA) and the exome from the TCGA-CESC cohort (n=163) are being analyzed. The Sarek (nf-core) pipeline was applied for functional annotation of mutations of INCA's patients. For all samples, mutational signatures were extracted using the SigProfileMatrixGenerator and SigProfileExtractor packages and results compared statistically for each HPV-infected tumor and clinical information.

RESULTS: The mutational profile from the TCGA-CESC cohort generated three de novo signatures, later decomposed for the COSMIC signatures. Among those, a contribution of SBS1, SBS5, SBS10a, SBS15 and both APOBEC signatures, SBS2 and SBS13, was observed. There was no statistically significant difference in the absolute number of mutations from SBS2 and SBS13 (APOBEC signatures) between different HPV types and status when a linear regression model adjusted for tumor staging and age was accessed. The analysis regarding INCA's samples revealed a distinct profile from the exome data, being the clock-like mutations SBS1 and SBS5 the two most frequent signatures, followed by APOBEC-associated signatures. Nonetheless, two signatures associated with defective DNA mismatch repair (SBS3 and SBS20), were also found in INCA cohort.

CONCLUSION: The frequency of APOBEC-associated signatures does not seem to differ among the different HPV groups in the TCGA cohort. The profile of mutational signatures differs between the INCA's WGS and TCGA exome data. The signatures specific to the exonic regions of the INCA genomes are currently being analyzed and will be compared to the TCGA results, along with the clinical aspects of these cohorts.

Keywords: cervical cancer; HPV; APOBEC.

PAPER CODE: 118

CHARACTERIZATION AND VALIDATION OF GLIOBLASTOMA CELL LINES HARBORING TP53 MUTATIONS

RAFAEL, M.M.1; MAGALHÃES, M.V.D.C.2; SILVA, M.L.D.S.3; MONTE-MÓR, B.3; MAIA, R.C.4; BERNARDO, P.S.4. 1. LABORATÓRIO DE HEMATO-ONCOLOGIA CELULAR E MOLECULAR, PROGRAMA DE HEMATO-ONCOLOGIA MOLECULAR, INSTITUTO NACIONAL DE CÂNCER (INCA) - RJ, BRASIL, UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO (UFRRJ), RIO DE JANEIRO - RJ - BRASIL; 2. PROGRAMA DE PÓS-GRADUAÇÃO STRICTO SENSO EM ONCOLOGIA, INSTITUTO NACIONAL DE CÂNCER (INCA) - RJ, BRASIL, RIO DE JANEIRO - RJ - BRASIL; 3. DIVISÃO DE LABORATÓRIOS ESPECIALIZADOS(DILABESP), LABORATÓRIO DE BIOLOGIA MOLECULAR, PROGRAMA DE TERAPIA CELULAR E GÊNICA, INCA-RJ, BRASIL, RIO DE JANEIRO - RJ - BRASIL; 4. LABORATÓRIO DE HEMATO-ONCOLOGIA CELULAR E MOLECULAR, PROGRAMA DE HEMATO-ONCOLOGIA MOLECULAR, INSTITUTO NACIONAL DE CÂNCER (INCA) - RJ, BRASIL, PROGRAMA DE PÓS-GRADUAÇÃO STRICTO SENSO EM ONCOLOGIA, INS, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Glioblastoma (GBM) is the most prevalent and aggressive malignant brain tumor in adults. Its pronounced molecular heterogeneity and remarkable infiltrative behavior contribute to treatment resistance and poor prognosis. p53 pathway plays a central role in tumor suppression by regulating genes involved in cell cycle arrest, DNA repair, and

apoptosis in response to oncogenic or genotoxic stress. Dysregulation of this crucial pathway is a key molecular feature of GBM pathogenesis. Around 35% of GBM cases harbor TP53 mutations, inhibiting its canonical function. Although TP53 mutations are generally correlated with a poor prognosis, our group has observed an increase in response to radiotherapy in TP53 mutant GBM cells, in vitro.

OBJECTIVES: Based on this finding, this study aims to characterize, and validate GBM cell lines expressing the TP53 R175H hotspot mutation to investigate how this mutation influences GBM response to radiotherapy.

METHOD: A172, a TP53 wild-type GBM cell line, was previously edited by CRISPR/Cas9 to insert TP53 R175H hotspot mutation. Selected clones from the edited cell pool were treated with ionizing radiation and analyzed by Western blotting to assess p53 protein levels in cellular stress context. Subsequently, clones lacking detectable p53 were further evaluated by Sanger DNA sequencing of exon 5 to identify potentially deleterious mutations in comparison to wild-type TP53 sequence.

RESULTS: So far, 21 clones were selected from the edited cell pool, and 13 of them were exposed to 8 Gy of ionizing radiation for subsequent analysis of p53 protein levels. Three clones exhibited a profile similar to the parental cell line, suggesting preservation of p53 pathway activation by ionizing radiation. Six clones showed absence of detectable p53 protein, possibly indicating the introduction of indels. Three clones displayed high p53 levels regardless of radiation treatment, consistent with insertion of the R175H mutation, which is characterized by accumulation of nonfunctional p53. DNA sequencing of exon 5 confirmed that clones 2D4, 2E4, 2E11, 1E10 and 2B5 have indels, promoting the absence of detectable protein. Selected clones carrying the R175H mutation will subsequently be exposed to ionizing radiation to determine whether the mutation modulates GBM resistance or sensitivity to treatment.

CONCLUSION: The absence of detectable levels of p53 in six clones is likely due to indels, resulting in protein loss. Furthermore, the gene expression of CDKN1A and

BAX, transcriptional targets of p53, will be assessed by RT-qPCR, and the cytotoxic effect of ionizing radiation will be evaluated via PARP and cleaved Caspase-3 expression by Western blot, as well as annexin V/PI staining by flow cytometry.

Keywords: Glioblastoma; TP53; radiation resistance.

PAPER CODE: 119

LONG-TERM DYNAMICS OF HIV-1 PROVIRAL RESERVOIRS FROM PATIENTS UNDER SUCCESSFUL ANTIRETROVIRAL THERAPY

BOTELHO, P.A.1; BOTELHO, O.M.1; GARRIDO, M.M.2; DA HORA, V.P.3; GOES, L.R.1; SEIXAS, B.L.M.A.1; SOARES, M.A.1. 1. PROGRAMA DE GENÉTICA E VIROLOGIA TUMORAL, INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL; 2. SERVIÇO DE DOENÇAS INFECCIOSAS, HOSPITAL FEDERAL DE IPANEMA, RIO DE JANEIRO - RJ - BRASIL; 3. LABORATÓRIO DE BIOLOGIA MOLECULAR, ESCOLA DE MEDICINA, UNIVERSIDADE FEDERAL DO RIO GRANDE, RIO GRANDE - RS - BRASIL.

INTRODUCTION: The major obstacle in developing a cure for immunodeficiency virus type 1 (HIV-1) infection lies in the establishment of a viral reservoir. Despite the success of antiretroviral therapy, the virus persists in latent reservoirs, integrated into the host genome of several cell types. These reservoirs are formed during the early stages of infection and function as the source of viral replication once treatment is interrupted. The immune deficiency triggered by HIV, along with viral-induced chronic inflammation, causes an increased risk of developing certain cancer types. To search for new therapeutic interventions and enhance treatment and prognosis of both HIV infection and its associated malignancies, an understanding of the mechanisms and dynamics driving viral persistence is imperative.

OBJECTIVES: In this regard, this project aims to evaluate the size and long-term dynamics of HIV-1 reservoirs in peripheral blood of patients undergoing successful antiretroviral therapy in two Brazilian cities.

METHOD: Patients from Rio de Janeiro (RJ) and Rio Grande (RS) were recruited and peripheral blood samples were collected. A subset of patients had a second and a third timepoint collected with an interval of two years between them. Total genomic DNA was extracted from PBMCs and HIV-1 proviral DNA was quantified using droplet digital PCR by detecting the LTR and pol viral genes. Each individual's total proviral load was estimated through normalization to endogenous genes RPP30 and deltaD.

RESULTS: Across all samples quantified so far, reservoir size showed a median of 86,112 LTR copies per 10^6 PBMCs and a median of 12,524 pol copies per 10^6 PBMCs. In T lymphocytes, a median of 115,577 LTR copies per 10^6 T cells and a median of 16,403 pol copies per 10^6 T cells were observed. Two of the patients analysed so far had longitudinal samples available and the dynamics revealed are in agreement with typical trends of total HIV DNA over time. In the first patient, a reduction of the reservoir in PBMCs was observed from the first to the second timepoint, followed by an increase at the third timepoint, both in LTR and pol. However, in the second patient, levels of LTR copies seemed stable, while a reduction of pol copies from the first to the second timepoint was noticed. For T cells, the levels of HIV DNA demonstrated different profiles, with the first patient exhibiting a decay of reservoir size and the second displaying an increase in the number of copies detected, both in LTR and pol.

CONCLUSION: Our preliminary results resemble those previously reported in literature, indicating a slow, dynamic, and fluctuating decay of proviral reservoirs. As expected, we observed higher levels of HIV DNA in T cells than PBMCs. The research conducted in this study will improve our understanding of HIV-1 reservoir size in peripheral blood of Brazilian patients living with HIV, their evolution during long-term antiretroviral therapy, and will identify viral and host factors associated with their size.

Keywords: HIV; reservoirs; antiretroviral therapy.

PAPER CODE: 123

Evaluation of the effects of ZNF429 transcription factor on ovarian cancer pathways

DE TOLEDO, N.E.1; BAPTISTA, G.1; DA FONSECA, G.C.F.C.1; TESSAROLLO, N.1; RAJÃO, M.1; VALVERDE, P.1; ARAÚJO JUNIOR, M.L.C.1; BESSA, C.1; BERNARDES, S.S.2; POSSIK, P.A.1; DA COSTA, N.O.M.1; PACCEZ, J.3; ZERBINI, L.4; BORONI, M.1. 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. UFMG, BELO HORIZONTE - MG - BRASIL; 3. ICESP, SÃO PAULO - SP - BRASIL; 4. CANCER GENOMICS GROUP, INTERNATIONAL CENTRE FOR GENETIC ENGINEERING AND BIOTECHNOLOGY (ICGEB), CAPE TOWN, SOUTH AFRICA, CIDADE DO CABO (AFRICA DO SUL) - SP - BRASIL.

INTRODUCTION: The KRAB-ZNFs (Krüppel box-associated zinc finger) is the largest family of epigenetic repressors. One of the members, ZNF429, is a poorly studied gene and its expression was associated with the survival of patients with high-grade serous ovarian cancer (HGSOC). Using clinical and gene expression data (RNA-seq) from The Cancer Genome Atlas repository, our group showed that higher ZNF429 expression is associated with a better prognosis in HGSOC. However, the underlying mechanisms mediated by ZNF429 remain unknown.

OBJECTIVES: To investigate ZNF429-mediated regulation, its targets, and related pathways in ovarian cancer using INCA tumor samples and cell lines with distinct ZNF429 expression levels.

METHOD: We analyzed ZNF429 gene and protein expression (RT-qPCR and immunohistochemistry) in ovarian tumor samples from INCA and in cell lines (control and genetically modified by CRISPR/Cas-9 system and lentiviral vector overexpressing the gene), and related the expression patterns with clinical outcomes. Additionally, we also performed ZNF429 knockdown by siRNA. Integrating ChIP-seq and RNA-seq data from control and modified cells, we will reveal the ZNF429 targets as well as the related pathways.

RESULTS: We assessed ZNF429 expression by real-time PCR and analyzed its association with patient survival. Samples were classified as high or low expressers,

revealing that high ZNF429 expression correlated with better overall survival in HGSOC ($p = 0.0096$, $HR = 0.4$, $95\% CI = 0.2-0.79$). Protein quantification by ImageJ and survival analysis confirmed this association for both nuclear and cytoplasmic ZNF429 ($p = 0.022$, $HR = 0.57$, $95\% CI = 0.34-0.95$; $p = 0.045$, $HR = 0.55$, $95\% CI = 0.29-1$). For functional studies, ZNF429 was cloned into pBabe-kasWT (HA-tagged) and subsequently into the lentiviral vector pLenti6.2 TagBFP. Lentivirus production and cell transduction for overexpression are underway, while CRISPR/Cas9 knockout cloning and transduction are in progress. Since the knockout was not yet successfully established, as an alternative approach, siRNA-mediated silencing efficiently reduced ZNF429 mRNA levels in SKOV3 cells within 24h, confirming effective knockdown.

CONCLUSION: This study has demonstrated that high expression of ZNF429 is associated with a favorable prognosis in patients with HGSOC, consistent with our preliminary analyses. Overall, these findings emphasize the importance of ZNF429 as a possible predictive biomarker in ovarian cancer. Its dual location and association with higher survival rates indicate that ZNF429 may be a critical regulator of tumor biology, necessitating additional exploration into its molecular processes and therapeutic potential.

Keywords: KRAB zinc fingers; ZNF429 ;ovarian cancer .

PAPER CODE: 124

The effect of Cyclin E1 overexpression on chromosome segregation and ultrafine DNA bridges formation in mammary epithelial cells

DE OLIVEIRA, V.P.; FERREIRA, P.D.S.; TEIXEIRA, L.K.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: The cell cycle is a fundamental process in biology due to its role in regulating cell proliferation and genome maintenance. Errors during DNA replication and chromosome segregation can lead to genomic instability, contributing to carcinogenesis. Cyclin E1 is a central protein involved in cell cycle control

by promoting DNA replication initiation. High levels of Cyclin E1 can cause genomic instability by inducing replication stress and chromosome segregation errors. However, it has not been evaluated whether Cyclin E1 overexpression induces the formation of ultrafine DNA bridges (UFBs). UFBs are histone-free bridges composed of intertwined DNA, associated with several proteins, that can originate from different chromosomal regions and impair chromosome segregation.

OBJECTIVES: Therefore, the main of this work is to evaluate if high levels of Cyclin E1 can affect cell cycle progression, impair chromosome segregation and induce UFBs formation.

METHOD: Cyclin E1 overexpression was induced in HME1 cells, a normal human mammary epithelial cell lineage, by retroviral or adenoviral transduction. Cells were analyzed for protein levels by Western blot and cell cycle profile by flow cytometry. Chromosome segregation and UFB formation were analyzed by immunofluorescence with antibodies for PICH (UFBs), CENP-A (centromeres) or FANCD2 (common fragile sites), and DAPI.

RESULTS: So far, the retroviral (Rv) model has been completely analyzed, while the adenoviral (Adv) model is in progress. We have observed that, compared with control cells, HME1 Adv-CCNE1 and HME1 Rv-CCNE1 cells exhibit higher levels of Cyclin E1 for at least 3 days and 30 days after transduction, respectively. HME1 cells with high levels of Cyclin E1 exhibit cell cycle profile alterations, showing a reduced frequency of cells in G1 phase and an increased frequency of cells in S and G2/M phases. HME1 Adv-CCNE1 cells exhibit increased frequency of abnormal anaphases. In contrast, we did not observe any differences in the frequency of abnormal anaphases after Cyclin E1 overexpression in the retroviral model. Regarding UFBs, we have observed that Cyclin E1 overexpression had different effects between the Adv and Rv models. In the adenoviral model, common fragile sites-associated UFBs were increased, while in the retroviral model, centromeric UFBs were decreased.

CONCLUSION: These findings suggest that Cyclin E1 overexpression alters the cell cycle progression, chromosome segregation, and formation and/or

resolution of centromeric and common fragile sites UFBs during anaphase. Future experiments will be needed to clarify the effect of Cyclin E1 overexpression in the formation of different types of UFBs and evaluate the impact of such events in gains and losses of chromosome regions.

Keywords: cell cycle; genomic instability; ultrafine anaphase bridges.

PAPER CODE: 131

PREVALENCE AND DIVERSITY OF HPV IN THE CERVIX AND ANAL CANAL OF WOMEN LIVING WITH HIV

DE OLIVEIRA, É.M.1; SILVA, J.A.D.D.C.E.1; LEAL, F.E.1; GUIMARÃES, I.C.C.D.V.2; LIBERATORI, J.1; LAMEIRA, R.2; PACHECO, M.G.D.A.1; EMERICK, C.1; SOARES, M.A.1; GOES, L.R.1; SIQUEIRA, J.D.1. 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. UFF, NITERÓI - RJ - BRASIL.

INTRODUCTION: Human papillomavirus (HPV) is the most common sexually transmitted infection worldwide and is associated with various cancers, including cervical and anal cancer. People living with HIV are at higher risk for HPV infection, intraepithelial neoplasia, and related cancers. The incidence of anal cancer has increased over the years, supporting screening in high-risk groups such as women living with HIV (WLWH). Studies suggest that cervical HPV screening may help identify women at risk for anal cancer, although this association remains unclear. Assessing HPV diversity and the relationship between cervical and anal HPV infection is essential for developing effective prevention and management strategies for anal cancer in this vulnerable population.

OBJECTIVES: This study aimed to characterize the presence and diversity of HPV in the anal canal and cervix of WLWH to better understand the dynamics of HPV infection at these two anatomical sites.

METHOD: Cervical and anal canal swabs were collected from WLWH recruited from hospitals in Rio de Janeiro, Brazil. HPV detection and genotyping were performed using the reverse hybridization method, and Cohen's kappa coefficient (k) was calculated to assess agreement between the results from both sites.

RESULTS: In total, 266 samples from 133 women were analyzed. HPV was identified in 42.8% (57/133) of cervical samples and 72.9% (97/133) of anal canal samples. Thirty-two distinct HPV types were detected. In the cervix, the most prevalent type was HPV 44/55, followed by types 62/81, 61, and 16. In the anal canal, the most prevalent type was HPV 44/55, followed by types 6, 62/81, and 42. HPV16 was found in seven cervical and eleven anal samples, while HPV18 was detected in four cervical and ten anal samples. Multiple HPV infections were observed in 29 cervical and 54 anal samples. In most cases, HPV status differed between the two sites within the same individual. Regarding HPV-positive status, only 37.6% had concordant results in both regions ($k = 0.1597$). A low concordance was also observed when comparing positivity for high-risk HPV types ($k = 0.1104$) and for multiple HPV infections ($k = 0.2332$). No concordance was observed for HPV16-positive samples ($k = -0.0761$).

CONCLUSION: These results suggest differences in susceptibility to HPV infection between the two anatomical sites and highlight the need for specific anal cancer screening in this population. To better understand these differences, we will explore the role of the immune response and microbiome in the acquisition, clearance and persistence of HPV infection at these sites. Understanding these interactions may help develop more targeted prevention and management strategies for HPV-associated diseases.

Keywords: HPV; HIV; Prevalence.

PAPER CODE: 132

CHARACTERIZATION OF THE INTRATUMORAL MICROBIOTA IN CERVICAL CANCER

RIBEIRO, E.J.; FELIX, S.P.; BRANT, A.C.; MOREIRA, M.A.M.; SOARES, M.A.; GOES, L.R.; SIQUEIRA, J.D.. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Human papillomavirus (HPV) infection is the primary risk factor for cervical cancer. Recently, the cervical bacterial community has been considered an additional risk factor for HPV infection and carcinogenesis. A decrease in lactobacillus proportions,

along with an increase in bacterial diversity, known as dysbiosis, has been associated with HPV persistence. In tumors, bacterial profiles have been linked to intratumoral immune responses and the efficacy of treatment across cancer types. Understanding the taxonomic and functional composition of the cervical cancer microbiome is essential to understand each microorganism's role and its contribution to cancer development and prognosis. These data can provide insights for developing strategies in prevention, screening, and treatment.

OBJECTIVES: The objective of this project is to characterize the microbiota in cervical cancer tumor tissue using shotgun sequencing.

METHOD: DNA was extracted from tumor samples of 29 patients at the Instituto Nacional de Câncer (INCA) and sequenced on the MGI Tech DNBSEQ-T7 platform. Sequences were quality-filtered and taxonomically assigned using the Kraken2 program with the RefSeq Standard-16 database. Relative abundances were normalized in R (vegan v.2.6-10). Taxonomic diversity was assessed and compared between samples of different histological types, tumor stages, and HPV genotypes using statistical analyses.

RESULTS: A total of 917 species and 359 genera of bacteria were identified. The most abundant species were *Escherichia coli*, *Bacteroides ovatus*, *Fusobacterium nucleatum*, *Sneathia vaginalis*, and *Bacteroides fragilis*. Stage II samples showed higher alpha diversity (Shannon Index), compared to stage I and stage III samples. In the multivariate analysis, a lower abundance of the genus *Parvimonas* was observed in squamous cell carcinoma samples and of the genus *Porphyromonas* in samples from non-smoking women, when compared to adenocarcinomas and smoking samples, respectively, whereas the genera *Solobacterium* and *Dorea* showed higher abundance in stage III samples.

CONCLUSION: The characterization of the intratumoral microbiota in cervical cancer revealed a diverse microbial community potentially linked to disease development and progression, with specific associations observed between certain microorganisms and clinical features. These findings highlight the need to evaluate the microbiome and its role in the tumor microenvironment

to develop more effective therapeutic and screening strategies.

Keywords: Shotgun sequencing; Intratumoral microbiome; Human papillomavirus.

PAPER CODE: 133

Gene expression profiles of chemotherapy resistance in ovarian cancer patients

BAPTISTA, G.1; TESSAROLLO, N.1; DE TOLEDO, N.E.1; DA FONSECA, G.C.F.C.1; SERAIN, A.F.1; GUIMARÃES, G.R.1; DE LANNA, C.A.1; PAIVA, V.B.1; MORREUW, L.D.C.1; DE PAULA, D.J.G.1; MACEDO, F.D.C.1; BESSA, C.1; DE MELO, A.C.1; BERNARDES, S.S.2; VIOLA, J.P.B.1; BORONI, M.1. 1. INSTITUTO NACIONAL DE CâNCER, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE - MG - BRASIL.

INTRODUCTION: Ovarian cancer is the most lethal gynecological malignancy. In Brazil, according to the National Cancer Institute (INCA), it ranks eighth in incidence, with an estimated 7,310 new cases per year for the 2023–2025 triennium. High-grade serous ovarian carcinoma (HGSOC) is the most common subtype, accounting for 85–90% of cases, often diagnosed at advanced stages with poor prognosis. Standard therapy in the Brazilian Public Health System is based on platinum and taxane compounds. Despite initial treatment response, over 70% of patients relapse within five years. Characterizing transcriptomic profiles associated with clinical outcomes is essential for the development of targeted therapies.

Objectives: To characterize transcriptomic profiles of HGSOC from Brazilian patients treated at INCA and to identify genes linked to chemotherapy response.

METHOD: Forty-nine HGSOC samples from patients treated with platinum-based chemotherapy were classified according to the progression-free interval. RNA libraries were prepared and sequenced on an Illumina platform. Data quality control and preprocessing were performed using FASTQC, Trimmomatic, and STAR. Differential expression and pathway enrichment analyses were conducted with DESeq2 and fgsea R

packages, while survival analysis was performed using the Survminer R package.

RESULTS: Differential expression analysis revealed marked transcriptomic differences between platinum resistant and platinum sensitive patient groups. Functional enrichment in this comparison highlighted key molecular pathways associated with chemoresistance, including extracellular matrix organization, PI3K-Akt, WNT, and KRAS signaling. Collagen-related genes, such as COL5A3 (INCA: $p=0.03$; TCGA: $p=0.07$) and VWF (INCA: $p=0.02$; TCGA: $p=0.02$), were significantly associated with poorer overall survival or shorter progression-free interval.

CONCLUSION: According to these findings, extracellular matrix remodeling and the activation of oncogenic pathways are involved in platinum resistance in HGSOC. Genes from this network are highlighted as possible biomarkers and therapeutic targets to overcome chemoresistance.

Keywords: Ovarian cancer; Chemotherapy resistance; Transcriptomics.

PAPER CODE: 136

ROLE OF TRANSCRIPTIONAL CO-FACTOR IRF2BP2 IN ACTIVATION AND DIFFERENTIATION OF CD8 T CELLS

OLIVEIRA-VIEIRA, B.1; PEIXOTO, B.C.1; ROSA-CAMPOS, F.1; PEREIRA, R.M.2; VIOLA, J.P.B.1. 1. NATIONAL CANCER INSTITUTE, RIO DE JANEIRO - RJ - BRASIL; 2. FEDERAL UNIVERSITY RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Introduction: IRF2BP2 (Interferon Regulatory Factor-2-Binding Protein-2) is a transcriptional co-factor involved in the regulation of gene expression in different biological contexts. Our group previously described IRF2BP2 protein as a NFAT1 co-repressor playing a role in the activation and differentiation of T lymphocytes. To evaluate the role of IRF2BP2 in the CD8 T cell response in vivo, we generated a conditional transgenic mice that overexpress IRF2BP2 in T lymphocytes, LCK-Cre Rosa26 Stop-flox IRF2BP2-IRES-EGFP (IRF2BP2 TKi).

OBJECTIVES: To evaluate the involvement of IRF2BP2 overexpression in CD8⁺ T cell activation and differentiation.

METHOD: Conditional IRF2BP2 TKi mice were used to evaluate CD8⁺ T cell activation and differentiation. To evaluate the effect of IRF2BP2 on CD8 T cell differentiation, we assessed effector and memory profile using in vitro skewed cell culture, with marker expression assessed by flow cytometry and qRT-PCR and cytokine production quantified by ELISA. For in vivo analysis, mice were infected with LCMV clone 13, and virus-specific CD8⁺ T cells were analyzed by flow cytometry after 20 days.

RESULTS: Naive IRF2BP2 TKi mice showed a reduction in T cell populations in peripheral organs and an increased percentage of CD8⁺CD44⁺ cells, suggesting a higher proportion of effector cells. In effector cultures, cells from IRF2BP2 TKi mice showed increased CD8⁺CD44⁺ populations and higher granzyme B and IFN- γ expression, while in memory cultures they exhibited an increase of percentage of CD25 and a decrease of CD127, effector and memory marker respectively and this cells demonstrated an increased IFN- γ and granzyme B. qRT-PCR analysis revealed elevated Tbet and Prdm1 expression in effector cells and an increase of Prdm1 in memory cells, this expression indicating positive regulation of effector molecules. In a chronic LCMV clone13 infection model, 20 days post-infection, virus-specific CD8⁺ T cells from IRF2BP2 TKi mice showed an increase of CD44 cells and decreased PD-1 expression, suggesting suggesting that these cells are more activated and less exhausted

CONCLUSION: IRF2BP2 enhances CD8⁺ T cell activation and effector differentiation, increasing granzyme B and IFN- γ expression and upregulating Tbet and Prdm1. In vivo, IRF2BP2 TKi mice show reduced exhaustion during chronic LCMV infection, indicating a role in promoting functional effector CD8⁺ T cells. Taken together, these data suggest a possible role for IRF2BP2 in regulating the balance between CD8 T cell activation and hyporesponsiveness.

Keywords: CD8 T Cell; IRF2BP2; Effector cells.

PAPER CODE: 139

EFFECT OF COMBINED TREATMENT BETWEEN TRASTUZUMAB AND RECOMBINANT JARARACIN OVER EPITHELIAL-MESENCHYMAL TRANSITION IN HER2+ BREAST CANCER CELLS

GONÇALVES, L.A.1; LOVATEL, V.L.2; SANT'ANNA, A.L.2; FERNANDEZ, T.D.S.2; ZINGALI, R.B.3; PEREIRA, D.D.A.2.

1. INSTITUTO NACIONAL DO CÂNCER (INCA), RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO NACIONAL DE CÂNCER (INCA), RIO DE JANEIRO - RJ - BRASIL; 3. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO (UFRJ), RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Breast cancer is the leading cause of death due to cancer in women worldwide. The HER2+ subtype is characterized by the overexpression of the Epidermal Growth Factor Receptor 2 (HER2), a member of the EGFR family. This subtype is treated with a target antibody named Trastuzumab. However, 70% of the patients with this subtype present resistance. Previously, we showed that the resistant cell type has a high expression of β 1 integrin and after Trastuzumab treatment, the expression increased. As integrins have a specific binding region to communicate with the ECM molecules, such as the RGD domain, we blocked β 1 integrin to analyze the effect over the EMT. For this we used a recombinant disintegrin, a polypeptide derived from the venom of the Bothrops jararaca snake, jararacin.

Objectives: Analyze the effect of β 1 integrin blocking with jararacin over EMT in HER2 positive breast cancer cell line after Trastuzumab treatment.

METHOD: HER2+ breast cancer cell line was treated with Trastuzumab or jararacin or with a combination of both. RNA was extracted, cDNA was synthesized, and RT-qPCR were performed for E-cadherin, Vimentin, and GAPDH using the BioRad SsoAdvancedTM Universal SYBR[®] Green Supermix on a QIAGEN Rotor-Gene thermocycler. To analyze the co-localization of E-cadherin and HER2 receptor and E-cadherin and β 1 integrin, we performed immunofluorescence.

RESULTS: Our RT-qPCR preliminary results showed that the HCC-1954 cell line resistant to Trastuzumab treatment, has a high expression of E-cadherin in both untreated and Trastuzumab treated conditions. While in jararacin and in combined Trastuzumab plus jararacin treatments, E-cadherin expression was reduced. Vimentin expression was low in untreated and in Trastuzumab treated conditions. In jararacin treatment, the Vimentin expression was high, while in the combined treatment the expression was higher. Immunofluorescence results showed that there is a co-localization of E-cadherin and HER2 and E-cadherin and β 1 integrin in the HCC-1954 untreated cell line.

CONCLUSION: Blocking β 1 integrin in HCC-1954 cells resulted in a decrease in E-cadherin and an increase in vimentin. However, for Epithelial-Mesenchymal Transition evaluation, other experiments are necessary. The co-localization of E-cadherin, HER2 and β 1 integrin suggests a crosstalk between these receptors.

Keywords: HER2+ Breast cancer; Trastuzumab; EMT.

PAPER CODE: 140

Transcriptomic Analysis of Cervical Cancer in Brazil

VEDOVATTO, S.; DE TOLEDO, N.E.; TESSAROLLO, N.; SCHERER, N.D.M.; FURTADO, C.; DE PAULA, D.J.G.; GOES, L.; MELO, Y.; DE MELO, A.C.; BORONI, M.; SOARES, M.A.; MOREIRA, M.Â.M.; LIMA, S.C.S.; PINTO, L.F.R.; VIOLA, J.P.B.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Cervical cancer (CC) remains a major public health challenge in Brazil, marked by high incidence and pronounced regional disparities in outcomes. Mortality rates are strongly associated with socioeconomic status, with higher values observed in states with lower household incomes. To help reduce these national inequalities, this study employs transcriptomic sequencing as a key approach to characterize the molecular landscape of CC. Through RNA sequencing, differentially expressed genes (DEGs) are analyzed in recurrent and non-recurrent tumors, providing insights into the biological processes driving disease progression.

OBJECTIVES: The main objective of this project is to establish a national multi-omic databank integrating transcriptomic, genomic, and methylomic data from 160 samples of cervical cancer and precancerous lesions.

METHOD: In this context, whole-genome (WGS) and transcriptome (RNA-seq) sequencing, as well as DNA methylation profiling, were conducted on cervical cancer and precancerous lesion samples. Libraries were prepared using Illumina and Zymo kits, and data were generated using the NovaSeq X Plus and iScan platforms. Transcriptomes underwent quality control, read alignment, gene expression quantification, differential analysis, and functional enrichment.

RESULTS: Multi-omic profiling, including WGS, RNA-seq, and methylome analyses, was performed on 160 cervical cancer and precancerous lesion samples. Data quality was satisfactory, with adequate coverage and signal intensity ensuring reliable downstream analyses. So far, only transcriptomic data have been processed. Gene expression comparison between recurrent and non-recurrent cervical cancer revealed distinct signatures for squamous cell carcinoma (SCC) and adenocarcinoma (AC). In SCC, upregulated lncRNAs (IL12A-AS1, CCDC14NL-AS1) and signaling genes (RASGEF1C, CPS1) indicate activated proliferation, while downregulated suppressors (NR0B2, KLK3, CDSN) suggest loss of apoptosis and adhesion. In AC, upregulated SOX10, FGF14, and MRC1 point to enhanced proliferation and immune evasion, whereas downregulated PRAF2, GRB7, ITLN1, and NLRP7 reflect impaired immune and tumor-suppressive control. These findings support the identification of biomarkers and therapeutic targets in cervical cancer.

CONCLUSION: Transcriptomic analysis revealed molecular alterations associated with cervical cancer recurrence, identifying genes involved in proliferation, migration, immune modulation, and matrix remodeling as potential biomarkers. Future integration of multi-omic data (transcriptomic, genomic, and methylomic) will further deepen our understanding of cervical cancer biology, enabling earlier identification of at-risk patients and the development of more personalized therapies.

Keywords: Cervical cancer; squamous cell carcinoma; adenocarcinoma.

PAPER CODE: 143

The role of DNA methylation in GATA6 regulation and its functional impact in pancreatic ductal adenocarcinoma

DE ABREU, J.S.S.; MORETÃO, F.R.; GUARALDI, S.; PINTO, L.F.R.; NICOLAU, P. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy with a five-year survival rate of around 10%. Most patients are diagnosed at advanced stages and receive only palliative chemotherapy. PDAC exhibits multiple molecular alterations driving carcinogenesis, with KRAS as the most frequently mutated oncogene. Less frequent mutations, including BRAF, also contribute to tumor progression. PDAC can be classified into classical and basal subtypes. The classical subtype shows epithelial features and high GATA6 expression, associated with better prognosis and therapeutic response, while the basal subtype exhibits mesenchymal traits and poor outcomes. GATA6 expression could be regulated by epigenetic mechanisms, including DNA methylation, and copy number alteration.

OBJECTIVES: To investigate the regulation of GATA6 by DNA methylation and copy number variation, and its association with KRAS and BRAF mutations in PDAC cell lines and tumor samples.

METHOD: PDAC cell lines (MiaPaca2, ASPC1, PANC1) will be treated with decitabine to assess GATA6 methylation (pyrosequencing), gene expression (RT-qPCR) and protein levels (Western blot). Functional assays for invasion (Transwell), apoptosis (Annexin V/PI), proliferation (crystal violet), and viability (MTT) will be performed in GATA6-silenced/overexpressing cells. RNA-seq will evaluate transcriptome changes, and GATA6 copy number will be assessed by qPCR, KRAS and BRAF mutations by sequencing.

RESULTS: Analysis of publicly available datasets (TCGA, GEO – GSE49149, GSE36924) identified three CpG sites with methylation levels negatively correlated with GATA6 expression. These sites were differentially methylated between PDAC and adjacent non-tumoral pancreatic tissues.

CONCLUSION: DNA methylation may regulate GATA6 expression in PDAC. High GATA6 expression appears to be associated with improved response to chemotherapy.

Keywords: GATA6; DNA methylation; Pancreatic ductal adenocarcinoma.

PAPER CODE: 146

THE EFFECT OF HRAS ON CHROMOSOME SEGREGATION IN MAMMARY EPITHELIAL CELLS

BARCELOS, D.D.S.; FERREIRA, P.D.S.. BRAZILIAN NATIONAL CANCER INSTITUTE (INCA), RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Breast cancer is the most common type of cancer among women worldwide. Among its molecular subtypes, triple negative breast cancer presents the worst prognosis, with high recurrence rates commonly associated with metastasis. This is mainly due to the lack of effective treatments against the disease and the high degree of tumor heterogeneity. Tumor heterogeneity is closely associated with chromosome instability. Alterations in specific oncogenes, such as the RAS gene family, can induce chromosome instability in cancer. Moreover, high expression of HRAS in breast tumors correlates with more aggressive disease, therapy resistance, and reduced survival. However, chromosome instability induced by HRAS in a mammary epithelial cell model has not been investigated yet.

OBJECTIVES: Therefore, the aim of this study is to evaluate the effect of HRAS on chromosome segregation in mammary epithelial cells.

METHOD: For the development of this study, immortalized human mammary epithelial cells (hTERT-HME1) were transduced with control retrovirus (HME1 Rv-CTL) or with retrovirus expressing the cDNA of the human oncogene HRASG12V (HME1 Rv-HRAS). Cell morphology was analyzed by optical microscopy. RAS protein levels were analyzed by Western blot. Cell cycle progression was analyzed by flow cytometry. Finally, chromosome segregation was analyzed by fluorescence microscopy using DAPI staining.

RESULTS: Our results demonstrate that HME1 Rv-HRAS cells exhibit morphological alterations, such as reduced cell size, loss of cellular projections and increased number of cytoplasmic vacuoles compared with control cells. However, quantitative methods have not been applied yet. Western blot analyses demonstrate that HME1 Rv-HRAS cells exhibit increased RAS protein levels compared with control cells. Flow cytometry analyses demonstrate that HME1 Rv-HRAS cells exhibit a lower frequency of cells in G1 phase and a higher frequency of cells in S and G2/M phases compared with control cells. Finally, chromosome segregation analyses demonstrate that HME1 Rv-HRAS cells exhibit an increased frequency of abnormal anaphases, characterized by the presence of anaphase bridges and lagging chromosomes, compared with control cells.

CONCLUSION: Taken together, these findings suggest that high expression of HRAS leads to alterations in cell cycle progression and to an increased frequency of abnormal anaphases in mammary epithelial cells. Future experiments will be performed as described for reproducibility. This work aims to contribute to the understanding of the effect of HRAS on chromosome instability in mammary epithelial cells.

Keywords: Breast Cancer; RAS Oncogene; Chromosome Instability.

PAPER CODE: 148

HISTOPATHOLOGICAL EVALUATION OF ESOPHAGEAL SQUAMOUS CELL CARCINOMA PROMOTED BY RECURRENT THERMAL INJURY IN C57BL/6 MICE

DOS SANTOS, T.D.O.; MELO, F.D.P.; JORGE, F.L.; DO NASCIMENTO, C.M.; VALVERDE, P.; PINTO, L.F.R.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Cancer incidence and mortality are increasing globally due to greater longevity and prolonged exposure to risk factors. Esophageal cancer is one of the most aggressive cancers of the gastrointestinal tract, ranking seventh in incidence and sixth in mortality worldwide, with a survival rate below 20%. In Brazil, it is the sixth most common cancer among men. Esophageal squamous cell carcinoma (ESCC) accounts for

approximately 90% of all cases and is strongly associated with alcohol and tobacco consumption. The ingestion of very hot beverages represents a third etiological factor, as it induces mucosal lesions and inflammation. Therefore, our group developed a mouse model to study thermal injuries and investigate their morphological and molecular alterations.

OBJECTIVES: The objective is to investigate the histopathological changes occurring during the process of initiation and promotion of esophageal tumors in C57BL/6 mice.

METHOD: Male C57BL/6 mice (2 months old) from the vivarium of the Coordenação de Pesquisa e Inovação (CPQI) of INCA were used. The animals received 10 or 20 ppm NDEA in drinking water and were exposed to water at 70 °C (0.3 mL, 3 times per week) by gavage. They were euthanized at 24 h, 14, 28, 56, and 112 days. The esophagus was analyzed macroscopically and microscopically, fixed in 10% formalin, embedded in paraffin, and stained with H&E.

RESULTS: The results indicate that combined exposure to NDEA and hot water intensifies histopathological changes in the esophageal epithelium of mice, producing more severe lesions than either exposure alone. Progressive atypical hyperplasia and moderate to severe dysplasia were observed. Macroscopically, the combination compromises the structure of the esophagus, making the mucosa more fragile and susceptible to neoplastic transformations. These findings emphasize the contribution of environmental factors, such as the consumption of very hot beverages, especially when combined with carcinogens, to the development of esophageal squamous cell carcinoma.

CONCLUSION: Combined exposure to NDEA and hot water intensified histopathological changes in the esophageal epithelium, leading to more extensive and severe lesions. The progression from hyperplasia to dysplasia depended on both exposure duration and the combined or isolated administration of initiating and promoting agents. The structural compromise of the esophagus reinforces the importance of environmental factors in esophageal carcinogenesis.

Keywords: Hot water; ESCC; mice.

PAPER CODE: 150

Molecular mapping in a mouse model of recurrent thermal injury-induced esophageal squamous cell carcinoma

MELO, F.D.P.1; JORGE, F.L.1; DOS SANTOS, T.D.O.1; VALVERDE, P.2; DO NASCIMENTO, C.M.1; PINTO, L.F.R.1. 1. INCA, RJ - RJ - BRASIL; 2. DIPAT, RJ - RJ - BRASIL.

INTRODUCTION: Esophageal cancer (EC) is the sixth most common tumor among men and the fifteenth among women in Brazil. In Western countries, the main risk factors for esophageal squamous cell carcinoma (ESCC) include alcohol consumption and tobacco use. In regions such as southern Brazil, Uruguay, and Argentina, the high incidence of ESCC is also linked to the consumption of hot beverages, such as chimarrão (large volumes and high temperatures). Based on these observations, our group developed the first animal model to study thermal injury-induced ESCC, using water at 70°C as a tumor promoter and N-nitrosodiethylamine (NDEA) as a tumor initiator. Mapping the different stages of tumor development in this model will contribute to a better understanding of the in vivo carcinogenic process underlying ESCC.

OBJECTIVES: To investigate the molecular alterations involved in the initiation and promotion processes of ESCC using multi-omics strategies in a mouse model of esophageal tumor induction.

METHOD: Two-month-old male C57BL/6 mice from the vivarium of the Coordenação de Pesquisa e Inovação (CPQI) at INCA were used. The animals were exposed to the tumor initiator NDEA at concentrations of 10 or 20 ppm in drinking water. Tumor promotion was induced by administering 0.3 mL of water at 70 °C by gavage 3x per week. The animals were divided into 4 experimental groups: 1) Filtered water; 2) Hot water; 3) NDEA and 4) NDEA+ hot water. The animals were euthanized at 24 hours, 14, 28, 56 and 112 days.

RESULTS: Macroscopic images of the esophagus after 56 days showed proximal region changes in the hot water group. In the NDEA group, a narrowing of the esophageal lumen was observed. In the NDEA + hot water group, there was a change in color and tissue

fragility. Macroscopic evaluation at 28 days showed narrowing of the esophagus exposed to NDEA, and while in the NDEA + hot water group, the beginning of the tissue color change was observed. H&E analyses at 56 days showed atypical hyperplasia in the hot water group, formation of rete pegs and hyperplasia in the NDEA group, while in the NDEA + hot water group, dysplasia of varying degrees (moderate-severe) with focal polypoid hyperplasia. At 28 days, dysplasia of varying degrees was observed in the NDEA and NDEA + hot water groups, while foci of hyperplasia appeared only in the combined treatment group.

CONCLUSION: The data indicate that macroscopic changes at 28 days are more subtle than those observed at 56 days, particularly in animals simultaneously exposed to the initiator and promoter. Histopathological findings demonstrate that the combined exposure accelerates lesion diversity and progression, supporting the successful establishment of esophageal carcinogenesis in this model.

Keywords: Chimarrão; Hot water; ESCC.

PAPER CODE: 152

EVALUATION OF THE IMPACT OF AUTOPHAGY ON THE THERAPEUTIC RESPONSE OF ESOPHAGEAL SQUAMOID CARCINOMA (ESCC) AND THE POTENTIAL MECHANISMS INVOLVED IN THIS PROCESS

LOPES, C.G.; DE BRITO, T.F.; PINTO, L.F.R.; DA COSTA, N.M.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Esophageal squamous cell carcinoma (ESCC) is a highly prevalent and lethal cancer often diagnosed too late for effective treatment. A key factor in the lack of therapeutic response in ESCC patients may be autophagy, a catabolic process involved in cellular homeostasis. While autophagy can act as an anti-tumor mechanism in early carcinogenesis, it often becomes pro-tumor in more established cancers. When triggered by stressful stimuli, autophagy can serve as a survival pathway, promoting tumor progression and contributing to chemotherapy resistance. The specific

role of autophagy in ESCC remains poorly understood. Gaining a better understanding of how autophagy promotes resistance to conventional therapies could significantly improve the prognosis for patients with this neoplasm.

OBJECTIVES: Thus, the project aims to investigate the role of the autophagic process in the therapeutic response of CEE and the mechanisms potentially involved in this process

METHOD: So far, we analyzed 48 autophagy genes using data from 86 ESCC samples from The Cancer Genome Atlas (TCGA) and RNA-seq data from the QUIMERA study, which evaluated the therapeutic response of ESCC patients at INCA. Our analysis correlated these genes with differentially expressed genes in ESCC, evaluated their ability to distinguish treatment responders from non-responders via ROC curve analysis, and assessed their impact on patient survival and clinicopathological parameters.

RESULTS: Clinical staging influences patient prognosis. Therefore, we evaluated the overall survival of ESCC patients from TCGA and observed that patients with advanced stages (III and IV) had worse survival outcomes compared to those with early stages (I and II). To investigate this further, we identified differentially expressed genes (DEGs) between these two groups of patients (advanced vs early stages). We found 145 DEGs, 133 of which correlated with 39 autophagy-related genes. Notably, the GDF15 gene showed a significant correlation with the autophagic gene GABARAPL2. Additionally, our analyses using data from the QUIMERA study revealed that autophagy is an overrepresented process in ESCC patients within this cohort, suggesting its importance in therapeutic response. We also found that the expression levels of GABARAPL2 and GDF15 could effectively differentiate between ESCC patients who responded better compared to the worst in quimera study (AUC=0,72).

CONCLUSION: Autophagy is associated with ESCC therapeutic response. Autophagic genes can predict patient outcomes, in particular GABARAPL2, which is correlated with GDF15 (a trophic factor associated with cancer treatment resistance). To investigate this further

and understand the mechanisms involved, we will conduct RNA sequencing in ESCC cell lines following autophagy modulation. Finally, we will validate these in silico and in vitro results using ESCC patient samples.

Keywords: AUTOPHAGY; ESCC; Therapeutic Resistance.

PAPER CODE: 153

PIK3R3 activates PI3K/AKT signaling contributing to drive esophageal squamous cell carcinoma progression representing a promising therapeutic target

CRUZ, J.V.R.1; NICOLAU, P.1; WAGNER, M.L.B.2; DA COSTA, N.O.M.1; PINTO, L.F.R.1. 1. INSTITUTO NACIONAL DE CâNCER, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Esophageal squamous cell carcinoma (ESCC) is the most common histological subtype of esophageal cancer, accounting for about 80% of cases, and is associated with a dismal prognosis: fewer than 20% of patients survive beyond 5 years. ESCC exhibits high heterogeneity, with TP53 mutations being the only genomic alteration consistently observed. However, our group recently identified PIK3R3 (a regulatory subunit of PI3K) overexpression in ESCC through DNA microarray analysis of 15 paired tumor/non-tumoral tissue samples from Instituto Nacional de Câncer (INCA) patients. PIK3R3 overexpression enhanced AKT phosphorylation, revealing a novel mechanism of PI3K/AKT pathway activation, and was independently correlated with poor patient prognosis.

OBJECTIVES: Our aim is to evaluate the functional role of PIK3R3 and the PI3K/AKT pathway in the progression of ESCC, and its potential as a therapeutic target.

METHOD: Using specific shRNA or expression vector transfection, we silenced/overexpressed PIK3R3 in TE-1 and TE-13 cells, derived from human ESCC, and conducted in vitro assays to assess migration, proliferation, clonogenic potential and spheroid

formation capacity. The morphology and cell cycle of these cells were also analyzed. In silico correlation analyses utilizing microarray data were performed to unveil genes potentially activated by or with PIK3R3, contributing to an aggressive phenotype.

RESULTS: In vitro functional assays demonstrated that PIK3R3 knockdown reduced proliferation, migration, clonogenicity, and spheroid formation, while its overexpression promoted the opposite effects in both TE-1 and TE-13 cells. Additionally, PIK3R3 silencing induced cell enlargement and G1 cell-cycle arrest, whereas overexpression increased S-phase progression. In silico analysis of PIK3R3-correlated genes identified associations with a senescent phenotype and highlighted RGS-5 and HEY-1, the most strongly correlated and differentially expressed genes in tumors, as possible regulators of PI3K/AKT signaling.

CONCLUSION: Collectively, our findings demonstrate that PIK3R3 drives ESCC aggressiveness by activating the PI3K/AKT pathway, modulating cell-cycle progression, and promoting tumorigenic phenotypes, positioning PIK3R3 as both a key biomarker and a promising therapeutic target for ESCC, offering new avenues for precision oncology approaches.

Keywords: Esophageal Squamous Cell Carcinoma; PI3K/AKT; Therapeutic Target.

PAPER CODE: 154

Institutionalizing Research Integrity at the Brazilian National Institute of Cancer: Governance, Education, and Collaboration in Action

RIBEIRO, M.D.; PINTO, L.F.R.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Research integrity (RI) is fundamental to ensuring credibility, transparency, and reproducibility in science. It encompasses not only adherence to ethical norms but also the establishment of governance mechanisms that promote a culture of responsible conduct in research. In Brazil, research institutions

have increasingly recognized the importance of implementing policies and educational initiatives that strengthen scientific accountability and public trust. INCA, has taken significant steps to align itself with international standards of research integrity. Since 2023, a coordinated institutional effort has been underway to formalize internal policies, develop educational programs, and establish structures dedicated to supporting and promoting integrity in research.

OBJECTIVES: This work analyzes INCA's initiatives, including the RI framework, Responsible Conduct of Research (RCR) course, mapping technical committees, and the institutional Good Research Practices guidelines.

METHOD: Multi-method approach combining document analysis, interviews, and qualitative data collection. It reviews internal documentation on integrity policies, governance, and the RCR course. Mapping of research integrity committees is ongoing, with interviews conducted at UFG, Fiocruz, and USP. The development of Good Research Practices guidelines follows iterative cycles involving researchers and ethics committees. This approach ensures alignment with national and international standards.

RESULTS: Preliminary results show significant progress in INCA's integrity governance. A key achievement was the mandatory graduate course Introduction to Research Integrity, held in 2024 for PPGO students. The course focused on responsible conduct, ethical decision-making, and good research practices. Feedback from 52 students and auditors showed strong satisfaction with the content's clarity and practical relevance. In 2024, INCA advanced its Office for Research Integrity, while collaborating with Fiocruz, UFRJ, and BRISPE. A key event was INCA's hosting of a satellite event for the VII BRISPE, which helped strengthen INCA's national and international engagement. The national mapping of research integrity committees continues, with interviews providing insights into organizational models and challenges. Meanwhile, INCA's Good Research Practices guidelines are in development, aligned with global standards, ensuring ongoing advancements in responsible research practices.

CONCLUSION: INCA's efforts showcase how institutional commitment drives progress in research integrity. By combining policies and training, INCA embeds integrity at all research levels. Aligning internal policies with global standards while adapting them locally, INCA supports transparency and accountability in research. Continued collaboration with BRISPE and WCRI will sustain this momentum, positioning INCA as a national and international reference in ethical research.

Keywords: research integrity; institutional governance; responsible conduct of research.

PAPER CODE: 155

Characterization of immune infiltrate and biomarkers associated with response to chemoradiotherapy in patients with esophageal squamous cell carcinoma

DE BRITO, T.F.; RIBEIRO, L.D.C.B.; NETO, P.N.; PINTO, L.F.R..
INSTITUTO NACIONAL DE CÂNCER (INCA), RIO DE JANEIRO
- RJ - BRASIL.

INTRODUCTION: Esophageal cancer is the 11th most common cancer worldwide. The two main histological subtypes of this cancer are esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). Although ESCC has been intensively studied in recent years, its etiology has not been associated with many genetic mutations. One exception is the TP53 gene, which is mutated in approximately 80% of ESCC patients. These findings show the potential of immune infiltration analysis to uncover meaningful prognostic markers. The different immune cells present in the tumor microenvironment can have diverse consequences, with some promoting greater tumor control, while others facilitate disease progression.

Objectives: This project aims to characterize the immune infiltrate in ESCC patients through deconvolution analysis of bulk RNA-Seq data and to identify biomarkers associated with treatment response.

METHOD: RNA-Seq libraries from 19 ESCC patients submitted to the QUIMERA clinical protocol were pre-processed with TrimGalore, FASTQC and MultiQC. TPM expression values for each gene were obtained using Salmon and used as input for the CIBERSORTx deconvolution analysis. Fractions for 22 immune cells for each patient were hierarchically clustered and correlated with clinical data. Groups were submitted to differential expression analysis using DESeq2.

RESULTS: Data deconvolution revealed a predominant immune profile consisting of CD4 T lymphocytes, macrophages, mast cells, and neutrophils. Cluster analysis identified two major patient groups corresponding to clinical stages II and III. When stratified by pathological response, responders (Rp_II and Rp_III) displayed distinct immune signatures compared to non-responders (NRp). Rp_II responders showed elevated neutrophil levels and reduced follicular helper T cells, regulatory T cells, and M2 macrophages. In contrast, Rp_III responders exhibited higher proportions of resting memory CD4 T lymphocytes. Differential gene expression analysis uncovered numerous genes associated with these immune profiles. Notably, PLA2G4B expression was significantly linked to overall survival ($p=0.039$) and effectively distinguished responders from non-responders ($AUC=0.84$), highlighting its potential as a predictive biomarker.

CONCLUSION: Clinical staging in ESCC is linked to distinct immune profiles and treatment responses. Stage II tumors show higher neutrophils and activated mast cells, while stage III tumors present more regulatory cells. Gene expression patterns further differentiate responders, with PLA2G4B emerging as a biomarker for survival and treatment efficacy ($AUC=0.84$).

Keywords: Esophageal Squamous Cell Carcinoma; Immune infiltrate; Transcriptomics.

PAPER CODE: 157

BACTERIOME ANALYSIS IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA

LOPES, M.D.S.A.1; SIMÃO, T.A.1; ALBANO, R.1; REISNER, R.G.2; SANTOS, P.T.S.3; PINTO, L.F.R.4; GUNTER, M.5; LIMA, S.C.S.2. 1. UERJ, RIO DE JANEIRO - RJ - BRASIL; 2. INCA, RIO DE JANEIRO - RJ - BRASIL; 3. BENEFICIÊNCIA PORTUGUESA - SP, SÃO PAULO - SP - BRASIL; 4. INCA / UERJ, RIO DE JANEIRO - RJ - BRASIL; 5. IARC, LYON - FRANÇA.

INTRODUCTION: Esophageal cancer ranks among the ten most common malignancies worldwide and in Brazil. In our country, esophageal squamous cell carcinoma (ESCC) accounts for more than 70% of diagnosed cases, being strongly associated with tobacco use, alcohol consumption, and intake of hot beverages, with its incidence particularly pronounced among socioeconomically vulnerable populations. In this context, socioeconomic status (SES), being a complex trait, can be measured by different proxies, such as education. However, other aspects cannot be ignored when evaluating its association with ESCC development. From a biological perspective, alterations in the esophageal bacteriome have been linked to ESCC risk, as bacterial richness and composition may be influenced by environmental exposures.

OBJECTIVES: This study aimed to characterize the esophageal bacteriome of ESCC patients and to evaluate its associations with SES, risk factors, and major oncological outcomes.

METHOD: The esophageal bacteriome was profiled through sequencing of the hypervariable V4 region of the bacterial 16S rRNA gene using Illumina MiSeq. Post-sequencing, reads were filtered and clustered into operational taxonomic units (OTUs). Statistical analyses were conducted in R with specialized packages and GraphPad Prism 5.

RESULTS: The tumor and non-tumor adjacent tissue bacteriome of 34 ESCC patients were analyzed, as only a limited number of samples yielded bacterial DNA

concentrations >1 ng/μL suitable for sequencing. No significant differences were observed in alpha or beta diversity between tissue types; however, 33 OTUs were differentially represented. Actinobacillus and Streptococcus were less represented in tumor tissue, while Neisseria was more abundant in tumors compared with adjacent non-tumor tissue. When stratified by education (<8 vs. ≥8 years), no significant differences were found in diversity metrics or discriminant OTUs. Nonetheless, patients with higher education exhibited a greater number of core microbiome in both tissue types, while Fusobacterium was exclusively detected in tumor samples of patients with lower education levels. These findings are being validated by real-time PCR in a larger cohort.

CONCLUSION: Our results suggest that the esophageal bacteriome undergoes changes during carcinogenesis and that SES may be linked to some of these alterations. Further analyses are required to validate these findings in larger patient cohorts and to assess the association of bacteriome profiles with key ESCC risk factors, treatment response, and overall survival.

Keywords: Bacteriome; Esophageal Squamous Cell Carcinoma (ESCC); socioeconomic status (SES).

PAPER CODE: 159

DNA Methylation-Based Biomarkers for Predicting Chemotherapy Response in High-Grade Serous Ovarian Cancer Patients

PAIVA, V.B.F.; TESSAROLLO, N.. BRAZILIAN NATIONAL CANCER INSTITUTE, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: High-Grade Serous Ovarian Cancer (HGSOC), the most prevalent and lethal subtype of epithelial ovarian cancer, is a major gynecological malignancy characterized by late diagnosis, histological heterogeneity, and frequent resistance to platinum-based chemotherapy. Mutations in genes such as TP53 and BRCA1/2, are frequent, leading to genomic instability and

aggressive clinical behavior. Despite a standard treatment of surgery combined with platinum-taxane regimens, recurrence remains common, and long-term outcomes are limited by the development of chemoresistance. DNA methylation, an epigenetic modification that regulates gene expression without altering the DNA sequence, has emerged as a promising biomarker, offering potential to improve patient stratification and personalize treatment.

OBJECTIVES: The objective of this study is to identify DNA methylation-based biomarkers capable of predicting chemotherapy response and stratifying patients with HGSOC.

METHOD: We will analyze 200 HGSOC patients treated at the Brazilian National Cancer Institute, classified by chemotherapy response based on the chemotherapy-free interval. Tumor DNA will undergo bisulfite treatment and genome-wide methylation profiling using the Infinium MethylationEPIC BeadChip V2. Data will be processed in R, applying differential methylation, functional enrichment, and epigenetic clock analyses, with predictive models developed using machine learning.

RESULTS: As this project is ongoing, we expect to generate a comprehensive DNA methylation profile for HGSOC patients and identify differentially methylated regions associated with therapy response. Integrating these epigenetic signatures with clinical data will enable the development of predictive models to stratify patients by chemotherapy sensitivity. Ultimately, the findings may provide novel biomarkers for patient stratification and advance precision oncology strategies in ovarian cancer.

CONCLUSION: In conclusion, this study will explore DNA methylation as a predictive biomarker in HGSOC, aiming to improve patient stratification, optimize treatment strategies, and advance personalized medicine.

Keywords: Ovarian Cancer; DNA Methylation; Chemotherapy Response.

PAPER CODE: 160

SNVs and INDELs profiles associated with Treatment Response Whole Genome Sequencing Data from a Brazilian Cohort of High-Grade Serous Ovarian Cancer

ESCOBAR, M.M.P.; TESSAROLLO, N.; SERAIN, A.; MORREUW, L.D.C.; DE PAULA, D.J.G.; BESSA, C.; DE MELO, A.C.; VIOLA, J.P.B.; BORONI, M.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: High-grade serous ovarian carcinoma (HGSOC) is the most frequent and lethal subtype of epithelial ovarian cancer, characterized by marked genomic instability and poor clinical outcomes. Despite advances in surgery and chemotherapy, resistance to platinum-based treatment remains a major challenge, limiting survival benefits. Molecular profiling in Brazilian patients is crucial to reveal distinct mutational patterns, identify actionable alterations, and improve precision medicine approaches for ovarian cancer management.

OBJECTIVES: To characterize somatic SNV and INDEL profiles in Brazilian HGSOC patients and investigate their association with response to platinum-based chemotherapy.

METHOD: A retrospective cohort of 65 Brazilian HGSOC patients (67 tumor samples) treated with platinum-based chemotherapy at INCA was analyzed. DNA data analysis was performed using nf-core/Sarek following GATK Best Practices. Variants were identified with three somatic callers in a tumor-only setting, annotated with Ensembl VEP, and classified per ClinGen guidelines. Enriched somatic variants were used to assess mutational signatures with SigProfiler.

RESULTS: Kaplan–Meier analysis confirmed significant survival differences among clinical response groups. The mutational landscape was dominated by single-nucleotide variants, with C>T transitions as the most frequent substitution. Missense and nonsense mutations prevailed, reflecting HGSOC genomic instability. TP53 was the most frequently mutated gene

(74%), consistent with its central role in tumorigenesis. Variants in homologous recombination deficiency (HRD)-related genes, including BRCA1 (23%), were enriched in platinum-sensitive groups, suggesting predictive potential for platinum response and PARP inhibitor eligibility. Mutational signatures SBS3 and SBS6 were identified, indicating HRD and mismatch repair deficiency processes.

CONCLUSION: This study represents the first comprehensive genomic characterization of Brazilian HGSOC patients by WES and WGS. The findings reveal key somatic alterations and mutational signatures associated with chemotherapy response, underscoring the value of genomic profiling in precision oncology and supporting the inclusion of diverse populations in cancer genomics research.

Keywords: Ovarian cancer; Whole genome sequencing; Whole exome sequencing.

PAPER CODE: 161

CHARACTERIZATION OF AN ANTI-INFLAMMATORY RESIDENT MACROPHAGE SUBPOPULATION ASSOCIATED WITH POOR PROGNOSIS IN HIGH-GRADE SEROUS OVARIAN CANCER

TESSAROLLO, N.1; FAGUNDES, A.A.1; BAPTISTA, G.1; DE TOLEDO, N.E.1; DE PAULA, D.J.G.1; MORREUW, L.D.C.1; CHAVES, C.B.P.1; DE MELO, A.C.1; RAMOS, R.N.2; VIOLA, J.P.B.1; BORONI, M.1. 1. INCA, BRAZIL - RJ - BRASIL; 2. INSTITUTO D'OR DE PESQUISA E ENSINO (IDOR) E DEPARTAMENTO DE CIÊNCIAS MÉDICAS DO HC-FMUSP, BRAZIL - SP - BRASIL.

INTRODUCTION: Ovarian cancer is the most lethal gynecologic malignancy. Among its epithelial subtypes, high-grade serous ovarian carcinoma (HGSOC) is the most prevalent, with a five-year survival rate below 30%. The complex interplay between tumor and immune cells, particularly tumor-associated macrophages (TAMs), drives treatment resistance, recurrence, and metastasis. In our recent study,

we identified a TAM population highly expressing SELENOP, enriched in HGSOC tumors with poor prognosis. SELENOP promotes tumorigenic pathways, including WNT signaling via LRP receptors.

OBJECTIVES: To investigate the potential interactions between SELENOP and its receptors LRP2, LRP5, LRP6, and LRP8 expressed in malignant cells and its association with cancer progression.

METHOD: To this end, we evaluated the relationship between LRP receptor expression and overall survival (OS) and progression-free interval (PFI) in both the TCGA and a Brazilian high-grade serous ovarian cancer cohort, as well as gene expression levels by qPCR.

RESULTS: The differentiation of PBMCs into macrophages in the presence of IL-10 led to elevated SEPP1 expression, and an anti-inflammatory profile. Next, we evaluate LRP receptors that could potentially mediate the cross-talk among these TAMs and malignant cells. In TCGA data, patients with high LRP8 expression exhibited significantly worse OS ($p = 0.022$, Log-Rank test). In contrast, in the Brazilian HGSOC cohort, no statistically significant association was found. However, a trend toward poorer PFI was observed among patients with higher LRP8 expression, and increased LRP8 levels were associated with stage II compared to stage IV tumors ($p = 0.018$). Co-culturing TAM supernatants with OVCAR-4 cells modulated the expression of genes involved in the WNT/ β -catenin signaling pathway and the receptor LRP8.

CONCLUSION: These findings suggest that the SELENOP-LRPs axis may play a role in HGSOC progression, corroborating the potential protumorigenic role of TAMs expressing high levels of SEPP1. Further functional experiments are needed to elucidate the contribution of these immune cells to tumor development and therapeutic resistance, advancing our understanding of immune regulation in ovarian cancer.

Keywords: High-grade serous ovarian cancer; macrophages; SELENOP-LRPs axis.

PAPER CODE: 162**Xylene and Formaldehyde Exposure in INCA Pathology Workers: Evaluation by Comet Assay and Micronucleus Test.**

BENEDITO, O.L.; ANCHETE, J.B.D.A.; ALVES, M.C.V.; DA POÇA, K.S.; ACCIOLY, M.T.D.S.. BRAZILIAN NATIONAL CANCER INSTITUTE, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: In pathology laboratories, occupational exposure to chemical substances such as formaldehyde (FA) and xylene constitutes important risk factors for genetic damage. FA, a tissue fixative and preservative, is classified as a human carcinogen by the IARC due to the increased incidence of nasopharyngeal cancer and leukemia in occupationally exposed populations. Xylene, a solvent used in tissue clearing and deparaffinization processes of paraffin-embedded samples, is toxic and exhibits neurotoxic properties, in addition to being associated with respiratory disorders. DNA alterations induced by these compounds have not yet been studied in DIPAT/INCA professionals who are occupationally exposed, making this an unprecedented study with comet assay (CA) and the micronucleus test (MN).

OBJECTIVES: To evaluate occupational exposure to xylene and FA among DIPAT/INCA professionals by analyzing the potential genotoxic effects through the comet assay and micronucleus test in whole blood.

METHOD: Informed Consent Form was obtained, and sociodemographic data collected using the REDCap platform. Blood samples from exposed (n=33) and comparison (n=20) groups were analyzed using two validated LabTox genotoxicity methods: (i) Comet Assay, in which 100 cells/sample were analyzed and classified according to the degree of DNA damage up to "hedgehog" comets; and (ii) Cytokinesis-block Micronucleus Test, quantifying micronuclei, nucleoplasmic bridges, and nuclear buds in 2,000 binucleated cells.

RESULTS: So far, eight slides per group have been analyzed in the CA and five in the MN, representing preliminary results. In the comet assay, the mean percentage of undamaged cells (class 0) was 99.4%

in the exposed group versus 96.9% in controls, while damaged cells (classes 1–4) were 0.23% vs. 2.36%, and "hedgehog" comets 0.46% vs. 0.75%, suggesting greater variability among controls, possibly due to the small sample size. Although there were no active smokers, three passive smokers were identified among controls and one among the exposed, which may also have influenced the results. In the micronucleus test, mean values were 16.2 micronuclei and 17.4 total micronuclei (tMN) in the exposed group, compared to 8.0 and 8.6 tMN in controls, with higher frequencies of nuclear buds (4.8 vs. 2.8) and nucleoplasmic bridges (3.2 vs. 0.2), indicating a trend of greater genotoxic effect among the exposed

CONCLUSION: The findings suggest higher susceptibility to DNA damage in the exposed group, although further sample analysis is needed.

Keywords: Genotoxicity; Occupational Exposure; DNA Damage.

PAPER CODE: 165**Analysis of epigenetic mechanisms involved in carcinogenesis of the squamous cell carcinoma in the upper aero digestive tract.**

CARDOSO, M.A.. CPQI/INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: The Upper Aero Digest Tract Cancers (UADTs) cause 700,000 deaths per year worldwide and include head and neck (HNSCC) and esophageal (ESCC) squamous cell carcinomas. An event that has been used to understand mechanisms involved in the carcinogenesis is DNA methylation. A small non-coding RNA pathway known as Piwi-interacting RNA (piRNA) has been associated with tumorigenesis, interfering in oncogene or tumor suppressor gene expression. How piRNA processing occurs and how PIWIL-piRNA complexes could regulate transposable elements or coding genes were poorly explored in cancer. piRNAs are able to direct PIWIL4 to a target transcript in a specific-sequence manner inducing epigenetic modifications on chromatin or increasing DNA methylation, having as consequence the transcription interruption.

OBJECTIVES: We intend to analyze epigenetic mechanisms in UADT's carcinogenesis, focusing on esophageal cancer cell response changes in the context of PIWIL4 gene expression modulation.

METHOD: PIWIL1-4 expression levels were detected by qPCR. We transfected the TE-1 esophageal cancer cell line with PIWIL4 shRNA-psiU6 for PIWIL4 knockdown. The PIWIL4-AS lncRNA overexpression has been made a transfecting expression vector in TE-1. Both conditions were used in the functional assays: cell migration, invasion, colony formation and proliferation. We intend to evaluate apoptosis by flow cytometry using Annexin and PI double staining. DNA methylation has been performed by pyrosequencing.

RESULTS: TE-1 cells show a significant increase in PIWIL4 expression comparatively to non-tumoral esophageal cell line (EPC hTERT). Analysing RNA-seq data previously published by our group, we identified PIWIL2 has an increased expression in tumoral tissues from ESCC patients comparatively to adjacent non-tumoral tissues. Using the WikiPathways cancer database and a 3821 gene list correlated with PIWIL4, we found only one pathway was enriched: Focal adhesion PI3K Akt mTOR signaling pathway, giving support to the idea that PI3K Akt mTOR pathway is very important to ESCC carcinogenesis, since our group had demonstrated that the FOXM1 or PIK3R3 overexpression was associated to a worst prognostic. Moreover, we performed global survival in 60 months using TCGA ESCC cohort data comparing groups with PIWIL4 low or high expression levels. We detected that there is a worst prognosis in patients with PIWIL4 low levels comparatively to PIWIL4 high expression levels (Log-rank p value=0,03; HR=2,17).

CONCLUSION: The PIWIL4 high levels have a protective impact on global survival in the TCGA ESCC cohort and there is a PIWIL4 high expression in ESCC cell lines versus EPC-hTERT. Taken together, we can hypothesize PIWIL4 as a tumor suppressor gene. However, it is essential to investigate in the context PIWIL4 gene expression modulation some functional cell aspects. Furthermore, PIWIL2 is also upregulated in INCA cohort RNA-seq data, suggesting that the piRNA pathway has some influence on ESCC carcinogenesis.

Keywords: Upper Aero Digest Tract Cancers; piRNAs; DNA methylation.

PAPER CODE: 167

Investigation of the regulatory mechanisms of the IRF2BP2 cofactor over CD4+ T cell activation and differentiation

TAN, L.B.; OLIVEIRA-VIEIRA, B.; VIOLA, J.P.B.. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: IRF2BP2 is a transcriptional cofactor that interacts with transcription factors and participates in the regulation of their target genes, including some that are relevant to CD4+ T cell biology such as NFAT1, IRF2 and KLF2. Mutant variants of IRF2BP2 have been described associated with deficiency of B and T cells in humans. When IRF2BP2 is overexpressed in T cells, animals show a sharp reduction in the number of CD4+ T cells in the lymph nodes and spleen and an increase in Tregs. Further, IRF2BP2 inhibits CD4 T cell activation in vitro, with lower expression of activation markers and cytokines. However, the molecular mechanisms involved in these processes are still unknown. We seek to identify IRF2BP2 regulated genes and their roles in promoting these phenotypic changes in CD4+ T cells.

OBJECTIVES: Characterize IRF2BP2 induced phenotypic alterations in activated CD4 T cells Discover and validate regulated genes required for alterations Compare the CD4 T cell response to B16 in IRF2BP2 KI mice Method: We use the IRF2BP2 KI Lck-Cre mouse model for overexpression of IRF2BP2 only in T cells. CD4 T cells are purified from these animals and differentiation (cytokines and TFs), proliferation (cells counts, dye dilution) and cell death (Annexin-V/7-AAD) after in vitro activation are compared to controls by flow cytometry and qPCR. Genes of interest will be identified by bulk RNAseq of activated cells and their roles will be confirmed by gene silencing and phenotypic reassessment.

RESULTS: IRF2BP2 KI Lck-Cre animals have very reduced numbers of CD4 T cells in their peripheral lymph nodes compared to control mice. Purified CD4+ T cells from IRF2BP2 KI Lck-Cre animals undergo deficient clonal

expansion and are strongly skewed towards a Th1-like phenotype in response to in vitro activation compared to controls, as assessed by interferon- γ production and Tbx21 expression. This happens as early as 24 hours after activation, even in the presence of Th2 and Th17 polarizing cytokines. IRF2BP2 KI CD4 T cells proliferation after activation is inhibited compared to controls as assessed by VPD dilution and undergo increased activation induced cell death, which together lead to diminished clonal expansion.

CONCLUSION: We were able to show that IRF2BP2 overexpression during the activation of CD4 T cells inhibits clonal expansion and has a strong effect on differentiation fate, skewing cells towards a Th1-like phenotype. This effect happens early during activation (~24h) suggesting that the involvement of TCR or cytokine signalling. In next steps, RNAseq data will allow us to identify genes of interest that are responsible for the observed phenotypic alterations and will be able to begin validating their roles.

Keywords: IRF2BP2; Lymphocytes; Differentiation.

PAPER CODE: 169

The Effect of CCNE1 and HRAS Oncogenes on the Induction of Genomic Instability in Breast Epithelial Cells

FERREIRA, P.D.S.; TEIXEIRA, L.K.. BRAZILIAN NATIONAL CANCER INSTITUTE (INCA), RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: One of the main characteristics of carcinogenesis is uncontrolled cell division, which can lead to uncontrolled cell proliferation. DNA replication and chromosome segregation can be interfered with by several factors during the cell cycle, leading to replication stress and chromosome instability, respectively. Activation of certain oncogenes, including CCNE1 and HRAS, can lead to genomic instability, favoring the carcinogenesis process. CCNE1 and HRAS play relevant roles in breast carcinogenesis, as breast cancer patients with activation of these oncogenes have a worse prognosis and shorter survival. However, the landscape of genetic alterations caused by the activation of these oncogenes in breast tumors are not fully understood yet.

OBJECTIVES: Therefore, the main objective of this project is to evaluate the role of human oncogenes CCNE1 and HRASV12 in the induction of genomic instability in breast epithelial cells.

METHOD: Immortalized normal human mammary gland epithelial cells (h-TERT-HME1) were transduced with control retrovirus (HME1-RV-CTL) or retroviruses expressing the cDNA of the human oncogenes CCNE1 (HME1-RV-CCNE1) or HRASV12 (HME1-RV-RAS) and evaluated on days 1, 30 and 60 after selection of transduced cells. RNA and protein levels were analyzed by qPCR and Western blot, respectively, cell cycle profiles were analyzed by flow cytometry, and metaphases chromosomes were analyzed by Giemsa staining.

RESULTS: HME1-RV-CCNE1 cells exhibit increased transcriptional and protein levels of CCNE1 compared to control cells and HME1-RV-RAS cells exhibit increased transcriptional and protein levels of RAS compared to control cells. High levels of CCNE1 or HRASV12 lead to activation of the replication stress response pathway, with increased levels of phosphorylated CHK1 compared to control cells. Analysis of metaphase chromosomes demonstrates that activation of CCNE1 or HRASV12 induces structural or numerical chromosome alterations, respectively. High levels of CCNE1 lead to premature sister chromatid separation, while high levels of HRASV12 lead to chromosome loss (hypoploidy) compared to the control cells. Preliminary analyses demonstrate that low concentrations of Hoechst 33342 efficiently label the different phases of the cell cycle and do not alter cell viability, enabling FACS-assisted cell sorting of cells in G0/G1 phases for future whole genome sequencing analysis.

CONCLUSION: Our results demonstrate that the CCNE1 and HRASV12 oncogenes induce genomic instability in distinct ways in human breast epithelial cells. This work aims to contribute to the elucidation of the genomic instability profile caused by each of these oncogenes during breast carcinogenesis.

Keywords: Breast cancer; Genomic instability; Oncogenes.

PAPER CODE: 170

OPNa overexpression promotes T-cell acute lymphoblastic leukemia cell differentiation

PEREIRA, I.D.O.1; BASTOS, A.C.S.D.F.2; COSTA, E.S.3; DE SÁ, M.E.C.1; GIMBA, E.R.P.4. 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. CNPEM, CAMPINAS - SP - BRASIL; 3. UFRJ, RIO DE JANEIRO - RJ - BRASIL; 4. UFF, RIO DAS OSTRAS - RJ - BRASIL.

INTRODUCTION: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematologic malignancy characterized by the accumulation of genetic lesions that lead to differentiation arrest and aberrant proliferation of immature T-cell progenitors. Reliable biomarkers to predict relapse risk, an unfortunate common outcome, are still lacking. Therefore, identifying new biomarkers differentially expressed in T-ALL is essential to better understand its malignancy mechanisms. We previously identified the Osteopontin-a (OPNa) isoform as the most frequent and highly expressed among the five known OPN splice isoforms (OPN-SI) in a cohort of T-ALL patients.

OBJECTIVES: In this context, this study aims to elucidate OPNa functional roles in the biology of T-ALL cells.

METHOD: Jurkat T-ALL cell line was transfected with an expression vector containing the OPNa complete cDNA sequence (pCR3.1/OPNa) or an empty vector (pCR3.1/EV). Images were captured using the ZOE Fluorescent Cell Imager microscope. The number, size, and morphology of Jurkat cell aggregates were evaluated using Fiji/ImageJ. Trypan blue cell staining and MTT assays evaluated cell viability. Cell-cycle DRAQ5 staining and evaluation of T-ALL differentiation markers were performed by flow cytometry.

RESULTS: Transfection of the Jurkat cell line with the expression vectors mentioned above generated cell lines overexpressing OPNa (Jurkat-OPNa) and the Jurkat-EV control cells. RT-qPCR assays showed that Jurkat-OPNa cells expressed approximately 500-fold higher levels of OPNa compared to Jurkat-EV, confirming ectopic overexpression. From two months post-transfection,

Jurkat-OPNa cells exhibited notable morphological changes, forming aggregates 30–60% smaller than those of Jurkat-EV, which retained the typical Jurkat morphology. Trypan blue and MTT assays performed before these changes showed no significant differences in viability between the two samples. Afterward, flow cytometry and cell cycle assay were performed. Flow cytometry revealed that Jurkat-OPNa cells showed loss of CD7 and gain of CD45 and CD3, indicating a shift toward a more differentiated phenotype. Cell cycle analysis showed a higher percentage of Jurkat-OPNa cells in the G2/M phase (65.7%) compared to controls (52.4%).

CONCLUSION: In conclusion, OPNa stable overexpression in Jurkat T-ALL cells progressively alters the morphology and aggregation properties of these cells. Moreover, we provide early evidence that long term OPNa overexpression could promote Jurkat T-ALL cell differentiation, besides promoting cell cycle progression in these cells. These findings highlight that OPNa overexpression could be a strategy to promote T-ALL cell differentiation with potential therapeutic approaches in this neoplastic disease.

Keywords: Phenotype alteration; Splicing isoforms; T-cell acute lymphoblastic leukemia.

PAPER CODE: 171

ANALYSIS OF DERMOSCOPIC CRITERIA FOR A POTENTIALLY MALIGNANT DISORDER: ACTINIC CHEILITIS

FROEDE, L.P.L.1; CURVELO, J.A.D.R.1; DA SILVA, B.N.M.1; ANTUNES, H.S.2; DE MELO, A.C.1; GOLDEMBERG, D.C.3. 1. INSTITUTO NACIONAL DO CÂNCER, RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO NACIONAL DO CÂNCER, RIO DE JANEIRO - RJ - BRASIL; 3. INSTITUTO NACIONAL DO CÂNCER, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Actinic cheilitis (AC) is a potentially malignant disorder with a risk of malignant transformation ranging from 10% to 30% into squamous cell carcinoma (SCC). SCC arising in the vermilion region of the lips is a high-risk tumor, with an 11% likelihood of metastasis compared to SCC located at other body sites.

OBJECTIVES: The aim of this study was to evaluate the features of AC through dermoscopy and correlate them with the clinical presentation, grading, and histopathological findings.

METHOD: Thirty patients with AC underwent digital dermoscopy of the oral region, with special focus on the lower lip. Additionally, clinical findings were graded according to the classification previously proposed by Poitevin (2017).

RESULTS: The vascular patterns observed were irregular linear vessels, dotted vessels, and hairpin vessels in Grades I, II; polymorphic vessels in Grades III, IV. Fine, white and/or diffuse scales were found in the early grades, while thick, yellowish, and focal scales were observed in the advanced grades. White and/or red amorphous areas were present in Grades I to IV. Ulceration was noted in Grade IV. Dysplasia and elastosis were observed across Grades I to IV. Mild to moderate dysplasia was found in Grades I and II, and moderate to severe dysplasia in Grades II, III, and IV.

CONCLUSION: Although mucosal dermoscopy is still in its early stages of development, its use for the evaluation of this region enables improved clinical assessment, better definition of biopsy sites, and effective monitoring of therapeutic response. The results support the importance of further investigation of these precursor lesions, even in the early stages, since this disorder may progress to severe dysplasia, microinvasive carcinoma, or even overtly invasive carcinoma.

Keywords: ACTINIC CHEILITIS; POTENTIALLY MALIGNANT DISORDER; DERMOSCOPIC.

PAPER CODE: 172

Characterization of the Cervical Cancer Microbiome

SIQUEIRA, J.D.; RIBEIRO, E.J.; FELIX, S.P.; BRANT, A.C.; MOREIRA, M.Â.M.; GOES, L.R.; SOARES, M.A.. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Cervical cancer (CC) is the fourth most common cancer among women. The main factor associated with the development of CC is the infection by the human papillomavirus (HPV), which is necessary but not sufficient by itself. The distribution of bacterial species present in the cervicovaginal mucosa is associated with the risk of HPV infection, the development of intraepithelial neoplasia, and CC. While the bacteriome has been widely studied, other microbiome components have been little explored in the literature. Understanding the microbiome composition and interactions in CC is essential to evaluate the role of each organism and may provide insights for prevention, screening and treatment strategies.

OBJECTIVES: Therefore, the aim of this study was to characterize the taxonomic and functional diversity of the microbiome and the interaction of its components in CC through shotgun sequencing.

METHOD: DNA isolated from 22 CC samples from patients followed up at the Instituto Nacional de Câncer was sequenced using the MGI Tech DNBSEQ-T7 platform. Sequences were filtered for quality and taxonomically classified using the Kraken2 program. Microbiome diversity and its associations with tumor stage, histological type, smoking status and HPV genotype were analyzed.

RESULTS: Samples showed an heterogenous distribution of bacteria, with 809 species from 326 genera identified. Higher alpha diversity was observed in stage II tumors compared to stage I, and beta diversity differed between current/former smokers and non-smokers. Regarding the viruses, 124 species and 72 genera were detected. The most part of them was bacteriophages, while papillomavirus, anellovirus, herpesvirus and other viral families were also identified. Alpha diversity of bacteriophages was higher in stage I cases when compared to stage II. Beta diversity of bacteriophages and vertebrate-infecting viruses did not differ by tumor stage, smoking status, tumor histological type or HPV infection.

CONCLUSION: The intratumoral microbiome of CC shows a diverse microbial community, which may be associated with disease development and progression. These results emphasize the importance of investigating the microbiome composition and its interactions within the tumor microenvironment to guide novel diagnostic and therapeutic strategies.

Keywords: Cervical cancer; Microbiome; Metagenomics.

PAPER CODE: 173

A PHOSPHO-CIRCUIT TO SYNERGISTICALLY MODULATE BRCA1-PALB2 INTERACTION

GOMES, T.T.1; ELIAS, A.B.R.1; NEPOMUCENO, T.C.2; SKAF, L.B.1; SUAREZ-KURTZ, G.1; MONTEIRO, A.N.2; CARVALHO, M.A.3. 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. H LEE MOFFITT CANCER CENTER, TAMPA - ESTADOS UNIDOS DA AMERICA; 3. IFRJ, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: BRCA1, BRCA2, and PALB2 are the main genes associated with breast cancer predisposition. They encode homonymous proteins that form a complex pivotal to the DNA Damage Response (DDR), a process essential for cellular homeostasis and cancer suppression. Mechanistically, BRCA1 is the key actor of Homologous Recombination (HR)-mediated DDR, orchestrating two main steps of it. First, the BRCA1 BRCT domain promotes the DNA end resection process by interacting directly with and stimulating CtIP. Then, the BRCA1 coiled-coil (CC) domain recruits PALB2-BRCA2 to DNA-damaged sites through a direct heterodimerization with the PALB2 CC domain. Since the 90s, it has been established that BRCA1 phosphorylation is crucial for genomic integrity maintenance; however, its mechanistic role remains unclear.

OBJECTIVES: As some of BRCA1 phosphorylation sites (PS) are near the PALB2-interacting region, we sought to evaluate their putative role in modulating PALB2 association.

METHOD: BRCA1/PALB2 interaction was evaluated by GST pulldown or co-immunoprecipitation using transfected models and/or stably protein reconstitution through retroviral knock-in. PS were mutated by site-directed mutagenesis to alanine or glutamic acid residues (mimicking a phospho-deficient or a phosphomimetic mutant, respectively). Recombinant proteins were produced in *E. coli* and purified using GST or HIS tags. Cell cycle synchronization was performed through a thymidine block.

RESULTS: Here, we show that specific BRCA1 PS cooperate to synergistically modulate PALB2 interaction without affecting BRCA1-CtIP association. Surprisingly, the analysis of direct BRCA1-PALB2 binding by in vitro pulldown using recombinant CC domains of BRCA1 and PALB2 shows that BRCA1 phosphorylation does not impact PALB2 interaction directly. We also demonstrated that BRCA1 phospho-dependent modulation of PALB2 association occurs independent of DNA damage, cell cycle phase, DNA binding, PALB2 homodimerization capacity, or PALB2 phosphorylation. Notably, a comparison of *E. coli* and human cells expressing recombinant BRCA1 CC domain revealed a decreased interaction specifically in the human-derived BRCA1 phospho-deficient mutant, uncovering an indirect inhibitory mechanism. Interestingly, we have also found that the BRCA1 BRCT domain is necessary to fully promote PALB2 interaction in a phospho-dependent manner, synergistically with BRCA1 phosphorylation, and, intriguingly, also indirectly.

CONCLUSION: We provide evidence for a mechanistic role of BRCA1 phosphorylation in the HR-mediated DDR. Taken together, our data uncover a regulatory phospho-circuit that modulates the interaction between BRCA1 and PALB2. We are currently evaluating the impact of BRCA1 phosphorylation on the HR-mediated DDR using the BRCA1-deficient breast cancer cell line HCC1937 stably reconstituted with BRCA1 (wild-type or mutant) as a model.

Keywords: Hereditary Cancer; BRCA1; DNA damage.

PAPER CODE: 181

Characterization of the Involvement of the Transcriptional Cofactor IRF2BP2 in CD8 T Cell Proliferation and Anti-Tumor Response.

RAMOS, B.V.S.P.; OLIVEIRA-VIEIRA, B.; VIOLA, J.P.B..
LABORATORY OF MOLECULAR IMMUNOLOGY, PROGRAM
OF IMMUNOLOGY AND TUMOR BIOLOGY, NATIONAL
CANCER INSTITUTE (INCA), RIO DE JANEIRO, BRAZIL., RIO
DE JANEIRO - RJ - BRASIL.

INTRODUCTION: The adaptive immune response is dependent on T lymphocytes and is essential for maintaining homeostasis, also playing an important role in tumor immune response. IRF2BP2 (Interferon Regulatory Factor 2 Binding Protein 2) is a transcriptional repressor of NFAT1 protein, which regulates T cell activation. Previous findings from our group indicated that IRF2BP2 overexpression alters CD4 T cell activation and reduces proliferation. However, its role in CD8 T cells remains poorly understood.

OBJECTIVES: To evaluate the involvement of IRF2BP2 in CD8 T lymphocyte proliferation, death and anti-tumor response.

METHOD: Transgenic IRF2BP2 T-KI T cell-specific overexpressing mice (LCK-Cre Rosa26 IRF2BP2-IRES-EGFP) were generated by our group and used with controls. CD8 T cells were purified, activated with anti-CD3/CD28 and polarized under effector differentiation for 5 days. CD8 T live and dead cells were counted and analyzed by flow cytometry (CD62L, CD44, Ki-67, Caspase-3). To assess the in vivo response, mice were inoculated with B16F10 melanoma and tumor growth measured every 5 days.

RESULTS: A reduction in the total number of CD8 T cells was observed in IRF2BP2 T-KI cultures compared to controls under both default and effector conditions at day 5. The percentage of dead CD8 T cells was higher in the KI group at days 3 and 4 in default conditions, and at days 3 and 5 under effector differentiation. Flow cytometry at day 0 showed no significant differences in Ki-67 or Caspase-3 expression between groups, and the

same was observed at day 5. In the in vivo melanoma model, no statistically significant difference in tumor growth was detected between IRF2BP2 T-KI and control mice.

CONCLUSION: Additional assays of proliferation and viability are required to better characterize the mechanisms underlying the changes in cell count, and further tumor microenvironment analysis is still needed to define the role of IRF2BP2 in antitumor immunity.

Keywords: T lymphocytes; CD8 T cells; IRF2BP2.

PAPER CODE: 185

Analysis of patients with rectal adenocarcinoma undergoing robotic resection after neoadjuvant treatment at INCA

BUSTOS, S.A.G.; NALIM, I.S.; DA SILVA, B.C.; SMALL, I.; VALADÃO, M.; ARAÚJO, R.O.D.C.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Robotic surgery is an innovative approach that offers potential advantages in the surgical treatment of rectal cancer. In the Unified Health System (SUS), long-term consolidated results related to this technique are still scarce. The use of robotics in surgical procedures may improve precision and patient recovery.

OBJECTIVES: To describe the clinical, surgical, and oncological outcomes of patients undergoing neoadjuvant therapy and robotic surgery at the National Cancer Institute (INCA) between 2012 and 2022.

METHOD: This is a retrospective study that analyzed 76 patients with locally advanced rectal adenocarcinoma who underwent neoadjuvant therapy with radiotherapy and chemotherapy, followed by robotic surgery using the Da Vinci platform. Of these, 13.2% underwent induction chemotherapy. Demographic, therapeutic, and histopathological data were evaluated, along with complication rates (classified by the Clavien-Dindo scale) and survival (analyzed by Kaplan-Meier) with a median follow-up of 94 months.

RESULTS: The median age of the patients was 59 years, with 58% being male. A complete pathological response was observed in 9.2% of cases. Complications of grade equal to or greater than CD occurred in 21% of patients, including anastomotic dehiscence (10.7%) and fistula/abscess (8.0%). Free margins were achieved in 97.3% of cases. TRG 1–2 was present in 28.7% of patients. Adjuvant chemotherapy was administered in 44.7% of cases. The 5-year overall survival rate was 62.1%, while the 5-year progression-free survival rate was 46.5%. Survival was lower in patients with ECOG 2 and in mesorectal excision grade I. There was no statistically significant difference between stages II and III. In our cohort, the local recurrence rate was only 1.3% (1 patient), while the other recurrences corresponded to distant disease progression, reflecting the need for improvements in systemic treatment strategies for high-risk cases.

CONCLUSION: Robotic surgery for rectal adenocarcinoma after neoadjuvant therapy showed high R0 rates, good oncological outcomes, and acceptable complications in the context of SUS. These findings reinforce the viability and safety of the technique, justifying the conduct of prospective comparative studies.

Keywords: Rectal adenocarcinoma; Robotic surgery; Neoadjuvant therapy.

PAPER CODE: 186

EVALUATION OF THE IMMUNE SIGNATURE IN RESPONSE TO ANESTHETIC AGENTS IN PYMT MICE SUBJECTED TO LAPAROTOMY

PINHO, A.C.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: The interaction between the immune system cells and tumors is highly complex, and the importance of immune function in tumor development and growth has been well demonstrated

in its dual role. The immune infiltrate, as well as neoplastic cells, is subject to the effects of various drugs, and its analysis allows for the optimization of strategies aimed at preserving its function as much as possible, thereby potentially favoring disease control.

Surgery remains one of the cornerstones of curative treatment for breast cancer; however, experimental models and retrospective clinical studies suggest that surgical trauma and perioperative management — including anesthetic drugs — may influence both the immune system and tumor cells.

OBJECTIVES: This project aims to evaluate the immune signature induced by lidocaine, fentanyl, and dexmedetomidine on mammary tumors in PyMT mice in the context of surgical trauma (laparotomy).

METHOD: Forty -seven female PYMT mice (B6 background) will be used. They will be evaluated weekly until tumor volume reaches between 200 and 600 mm³, the anesthetic-surgical procedure will be performed after allocation in seven groups according to the anesthetic agent injected intraperitoneally: PBS; Lidocaine 2 and 4mg/Kg; Fentanyl 0,2 and 0,4µg/Kg; Dexmedetomidine 50 and 100µg/Kg. Euthanasia will occur on POD 7, and the tumors will be analyzed by flow cytometry,

RESULTS: Twenty eight animals have already been managed, and the project is currently in the phase of flow cytometry analysis.

CONCLUSION: The distinctive feature of this study is the use of a spontaneous carcinogenesis model that closely resembles tumor progression in humans. The expectation is that the flow cytometry analyses will be completed by the time of the upcoming International Meeting in Oncology Research.

Keywords: Tumor microenvironment; Anesthetic drugs; Immune system.

PAPER CODE: 189**The cooperation between CALRdel52 and TP53 mutations increases the number of immature hematopoietic progenitors and enhances thrombopoietin-independent megakaryopoiesis**

BENEDITO, S.1; BERNARDO, P.S.2; ARKOUN, B.3; SILVA, M.L.D.S.4; YAHIA, S.B.H.3; BONAMINO, M.H.5; OYARZUN, C.M.6; EL-KHOURY, M.7; ROSA, E.3; STEFANO, A.D.3; MARTY, C.3; RASLOVA, H.3; DEBRE, I.A.3; VAINCHENKER, W.8; PLO, I.3; MONTE-MÓR, B.4. 1. INSTITUTO NACIONAL DE CâNCER, RIO DE JANEIRO, BRAZIL, INSERM, UMR 1287, GUSTAVE ROUSSY, VILLEJUIF, FRANCE, UNIVERSITÉ PARIS-CITÉ, INSERM UNITÉ MIXTE DE RECHERCHE 1287, PARIS, FRANCE, GUSTAVE ROUSSY, INSE, RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO NACIONAL DE CâNCER, RIO DE JANEIRO, BRAZIL, RIO DE JANEIRO, INSERM, UMR 1287, GUSTAVE ROUSSY, VILLEJUIF, FRANCE, INSERM, UMR 1287, GUSTAVE ROUSSY, VILLEJUIF, FRANCE, RIO DE JANEIRO - RJ - BRASIL; 3. INSERM, UMR 1287, GUSTAVE ROUSSY, VILLEJUIF, FRANCE, INSERM, UMR 1287, GUSTAVE ROUSSY, VILLEJUIF, FRANCE, UNIVERSITÉ PARIS-SACLAY, INSERM UNITÉ MIXTE DE RECHERCHE 1287, VILLEJUIF, FRANCE, PARIS - FRANÇA; 4. INSTITUTO NACIONAL DE CâNCER, RIO DE JANEIRO, BRAZIL, RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL; 5. INSTITUTO NACIONAL DE CâNCER, RIO DE JANEIRO, BRAZIL, RIO DE JANEIRO, VICE-PRESIDÊNCIA DE PESQUISA E COLEÇÕES BIOLÓGICAS (VPPCB), FUNDAÇÃO INSTITUTO OSWALDO CRUZ (FIOCRUZ), RIO DE JANEIRO, BRAZIL, RIO DE JANEIRO - RJ - BRASIL; 6. INSERM, UMR 1287, GUSTAVE ROUSSY, VILLEJUIF, FRANCE, INSERM, UMR 1287, GUSTAVE ROUSSY, VILLEJUIF, FRANCE, UNIVERSITÉ PARIS-SACLAY, INSERM UNITÉ MIXTE DE RECHERCHE 1287, VILLEJUIF, FRANCE, RIO DE JANEIRO - FRANÇA; 7. INSERM, UMR_S 938, SORBONNE UNIVERSITÉS, CDR SAINT-ANTOINE, PARIS, FRANCE, PARIS - FRANÇA; 8. NSERM, UMR 1287, GUSTAVE ROUSSY, VILLEJUIF, FRANCE, INSERM, UMR 1287, GUSTAVE ROUSSY, VILLEJUIF, FRANCE, UNIVERSITÉ PARIS-SACLAY, INSERM UNITÉ MIXTE DE RECHERCHE 1287, VILLEJUIF, FRANCE, PARIS - FRANÇA.

INTRODUCTION: Myeloproliferative neoplasms (MPNs) are clonal diseases of the hematopoietic stem cell, characterized by increased proliferation of myeloid

lineages due to driver mutations in JAK2, CALR, or MPL genes. These mutations lead to constitutive activation of the JAK/STAT pathway and myeloproliferation. In MPN patients, TP53 gene mutations, deletions, or loss of heterozygosity are associated with a higher risk of transformation to acute myeloid leukemia. However, the mechanisms remain poorly understood, especially in MPN with CALR mutations.

OBJECTIVES: Our objective was to investigate the cooperation between CALR and TP53 mutations in hematopoiesis using induced pluripotent stem cells (iPSCs).

METHOD: iPSC lines from a healthy individual (CALRWT) and an MPN patient (CALRdel52) were edited in the TP53 gene by CRISPR/Cas9. In the clones selected, mutations were evaluated by DNA/cDNA sequencing, p53 protein levels were assessed by Western Blot, and target gene expression was measured by qPCR. iPSC-derived hematopoietic cells were analyzed by flow cytometry, and progenitor potential was evaluated in semi-solid and liquid media. scRNA-seq was used to identify cell types and molecular pathways.

RESULTS: CALRWT and CALRdel52 iPSCs carrying TP53 mutations (TP53mut) in one or both alleles were generated. In TP53WT, p53 was stabilized and target genes were upregulated upon nutlin treatment, while its function was severely inhibited in TP53mut. After differentiation, biallelic TP53mut doubled myeloid and erythroid colony formation in CALRWT and CALRdel52 iPSCs. CALRdel52 increased megakaryocytic (MK) colonies compared to CALRWT. Biallelic TP53mut and CALRdel52 enhanced the number and size of TPO-dependent and -independent MK progenitors and produced more TPO-independent MKs in liquid culture. By day 20 of differentiation, CALRdel52/biallelic TP53mut iPSCs showed immature MKs and an increase in myeloid progenitors, including large CFU-GEMM and progenitors that generated cytokine-independent erythroid, myeloid, and MK cells. scRNA-seq showed that biallelic TP53mut increased hematopoietic progenitors by 25% in CALRWT, while CALRdel52 were mainly MK progenitors regardless of TP53 status.

CONCLUSION: The cooperation between CALRdel52 and TP53 mutations increases the generation of immature hematopoietic progenitors and enhances TPO-independent megakaryopoiesis. The hematopoietic effects are more pronounced with complete TP53 inactivation, highlighting the role of p53 loss-of-function in promoting immature hematopoiesis.

Keywords: TP53; Acute myeloid leukemia; Neoplasms Myeloproliferatives.

CLINICAL RESEARCH AND TECHNOLOGICAL DEVELOPMENT (CR)

PAPER CODE: 5

Low-power laser in the prevention of oral mucositis and oropharyngeal pain in patients undergoing allogeneic hematopoietic stem cell transplantation.

ALVES, L.D.B.; MENEZES, A.C.D.S.; TAGLIABUE, J.F.; DA COSTA, A.M.D.; ANTUNES, H.S.; DE MELO, A.C.. INSTITUTO NACIONAL DE CâNCER, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Low-power laser (LPL) is the main strategy for preventing oral mucositis and oropharyngeal pain, relevant toxicities in the context of hematopoietic stem cell transplantation (HSCT). Current LPL protocols perform isolated applications. The red spectrum (660nm) is the most used in intraoral structures, and the infrared (808nm) in extraoral ones due to its greater tissue penetration power. Recently, LPL devices have enabled simultaneous dual irradiation (of red and infrared wavelengths), creating the need to assess their efficacy.

OBJECTIVES: To compare the effects of two LPL protocols in the prevention of oral mucositis, oropharyngeal pain, dysphagia, use of parenteral nutrition, and opioids in patients undergoing allogeneic HSCT.

METHOD: This is a phase III, randomized, triple-blind clinical trial with adult patients undergoing allogeneic HSCT, assessed by intention-to-treat. The LPL protocol is defined by randomization in Group A (extraoral applications at 808nm and intraoral at 660nm) or Group B (extraoral and intraoral applications with simultaneous dual emission of 660nm and 808nm wavelengths). LPL is performed from the beginning of conditioning to HSCT engraftment or failure. Blind dentists assess outcomes of interest.

RESULTS: The sample size calculation foresees the inclusion of 74 patients, and to date, 66 patients have been enrolled; 6 discontinued, and 62 completed the protocol. 34 patients were allocated to Group A and 32 to Group B. Most were male (59.1%), with a mean age of 39.6 years, diagnosed with acute lymphocytic or myeloid leukemia (28.8% each), and without other comorbidities (57.6%). HSCT recipients were mostly related (72.7%) and haploidentical (70.8%). The conditioning protocol prescribed in most cases consisted of a combination of fludarabine and total body irradiation (22.7%). For graft-versus-host disease prophylaxis, methotrexate, cyclosporine, and anti-thymocyte globulin (18.2%) were prescribed.

CONCLUSION: Considering the sample size estimate of 74 patients, the study reached 89.1% of patients within a twenty-six-month period. The inclusion average was 2.5 patients per month. The enrollment of the remaining 8 patients is estimated to be completed in January 2026, meeting the study's anticipated schedule.

Keywords: Hematopoietic Stem Cell Transplantation; Low-Power Laser; Oral Mucositis.

PAPER CODE: 6

PHASE II STUDY OF A COPAIBA-BASED MOUTHWASH IN MUCOSITIS PREVENTION IN ORAL CAVITY CANCER PATIENTS UNDERGOING RADIOTHERAPY

MENEZES, A.C.D.S.; ANTUNES, H.S.; DE MELO, A.C.; ALVES, L.D.B.; DA COSTA, A.M.D.; TAGLIABUE, J.F.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Radiotherapy is used in oral cavity cancer treatment, either alone or in combination with chemotherapy and surgery. In addition to its effectiveness against the tumor, it also causes significant toxicity in adjacent tissues, with oral mucositis being the most notable adverse effect. Current scientific evidence supports preventive management with low-level laser therapy; however, this is contraindicated in the tumoral area due to the risk of cellular metabolism stimulation. Therefore, alternative methods for prevention in this region are necessary. Based on preliminary results from a phase I study conducted at INCA, a phase II study is proposed to evaluate the effectiveness of a copaiba-based mouthwash in preventing oral mucositis in patients undergoing radiotherapy for oral cavity tumors.

OBJECTIVES: To evaluate the incidence of oral mucositis (ulceration) in the tumor area in patients with oral cancer undergoing radiotherapy and using a copaiba-based mouthwash.

METHOD: This is a phase II, double-blind, randomized study. Patients will be randomly assigned to two groups, with 20 patients in each: Group A (copaiba mouthwash 4x/day) and Group B (placebo mouthwash 4x/day); both groups will receive daily prophylactic laser therapy. Randomization will be stratified in a 1:1 ratio based on treatment type and conducted by an unblinded team member. Patients will receive a clinical evaluation every day during the treatment period

RESULTS: Patient enrollment has begun; however, no patients have been included in the protocol to date. Data analysis will be descriptive: continuous variables will be presented as mean and standard deviation or median, maximum, and minimum (for non-parametric data); categorical variables will be presented as absolute and relative frequencies. The mean tumoral region ulcerated area (according to the SONIS method) will be compared between Groups A and B using Pearson's chi-square test. A p-value < 0.05 will be considered statistically significant. A total of 20 participants will be included in each group. Sample size calculation: Type I error = 0.05; Type II error = 0.20. Estimated proportions of ulcerated area in the tumor region: Group A (copaiba) = 0.50; Group B (placebo) = 0.90.

CONCLUSION: Data analysis will be conducted after all patients have completed the treatment as per the study protocol.

Keywords: Radiotherapy; Copaiba; Oral mucositis.

PAPER CODE: 7

PREVALENCE OF HISTOLOGICALLY-CONFIRMED, ANAL HSIL (H-HSIL), IN 150 WOMEN LIVING WITH HIV (WLWHIV) IN BRAZIL: FINAL RESULTS OF THE AMC FELLOWSHIP.

SILVA, J.A.D.D.C.E.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: The rising incidence of anal cancer among people living with HIV (PLHIV) has become a growing public health concern. While significant advances have reduced HPV-related cervical cancer rates, anal cancer incidence continues to increase, particularly among women. Globally, an estimated 18,000 new anal cancer cases are diagnosed each year, disproportionately affecting women. In Brazil, a country of over 200 million people, the prevalence of anal high-grade squamous intraepithelial lesions (h-HSIL) in women living with HIV (WLWHIV) remains poorly characterized, despite their elevated risk.

OBJECTIVES: To determine the prevalence of HRA-confirmed anal HSIL in WLWHIV from a Brazilian cohort. To determine the prevalence of anal high-risk HPV types.

METHOD: We are conducting a cross-sectional study of 150 WLWHIV aged ≥35 years, recruited sequentially from a cohort at the Brazilian National Institute of Infectious Diseases. This study is part of the AIDS Malignancy Consortium (AMC) Career Development Program (2023–2025). Clinical procedures include anal and cervical cytology, HPV genotyping, and high-resolution anoscopy (HRA) with biopsy performed by a certified specialist. Demographic, clinical, behavioral, and HIV-related data are also collected.

RESULTS: To date, 115 participants have been enrolled and evaluated. Among them, 50% were diagnosed with anal h-HSIL. The most prevalent, high risk, HPV type

were 16 for both anal and cervical with 25% and 27%, respectively. The average age of participants is 52.1 years; the oldest is 73. Among women with h-HSIL, 57% received treatment with the Bovie Hifricator. We are also collecting demographic information; HIV-related variables; sexual behavior; risk factors; clinical variables; and vaccination status.

CONCLUSION: Preliminary findings reveal a high prevalence of anal HPV infection and h-HSIL in Brazilian WLWHIV. These results underscore the urgent need for anal cancer screening and prevention strategies tailored to this high-risk population.

Keywords: anal cancer; HSIL; High resolution anoscopy.

PAPER CODE: 21

Wilms' tumor: Clinical features and molecular characterization

DE BRITO, F.M.O.1; AMORIM, L.P.2; AZEVEDO, L.B.D.B.E.3; DOS SANTOS, A.C.E.3; GOMES, R.4. 1. FEDERAL UNIVERSITY OF RIO DE JANEIRO (UFRJ); NATIONAL CANCER INSTITUTE (INCA), RIO DE JANEIRO - RJ - BRASIL; 2. FLUMINENSE FEDERAL UNIVERSITY (UFF), RIO DE JANEIRO - RJ - BRASIL; 3. NATIONAL CANCER INSTITUTE (INCA), RIO DE JANEIRO - RJ - BRASIL; 4. FLUMINENSE FEDERAL UNIVERSITY (UFF); NATIONAL CANCER INSTITUTE (INCA), RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Wilms tumor (WT) is the most common renal neoplasm in childhood, accounting for about 7% of all pediatric tumors. In Brazil, the incidence of WT in patients between 0 and 14 years old ranges from 5 to 18 cases per million, with approximately 80% of patients being diagnosed before 5 years of age. Around 10% of all diagnosed cases are thought to be caused by the presence of pathogenic variants in the WT1 and WTX genes. In this situation, bilateral or multicentric tumors and early age at diagnosis are more common, although cases of unilateral and unifocal tumor do not exclude genetic susceptibility.

OBJECTIVES: This study describes the clinicopathological features of Wilms tumor patients

referred to INCA's genetic counseling service and analyzes WT1 and WTX genes to assess the prevalence of genetic variants.

METHOD: This study included 50 patients clinically or histopathological diagnosis of Wilms tumor treated at INCA (2005–2023) who tested negative for pathogenic TP53 variants. Clinical, pathological, and family history data were retrieved from medical records. DNA extracted from peripheral blood was amplified by PCR and sequenced by the Sanger method using primers for WT1 and WTX genes. Variant nomenclature and classification was performed according to HGVS and CanVIG-UK guidelines, respectively.

RESULTS: Among the 50 patients evaluated, 68% were aged 5 years or less, and 12% were diagnosed with bilateral tumors. The presence of metastases was observed in 31.3% of all patients. Regarding clinical manifestations, it was observed that 18% of patients had congenital hand dysmorphia, 10% had café-au-lait spots, and 10% had facial malformations. Furthermore, syndromes associated with WT were recorded in five patients: Denys-Drash (n=2), Frasier (n=2), and Simpson-Golabi-Behmel (n=1).

To date, Sanger sequencing has been completed for 87.5% of the target regions of the WT1 gene and 100% of the target regions of the WTX gene. One synonymous variant in the WTX gene was identified, present in four patients, eight variants located in the intronic regions of the WT1 gene, and one pathogenic variant identified in a single patient.

CONCLUSION: These results showed that the frequency of pathogenic variants, in the absence of selection criteria, is uncommon, although most of our patients were diagnosed at an age suggestive of genetic susceptibility (≤ 5 years). The clinical manifestations identified in the patients of this cohort may indicate the presence of pathogenic variants in genes related to other hereditary syndromes that include nephroblastoma as one of their phenotypes.

Keywords: Nephroblastoma; WT1; hereditary cancer.

PAPER CODE: 22**Evaluation of the Microbiome in Oral Cancer Patients Undergoing Radiotherapy**

TAGLIABUE, J.F.; ALVES, L.D.B.; MENEZES, A.C.D.S.; DA COSTA, A.M.D.; RANGEL, C.E.D.S.; PIRES, F.R.; GONÇALVES, L.D.S.; ANTUNES, H.S.; DE MELO, A.C.. INSTITUTO NACIONAL DE CÂNCER (INCA), RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Oral mucositis is a common and debilitating complication in patients undergoing cancer therapies, especially radiotherapy. Its etiology is multifactorial, involving both direct and indirect damage to mucosal cells, and its incidence varies depending on the type of treatment and its cytotoxic potential. An imbalance in the oral microbiome can worsen the condition and has become the focus of recent studies.

OBJECTIVES: To evaluate the oral microbiome of patients with oral cancer undergoing radiotherapy through salivary analysis conducted before and at the end of the radiotherapy treatment.

METHOD: This retrospective cohort study was conducted at a single institution, evaluating the oral microbiome in a convenience sample of 28 patients. Saliva samples were collected pre- and post-radiotherapy in a prior study and properly stored. Analyses were carried out in collaboration with Estácio de Sá University. Total DNA was extracted using a QIAGEN microbiome kit, following its protocol, and three dentists verified the procedures. The analysis involved DNA extraction and metagenomic sequencing.

RESULTS: The study is currently ongoing, awaiting final data analysis by the bioinformatics team. After DNA extraction and quantification, 25 out of the 28 samples showed favorable results for genomic sequencing and were sent to the StarSeq laboratory in Germany. The other 3 samples were excluded from the subsequent step due to insufficient amount of saliva in the sample (1) and unsatisfactory result in DNA quantification (2). The metagenomic sequencing was then performed by next generation on the Ion Torrent platform targeting

the 16S rRNA small subunit gene, which contains several hypervariable regions that differ among microbial species, making these regions a molecular marker for species identification. The final data will be compared with the metagenomic database, obtaining the microbial composition of each sample. The clinical and demographic variables were collected and registered into REDCap®, and a descriptive analysis will be carried out.

CONCLUSION: It is expected that an association will be found between the incidence and severity of oral mucositis and alterations in the baseline salivary microbiome. Through this approach, the study may contribute to uncovering and understanding microbiologically mediated functions in saliva that have not yet been described, as well as to proposing new strategies to modulate the role of saliva in health and disease, particularly in oral mucositis.

Keywords: MICROBIOME; HEAD AND NECK CANCER; RADIOTHERAPY.

PAPER CODE: 24**Evaluation of response in metastatic prostate cancer patients treated with low doses of abiraterone**

RAMOS, M.J.. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Abiraterone acetate is an androgen biosynthesis inhibitor used to treat patients with metastatic prostate cancer (mPC). Food intake markedly increases its absorption, leading to higher serum levels when taken with high-fat meals. Therefore, the standard recommendation is 1000 mg once daily taken fasting. A phase II non-inferiority trial demonstrated that a lower dose of 250 mg administered with a low-fat meal achieved similar efficacy, supporting its use as a cost-saving alternative. This approach has been adopted to expand access within the Brazilian Public Health System, although concerns remain regarding pharmacokinetic variability, the lack of long-term outcomes, and the absence of local data.

OBJECTIVES: To compare the minimum serum concentration of abiraterone at 1000 mg and 250 mg doses and evaluate clinical outcomes in patients with mCP.

METHOD: A pre- and post-intervention clinical trial will include 30 patients without prior chemotherapy in the mCRPC setting to determine the minimum abiraterone serum levels across 3 dosing phases: 250 mg with food, 1000 mg fasting, and 250 mg with food. Group comparisons will be performed using ANOVA. An ambispective cohort from 2020 to 2027 will assess clinical outcomes. Kaplan- Meir and log-rank tests will be used to estimate PFS-PSA and OS. A waterfall plot will be used to illustrate PSA reduction.

RESULTS: The acquisition of Brazilian data on therapeutic response in the studied population will provide greater assurance for the clinical practice already implemented and may support therapeutic decisions in other centers. Such initiatives may reduce treatment costs and improve patient access.

CONCLUSION: -

Keywords: Low-dose abiraterone; Metastatic prostate cancer; Therapeutic response.

PAPER CODE: 29

Critical analysis of mouth self-examination methodologies as an educational strategy for awareness and screening of oral cancer: a systematic review

BARROSO, R.D.S.A.; DA SILVA, B.N.M.; ELLER, E.M.; FURTADO, L.D.S.; CURVELO, J.A.D.R.; DE MELO, A.C.; GOLDEMBERG, D.C.. NATIONAL CANCER INSTITUTE, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Oral cancer ranks 16th place in global incidence and is responsible for 389,000 new cases and 188,000 deaths annually, showing high rates among young women, especially on the tongue. Tobacco and alcohol remain the main risk factors, but cases in non-smokers and non-drinkers suggest other

causes. Diagnosis often occurs late, keeping the five-year survival rate at 50–55%. Mouth self-examination emerges as a simple, low-cost educational tool for the early detection of oral cancer. However, systematic reviews on oral cancer screening strategies have revealed that studies addressing self-examination did not present consistent evidence to support its actual effectiveness.

OBJECTIVES: This study aimed to analyze critically and systematically the methodologies and protocols for mouth self-examination used for the early detection of oral cancer available in the literature.

METHOD: This systematic review, following PRISMA guidelines, analyzed studies on mouth self-examination for early detection of oral cancer or potentially malignant disorders. Searched databases included PubMed, Scopus, and Web of Science. Eligible studies were observational, cross-sectional, multicenter, pilot or RCTs. Data on study design, patient characteristics, MSE protocols, and implementation strategies were extracted. Study quality was assessed using QUADAS-2.

RESULTS: Thirty-six articles were identified, and 8 correspond to the criteria for the systematic review. Most studies were conducted in India, two in Brazil, one in England and one in Spain. Seven studies recruited high-risk individuals, such as smokers, alcohol users, or patients with Fanconi anemia. All of them described educational strategies for mouth self-examination, but only few detailed the materials used. Clinical evaluation varied in examiner training, diagnostic validation, and use of histopathology. Unequal attention to information accessibility highlighted gaps in standardization. Quality assessment showed low risk of bias in patient selection, but uncertainties in test interpretation and applicability. Four studies had high applicability concerns, and one showed high risk in flow and timing due to unjustified exclusions.

CONCLUSION: The results highlight the need to standardize educational methodologies for mouth self-examination, ensuring that materials are clear, accessible, and culturally adapted. The adoption of well-structured

health education strategies promotes awareness of risk factors and preventive habits, strengthening collective knowledge and the population's autonomy to recognize warning signs and adopt continuous oral health care practices.

Keywords: Mouth Neoplasms; Mouth Self-examination; Early diagnosis .

PAPER CODE: 41

Endometrial cancer recurrence in p53 abnormal tumors without myometrial invasion

PAULINO, E.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Endometrial cancer (EC) is the most common gynecologic malignancy worldwide, accounting for approximately 4.3% of all cancers in women. Although most cases are endometrioid adenocarcinomas, which generally confer a favorable prognosis, non-endometrioid subtypes such as serous and clear cell carcinomas are associated with aggressive clinical behavior and disproportionate mortality. Clarifying the natural history, recurrence risk, and benefits of multimodal therapy in non-invasive high grade EC histologies is essential to refine risk stratification and align clinical management with the molecular underpinnings of the disease.

OBJECTIVES: Evaluated the impact of p53-abnormal (p53abn) status on recurrence patterns and outcomes among patients with non-invasive, high-grade endometrial cancer (EC)

METHOD: Eligible patients had high-grade histologies—including endometrioid grade 3 (ECG3), serous (USC), clear cell (CC), carcinosarcoma (CS), mixed, or undifferentiated tumors—without myometrial invasion. Clinical, surgical, and pathological characteristics, as well as recurrence and survival data, were reviewed. Immunohistochemical analysis of p53 expression was performed, and p53 status was correlated with clinical outcomes, particularly recurrence rate

RESULTS: Fifty-nine patients met the inclusion criteria, with a mean age of 65 years. Most underwent total hysterectomy with or without bilateral salpingo-oophorectomy (59.3%); 40.7% also received lymphadenectomy or omentectomy. Histologic distribution was mainly endometrioid (39.0%) and serous (39.0%). Seven patients (11.9%) received chemotherapy and 15 (25.4%) received radiotherapy. Three patients had already disease outside uterus. Among 56 patients with FIGO stage IC disease, recurrence occurred in 16.1%, predominantly at extra-pelvic sites (33.3% abdominal, 22.2% distant). Five-year recurrence was 23.2% for p53abn tumors versus 0% for wild-type, and 12.5% of patients died—mostly within the p53abn subgroup

CONCLUSION: The findings suggest that even in non-invasive high-risk EC, p53 abnormalities confer higher recurrence risk and poorer outcomes, while wild-type tumors show excellent prognosis. These results support individualized treatment approaches and highlight the need for larger, prospective validation studies

Keywords: Endometrial Cancer; p53 abnormal;non-invasive.

PAPER CODE: 45

Clinical, histopathological, and molecular characterization of ovarian mucinous carcinomas in Brazil

ROMA, N.R.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Ovarian mucinous carcinoma (MOC) represents approximately 3% of ovarian cancers. Its diagnosis is challenging due to immunohistochemical overlap with metastatic tumors. Platinum-taxane chemotherapy remains the gold standard, though resistance is frequent. Stage I patients show five-year survival rates near to 90%, while advanced stages carry poor outcomes. Targeted therapies (KRAS, HER2) and antibody-drug conjugates (TROP2, FRα) are under investigation to improve prognosis.

OBJECTIVES: To describe the clinical, histopathological, immunohistochemical, and molecular features of ovarian mucinous carcinoma cases treated at INCA from 2014 to 2024.

METHOD: Electronic records of primary MOC cases (ICD-C56) were reviewed, excluding prior malignancies and metastasis. Slides were re-evaluated by a collaborating pathologist. Immunohistochemistry will be performed to confirm the diagnosis and to evaluate the expression of therapeutic targets, along with genomic analysis of KRAS mutations. Statistical analyses will be performed using R software, applying association tests and survival models with a significant level of $p < 0.05$.

RESULTS: Of 105 cases identified through the C56 code search, 47 were excluded due to a history of previous malignancy, mixed histology, or metastatic origin (mainly appendix and colon). Twenty cases had their diagnosis revised after slide review, resulting in 38 confirmed MOC cases. The median age was 52 years, and the mean tumor size was 13.8 cm. Capsular invasion was not reported macroscopically in 13 cases but was microscopically confirmed in six. One case was described as expansile, and slide review identified four cases with infiltrative growth. Fifteen underwent IHC, with CK7, CK20, and CDX2 positivity being most frequent. Most patients showed diseases limited to the ovary. Eleven deaths occurred.

CONCLUSION: Findings align with international literature regarding age and stage distribution. Ongoing IHC and molecular analyses (FRα, TROP2, HER2, KRAS) aim to refine tumor classification and explore potential therapeutic targets.

Keywords: Ovarian cancer; Ovarian mucinous carcinoma; Immunohistochemistry.

PAPER CODE: 47

DETERMINANTS OF POOR QUALITY OF LIFE IN BREAST CANCER PATIENTS UNDERGOING CURATIVE CHEMOTHERAPY

DE ALCÂNTARA, R.R.; FERNANDES, M.C.; FABRO, É.A.N.; COSTA, R.M.; TORRES, D.M.; DE AGUIAR, S.S.; MARCONI, E.M.; LENZI, J.; BERGMANN, A.. BRAZILIAN NATIONAL CANCER INSTITUTE, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Breast cancer (BC) is considered one of the most prevalent diseases worldwide and is the most common type of cancer among women in Brazil. Neoadjuvant chemotherapy (CT) is one of the available treatment options; however, it is associated with significant adverse events. Health-related quality of life (HRQoL) is a crucial factor in assessing treatment effects and patients' functional capacities throughout their lives. Evaluating HRQoL in cancer patients prior to the initiation of oncologic treatment is essential to enhance treatment outcomes from the patient's perspective and allows for classification of the treatment's impact on HRQoL. This, in turn, helps the multidisciplinary team to plan interventions that minimize potential negative effects.

OBJECTIVES: To analyze factors contributing to poorer health-related quality of life (HRQoL) in women with BC before the first infusion of chemotherapy (CT).

METHOD: Cross-sectional study with women (≥ 18 years) with stage I–IIIC breast cancer, evaluated on the first day of curative chemotherapy at the Brazilian National Cancer Institute. Part of the trial “Photobiomodulation for the Prevention of Peripheral Neuropathy” (Ethics No. 5737539). HRQoL was assessed using the Portuguese FACT-G (Functional Assessment of Cancer Therapy/General). Sociodemographic and clinical data were collected from records. Multiple linear regression was used for analysis.

RESULTS: A total of 186 women were included. Most were under 54 years old, had ≥ 8 years of education and presented with advanced-stage disease (stage $\geq$ III). The lowest HRQoL scores were observed in the emotional and functional well-being subscales. Factors associated with worse quality of life in the physical well-being and total FACT-G scores were: reporting poor/very poor health status, partially performing or not performing household activities, and having stage $\geq$ III disease. For functional well-being, the associated factors were poor/very poor health status and limited or no performance

of household activities. For emotional well-being, only poor/very poor health status was associated with worse HRQoL. No factors were associated with worse HRQoL in the social/family well-being subscale.

CONCLUSION: Factors contributing to poorer HRQoL before starting CT included poor/very poor self-rated health, partial or no domestic activity and advanced disease stage ($\geq$ III).

Keywords: Breast cancer; Health-related quality of life; Chemotherapy.

PAPER CODE: 48

EVALUATION OF PROPHYLACTIC PHOTOBIO-MODULATION THERAPY IN PATIENTS WITH OSTEOSARCOMA UNDERGOING HIGH-DOSE METHOTREXATE

DA COSTA, A.M.D.; MENEZES, A.C.D.S.; ALVES, L.D.B.; TAGLIABUE, J.F.; GOLDEMBERG, D.C.; ANTUNES, H.S..
INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Osteosarcoma (OS) is the most frequently diagnosed malignant bone tumor during childhood and adolescence. Treatment consists of high-dose methotrexate (MTX) chemotherapy combined with doxorubicin and cisplatin, in addition to definitive surgery. However, adverse effects pose a challenge to clinical practice. Oral mucositis (OM) is considered one of the most debilitating complications for patients. Several studies have demonstrated the effectiveness of low-level laser therapy (LLLT) in preventing OM through tissue photobiomodulation. Due to the high incidence of OM in pediatric patients with OS undergoing high-dose MTX, there is a need to establish a prophylactic LLLT protocol for this population.

OBJECTIVES: To compare the efficacy of two LLLT protocols for the prevention of OM in pediatric patients with OS

METHOD: Phase 3, randomized trial with patients diagnosed with OS and undergoing chemotherapy. In both existing groups, prophylactic PBMT is performed

on the day of MTX infusion and maintained until the serum concentration reaches ≤ 0.3 mmol/L. In group 2, it is also applied on the days of doxorubicin and cisplatin infusions. A dose of 1 J/point is used ($\lambda = 660$ nm). Patients are evaluated at each infusion week. The sample size calculation estimates 48 patients, with 24 in group 1 and 24 in group 2.

RESULTS: A total of 32 patients have been included so far: 16 in group 1 and 16 in group 2. There was a predominance of male patients (62.5%), brown color (46.9%), age between 12 and 18 years (62.5%), non-metastatic disease (62.5%), and tumor location in both lower and upper limbs (43.7%). Of these, 12 patients were discontinued, and 6 experienced serious adverse events. An interim analysis is not planned for the study.

CONCLUSION: A lower incidence of OM is expected in patients in group 2, as well as reduced severity of the condition and improved recovery time.

Keywords: Osteosarcoma; Methotrexate; Low-level laser therapy.

PAPER CODE: 50

THE INFLUENCE OF NEOADJUVANT CHEMOTHERAPY ON THE CARDIOPULMONARY CAPACITY OF WOMEN WITH BREAST CANCER

DE SANT'ANNA, M.V.M.1; MARCONI, E.M.1; SARAIVA, S.A.1; TORRES, D.M.1; FABRO, E.A.N.1; SANT'ANNA JUNIOR, M.2; AGUIAR, S.S.1; BERGMANN, A.1; COSTA, R.M.1. 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. IFRJ, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Neoadjuvant chemotherapy (NACT) is one of the main modalities of oncologic treatment and may cause toxicities that affect patients' cardiopulmonary capacity (CPC). Despite advances in diagnosis and therapy, surgical procedures remain widely used. Such procedures can lead to respiratory dysfunctions, even without direct pulmonary involvement, due to anesthesia and central nervous system depression, ultimately impacting CPC - an important marker of morbidity and mortality in breast cancer.

OBJECTIVES: To evaluate the impact of NACT on the CPC of women with breast cancer using the 6-Minute Step Test (6MST).

METHOD: Cohort study, part of a project, including women aged 18 or older. CEP INCA 4.576.731. Sociodemographic and clinical data were collected. CPC was assessed using the 6MST before NACT and 30 days post-surgery. The test will collect a number of step repetitions and maximum heart rate. Descriptive analysis involved measures of central tendency and dispersion for continuous variables and frequency distribution for categorical variables. A paired t-test was applied for 6MST, with significance $p < 0.05$.

RESULTS: Preliminary results include 42 women who completed the 30-day post-surgery evaluation. The mean age was 52.5 (± 11.02) years; 71.4% self-identified as Black or Brown, 64.3% reported not having a partner, and 88.1% had more than eight years of education. Clinical data revealed, 86.8% were overweight or obese, and 90.5% had advanced clinical staging ($>IIB$). For treatment, 78.6% received anthracyclines or doxorubicin followed by paclitaxel or docetaxel chemotherapy and 90.5% underwent ≥ 8 chemotherapy sessions. The mean repetitions in the 6MST were 102.6 (± 34.7) steps pre-chemotherapy and 92.8 (± 25.6) steps 30 days post-surgery, showing a mean difference of 9.8 (± 26.6 ; $p = 0.022$). The maximum heart rate during the test was 113.0 (± 21.1) bpm pre-chemotherapy and 121.0 (± 21.9) bpm 30 days post-surgery, there was difference before and after ($p = 0.079$).

CONCLUSION: Following NACT and the postoperative period in breast cancer treatment, a significant decline in cardiopulmonary capacity (CPC) was observed. However, the change in heart rate response to exercise remained within normal limits.

Keywords: breast cancer; neoadjuvant therapy; cardiorespiratory fitness.

PAPER CODE: 52

Feasibility, safety, and efficacy of whole-body vibration exercise during chemotherapy in women with

gynecological cancer: a randomized clinical trial

MARCONI, E.M.; MORAIS, F.B.B.; MAGALHÃES, A.C.D.; MARCHON, R.M.; DE AGUIAR, S.S.; JÚNIOR, J.F.; BERGMANN, A.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Os cânceres de colo do útero, endométrio e ovário são, respectivamente, os 8º, 15º e 18º tipos de câncer mais comuns no mundo. As alterações físicas e emocionais decorrentes do diagnóstico e do tratamento interferem na capacidade funcional (CF) e na qualidade de vida relacionada à saúde (QVRS) dessas mulheres. O exercício físico é recomendado para prevenção, tratamento e remissão da doença. No entanto, muitos pacientes não conseguem manter ou iniciar esse hábito devido a alterações metabólicas e físicas decorrentes da doença ou de tratamentos como a quimioterapia (QT). O exercício de vibração de corpo inteiro (EVCI) é uma atividade física de fácil execução e com baixa percepção de esforço.

OBJECTIVES: Analisar a previsão, segurança e eficácia do uso de WBVE durante CT em mulheres com câncer ginecológico na prevenção e minimização da fadiga e na melhoria da FC e da qualidade de vida relacionada à saúde.

METHOD: Ensaio clínico randomizado com mulheres submetidas à TC, ≥ 18 anos. Grupo de intervenção (GI): WBVE com frequências: 10-35 Hz e amplitude: 2 mm (tempo total: 10 min). Grupo controle (GC): protocolo por instituição. Os estágios foram: fadiga, CF e QVRS. Este projeto faz parte de um projeto maior (CAAE-67391223.4.0000.5274 e REBEC -RBR-7d3yxz3). As avaliações foram realizadas: antes da TC (D1); no terceiro dia de infusão da TC (D2); no último dia da TC (D3); envio (D4).

RESULTS: Foram incluídos 84 pacientes ($n = 72$ - TC completa). A maioria dos participantes tinha ≥ 60 anos (58%) e era mestiça (44%). Um paciente relatou um efeito adverso WBVE sem necessidade de interrupção (WBVE ou TC). Não houve diferença estatisticamente significativa em relação à CF. Em relação aos domínios da QVRS, observou-se que entre D1-D2 houve melhora na função emocional em ambos os grupos (maior no GI $p < 0.001$), e o sintoma de diarreia piorou no GC

($p=0,007$). D1-D3 houve melhora na função emocional em ambos os grupos (maior no GC $p=0,002$). D1-D4 houve melhora na função geral no GI ($p=0,033$). Em relação à fadiga (D1-D2), houve piora da fadiga física no GI ($p=0,033$) e melhora na interferência diária no GI ($p=0,010$). D1-D3 houve melhora na fadiga emocional no GC ($p=0,029$) e no GI melhorou na fadiga cognitiva ($p=0,012$) e na interferência diária ($p=0,016$). D1-D4, não houve diferença nos domínios da fadiga.

CONCLUSION: Embora este estudo não esteja completo, o WBVE pode ser considerado uma modalidade de exercício viável e segura durante um TC. Os benefícios da WBVE foram distribuídos durante todo o tratamento com TC em relação à qualidade de vida relacionada à saúde (QVRS) nos domínios função emocional, função geral e domínio de sintomas de diarreia, e em relação à fadiga nos domínios fadiga cognitiva e interferência diária.

Keywords: vibration; exercise therapy; chemotherapy.

PAPER CODE: 61

Efficacy of a Self-Management Strategy for Breast Cancer-Related Lymphedema in the Volume Reduction Phase: A Pilot Study

DA SILVA, J.M.P.1; SOARES, N.B.1; VEIGA, M.A.C.1; FABRO, E.A.N.2; COSTA, R.M.2; MARCONI, E.M.1; DE AGUIAR, S.S.1; BERGMANN, A.1. 1. INSTITUTO NACIONAL DE CÂNCER (INCA), RIO DE JANEIRO - RJ - BRASIL; 2. HOSPITAL DO CÂNCER III/ INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Lymphedema is the main long-term complication of breast cancer treatment, resulting in an increase in the volume of the upper limb on the same side as the treatment, which can lead to both physical and psychosocial problems. Therefore, its control is essential to ensure a better quality of life. Compression therapy is the main component of lymphedema treatment, with compression bandaging applied by the physiotherapist being the most recommended approach during the

volume reduction phase. Self-bandaging appears to be a promising strategy, as it enables the individual to take an active role in their treatment and provides greater independence.

OBJECTIVES: To evaluate the effectiveness of compression therapy applied by the physiotherapist vs self-applied during the volume reduction phase of the upper limb in women with breast cancer-related lymphedema.

METHOD: A randomized clinical trial including women with breast cancer-related lymphedema in the volume reduction phase. The intervention consisted of compression bandaging applied by the physiotherapist (Group A) versus self-applied by the patient (Group B). Assessments were performed at baseline and after 30 days using upper limb perimetry and interviews. The primary outcome was the percentage of excess volume between the limbs. Data were analyzed with 95% CI and $p<0.05$ for statistical significance.

RESULTS: Twelve participants were included between September and December 2024. The mean age was 57.62 (± 10.5) years, and the mean number of axillary lymph nodes removed was 13.9 (± 7.5). Among them, 66.7% had undergone mastectomy, and 81.8% presented with grade II lymphedema, with a baseline mean percentage of excess limb volume between upper limbs of 23.5% ($\pm 12.8\%$). The mean reduction in excess volume percentage over 30 days was 41.1% (± 16.2) in the group with compression bandaging applied by the physiotherapist versus 26.5% (± 15.5) in the self-bandaging group, with no significant difference between groups ($p=0.142$).

CONCLUSION: Self-bandaging in women with breast cancer-related lymphedema was feasible and not inferior to physiotherapist-applied bandaging, achieving an acceptable reduction in excess volume within 30 days.

Keywords: breast cancer-related lymphedema; compression therapy; physiotherapy.

PAPER CODE: 64

Efficacy of photobiomodulation in preventing and managing adjuvant radiotherapy-related pain in breast cancer: a randomized clinical trial

JÚNIOR, J.F.1; SOARES, N.B.1; MARCONI, E.M.1; DE AGUIAR, S.S.1; DE ALCÂNTARA, R.R.1; DE MELLO, I.C.R.2; XARÃO, L.S.3; FRANCO, L.F.D.R.4; DE MELO, A.C.1; BERGMANN, A.1. 1. BRAZILIAN NATIONAL CANCER INSTITUTE (INCA), RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE VEIGA DE ALMEIDA, RIO DE JANEIRO - RJ - BRASIL; 3. CENTRO UNIVERSITÁRIO AUGUSTO MOTTA, RIO DE JANEIRO - RJ - BRASIL; 4. CENTRO UNIVERSITÁRIO DAS FACULDADES ASSOCIADAS DE ENSINO DE SÃO JOÃO DA BOA VISTA - UNIFAE, SÃO JOÃO DA BOA VISTA - SP - BRASIL.

INTRODUCTION: Cancer is one of the main public health problems and a leading cause of death worldwide. In Brazil, breast cancer (BC) is the most common cancer among women. Adjuvant radiotherapy (RT) is indicated to increase local disease control but may cause edema, nerve damage, lymphedema, reduced shoulder mobility, radiodermatitis, and pain. Individuals undergoing adjuvant RT have a 1.5-fold higher risk of pain, which can negatively affect sleep and health-related quality of life (HRQoL). Photobiomodulation (PBM) with low-level laser therapy, using red light (RL, 620–700 nm) and near-infrared (NIR, 700–1440 nm) wavelengths, has emerged as a promising non-pharmacological approach for preventing and managing RT-related pain.

OBJECTIVES: To evaluate the efficacy of PBM in preventing and managing adjuvant RT-related pain in people with BC.

METHOD: A randomized clinical trial will include 148 people with BC undergoing adjuvant RT at the Brazilian National Cancer Institute (Ethics approval No. 7.228.376). Participants will be randomized to an intervention group (active PBM, RL/NIR - 630/850 nm, 3 Joules) or a control group (sham PBM), applied three times weekly during RT. Outcomes: pain (PainDETECT, McGill), sensitivity (esthesiometry), HRQoL (EORTC QLQ-C30/BR23) and sleep (Pittsburgh). Analyses will follow the intention-to-treat principle.

RESULTS: Recruitment began in July 2025, and to date, 92 patients have been screened for eligibility. After applying the exclusion criteria, 51 participants were enrolled in the study and are currently under follow-up. It is expected that the PBM group will show lower pain intensity and prevalence, improved cutaneous sensitivity, and better HRQoL and sleep quality scores compared with the control group. Preliminary analyses may indicate a protective effect of PBM on the development and persistence of pain during and after adjuvant RT in people with BC.

CONCLUSION: It is expected that PBM will help prevent and reduce pain related to adjuvant RT in people with BC. If confirmed, these findings will support PBM as a complementary non-pharmacological strategy in the care of patients with BC undergoing adjuvant RT.

Keywords: Cancer pain; Radiotherapy; Photobiomodulation therapy.

PAPER CODE: 75

Quantitative Measurements of the Posterior Pharyngeal Wall in Videofluoroscopy: A Scoping Review

CERQUEIRA, R.B.A.; DE FREITAS, A.S.; DIAS, F.L.. BRAZILIAN NATIONAL OF CANCER INSTITUTE, RIO DE JANEIRO - RJ - BRASIL.

Introduction: Swallowing is a complex process that depends on the coordination and structural integrity of multiple muscle groups, including the pharyngeal constrictors. The posterior pharyngeal wall, composed of these muscles, plays a crucial role in the propulsion of the bolus and in the efficiency of the pharyngeal phase. Therefore, understanding the quantitative measurements applied to the analysis of the posterior pharyngeal wall can aid in the early identification of swallowing disorders and in directing more specific therapeutic interventions.

OBJECTIVES: To describe the methods of quantitative measurements used for the evaluation of the posterior pharyngeal wall in videofluoroscopic swallowing exams in adults.

METHOD: This is a scoping review. Searches were conducted in the PubMed/MEDLINE, BVS/LILACS, and Cochrane Library databases, with no restrictions on date or language, using descriptors and keywords related to 'posterior pharyngeal wall,' 'pharyngeal constriction,' and 'videofluoroscopy.' Studies that presented quantitative measurements of the posterior pharyngeal wall, used videofluoroscopy, and involved adult participants with or without dysphagia were included.

RESULTS: To date, 538 records have been identified (PubMed = 512; BVS/LILACS = 7; Cochrane = 19), with six duplicates removed. Title and abstract screening by two independent reviewers resulted in 33 articles currently under discussion in the full-text review. Preliminary findings indicate heterogeneity in the measurements used, with the Pharyngeal Constriction Ratio (PCR) and measurement of the thickness of the posterior pharyngeal wall emerging as the most frequently used parameters. Methodological variations among the studies suggest a lack of consensus regarding measurement protocols and interpretation of results.

CONCLUSION: There is a noticeable diversity of methods, small sample sizes, and a lack of standardization in the quantitative measurements of the posterior pharyngeal wall obtained through videofluoroscopy. This review will contribute to systematizing current knowledge, identifying gaps in the literature, and supporting future standardization of swallowing biomechanics analysis.

Keywords: swallowing; head and neck ; kinematics.

PAPER CODE: 79

FACTORS ASSOCIATED WITH WORSE FUNCTIONAL CAPACITY IN WOMEN WITH GYNECOLOGICAL CANCER UNDERGOING CHEMOTHERAPY: A CROSS-SECTIONAL STUDY

MAGALHÃES, A.C.D.; MORAIS, F.B.B.; JÚNIOR, J.F.; MARCHON, R.M.; MARCONI, E.M.; DE AGUIAR, S.S.; BERGMANN, A.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Gynecological cancers include cervical, endometrial, and ovarian cancers, ranking among the 8th, 15th, and 18th most prevalent cancers, respectively. Women with this diagnosis often experience functional impairments such as decreased muscle mass and strength and balance impairment. This can lead to changes in activities of daily living, potentially leading to dependence due to a decline in functional capacity even before chemotherapy (CT) begins. Therefore, it is important to understand the functional profile of patients to prevent complications and comorbidities arising from the natural process of disease progression or the course of treatment.

OBJECTIVES: The objective of this study was to evaluate the factors associated with poor functional capacity in patients indicated for CT for gynecological cancer.

METHOD: This is a cross-sectional study with patients >18 years old, diagnosed with gynecological cancer, with indication for CT with carboplatin and paclitaxel. The functional profile was assessed by handgrip strength (dynamometry), functionality (sit-to-stand test) and static balance (Fournier test). This study is part of a larger Project (CAAE: 67391223.4.0000.5274) and the Brazilian Registry of Clinical Trials (RBR-7d3yxz3).

RESULTS: Ninety-four patients with a mean age of 60.4 (± 10.9) years, with endometrial (n=65; 69.1%), ovarian (n=20; 1.3%) and cervical (n=9; 9.6%) cancer were included. Regarding skin color, the majority considered themselves non-white (n=64; 68.8%). Regarding marital status, the majority lived without a partner (n=60; 64.5%). The level of education was 49 (52.1%) women who studied less than 8 years and 32 (34%) who studied more than 8 years and 13 (13.8%) did not inform. Regarding the functional profile, most presented lower-than-expected handgrip strength (n=79; 84%), the sit-to-stand test time was 17.9 seconds (± 7.6), and balance was altered in 58 (61.7%) women and preserved in 36 (38.3%). Analysis of factors associated with poorer functional capacity is ongoing.

CONCLUSION: The patients evaluated presented an altered functional profile regarding balance and handgrip strength. Analysis of associated factors is still ongoing.

Keywords: muscle strength; female genital neoplasm; postural balance.

PAPER CODE: 80

Evaluation of the Cytotoxic Potential of Bothrops jararaca Phospholipases A₂ for the Treatment of Head and Neck Tumors

AGUIAR, I.S.. INCA, BELFORD ROXO - RJ - BRASIL.

INTRODUCTION: Head and neck cancer refers to malignant tumors that affect the upper aerodigestive tract. It represents one of the major causes of morbidity and mortality from malignant neoplasms in Brazil, as it is often diagnosed at advanced stages. In this context, the search for new and innovative therapeutic targets to treat this disease is essential. The use of certain biological resources as therapeutic targets may enable the development of novel antitumor treatments. This study aims to evaluate the cytotoxic potential of two Phospholipases A₂ (PLA₂s) obtained from the venom gland of Bothrops jararaca, cloned into expression vectors for the treatment of head and neck tumor cell lines.

OBJECTIVES: The main objective of this study is to evaluate the cytotoxic potential of Phospholipases A₂ extracted from Bothrops jararaca venom gland in head and neck tumor cell lines.

Method: Plasmid DNA containing Phospholipase A₂ coding sequences was extracted and enzymatically digested to confirm the enzyme's presence. Bacterial transformation, plasmid extraction, digestion, and gel electrophoresis were performed. The DNA was transfected to confirm recombinant protein expression and analyze cytotoxic effects in head and neck cell lines using viability assays and Western blotting.

RESULTS: Enzymatic digestion demonstrated that the Phospholipases A₂ were inserted into the plasmid because of previous molecular cloning. Fluorescence microscopy confirmed transfection in Vero cells by detecting the fluorescence of the EGFP reporter gene previously inserted into the control plasmid pCi.Neo EGFP, compared with the plasmids containing the

coding sequences of the Phospholipases A₂. However, detection of the recombinant PLA₂ protein expression after transfection is still in progress, as well as the cell viability assays in head and neck tumor lines and the analysis of cell death pathways affected by the Phospholipases A₂.

CONCLUSION: The methods employed for bacterial transformation, plasmid DNA extraction, enzymatic digestion, and gel electrophoresis proved to be efficient and appropriate for the proposed objectives, confirming the integrity and expected size of the plasmids. It is worth noting that both the cell viability assays in head and neck tumor lines and the analysis of cell death pathways are still in progress so that these stages of the project can be completed.

Keywords: phospholipases A₂; head and neck tumors; Bothrops Jararaca.

PAPER CODE: 84

Common Germline Genetic Variation and Susceptibility to Prostate Cancer in the Brazilian Population: A Case-Control Study

SPÍNOLA, .1; ROSA, A.A.2; LOTT, F.2; MASSON, P.2; VIOLA, J.P.B.3; PEDROSA, J.A.2; VALLE, S.F.4; CHAGAS, L.V.4; ABREU, D.2; MELO, C.E.2; CAMPOS, F.S.2; DIAS, A.4; DE MELO, A.C.4. 1. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL; 2. NATIONAL CANCER INSTITUTE (INCA), UROLOGIC ONCOLOGY SERVICE, RIO DE JANEIRO - RJ - BRASIL; 3. NATIONAL CANCER INSTITUTE (INCA), POSTGRADUATE PROGRAM IN ONCOLOGY (PPGO), RIO DE JANEIRO - RJ - BRASIL; 4. NATIONAL CANCER INSTITUTE (INCA), DIVISION OF CLINICAL RESEARCH AND TECHNOLOGICAL DEVELOPMENT (DIPETEC), RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Prostate cancer is one of the most common cancers among Brazilian men and represents a significant public health problem. Factors such as age, ethnicity, and family history are the main known risk determinants, with approximately 57% of population variability attributable to genetic inheritance. Almost all knowledge in genetic research relates to Caucasian

populations. The Brazilian population, due to its high degree of miscegenation, offers a unique setting for studying the genomic contribution to susceptibility to prostate cancer. Investigating the role of germline genetic variants, especially in black individuals, can provide important insights for improving screening, early diagnosis, and individualized risk stratification.

OBJECTIVES: To investigate germline genetic variants associated with prostate cancer and compare polygenic risk scores between Brazilian cases and controls.

METHOD: Prospective case-control study conducted at INCA with 2,300 men (1,150 cases and 1,150 controls), matched by age, ethnicity, and family history. DNA extracted from saliva sample are analyzed by next-generation sequencing (NGS) and Illumina GWAS assay (Oncoarray, 533,000 variants). The association between genotypes and risk is estimated by odds ratio, with Bonferroni correction. Polygenic risk scores are compared via ANOVA, using SPSS, STATA, and R software.

RESULTS: To date, 823 samples have been collected from prostate cancer patients and 273 controls. The mean age of patients with prostate cancer collected to date was 66 years and the mean BMI was 26.9 kg/m². There was a high prevalence of hypertension (64.4%), alcohol consumption (49.3%), and smoking (41.3%). Most patients were identified as black (61.9%), followed by non-white (27.4%) and white (10.7%). About 29.3% reported a family history of the disease. Histopathological analysis revealed a more aggressive disease profile, with 52% of cases classified as high risk (ISUP ≥ 4 or 5). Among the patients evaluated, 40.9% had recurrence after treatment and 37 developed metastases, reflecting a cohort with a predominance of advanced cases and a poor prognosis.

CONCLUSION: The preliminary data provide a comprehensive histopathological and prognostic analysis of prostate cancer. The recruitment phase is almost complete, and results analysis stage is expected to be finalized in April 2026. This will make important contributions to characterizing the genetic and germinative profile of prostate cancer in an ethnically

diverse population. Furthermore, as we have a profile of patients with aggressive disease, we will also be able to characterize lethal prostate cancer.

Keywords: Prostate cancer; Germline genetics; polygenic risk; .

PAPER CODE: 86

STUDY OF THE FREQUENCY OF ADVERSE EVENTS FROM IMMUNE CHECKPOINT INHIBITORS IN MELANOMA TREATMENT AND THEIR ASSOCIATION WITH RESPONSE

ROISMAN, L.E.D.B.P.K.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: The introduction of targeted therapy and immune checkpoint inhibitors (ICIs) has revolutionized the treatment of advanced melanoma, promoting significant survival gains. ICIs reactivate the T-lymphocyte-mediated antitumor immune response. The use of ICIs is associated with the development of immune-mediated adverse events (imAE), of which cutaneous toxicity is the most prevalent. Studies suggest a possible association between the development of imAEs and better prognosis in melanoma. However, there is a scarcity of data in the Brazilian population regarding ICI-associated cutaneous toxicity. This study aims to fill this gap through a cohort study conducted at the Brazilian National Cancer Institute (INCA).

OBJECTIVES: To evaluate the frequency of cutaneous adverse events from ICIs, including description of the clinical and demographic profile of patients who develop cutaneous imAEs (cimAEs).

METHOD: This is an ambidirectional cohort study investigating cimAEs of ICI in patients with unresectable stage III or stage IV melanoma. A convenience sample is being used. The cimAEs and clinical-demographic profile will be described by frequencies and measures of central tendency. Associations between cimAEs, vitiligo-like depigmentation, and response to ICIs will be evaluated by Relative Risk and 95% CI, with Pearson's chi-square test. Analyses will be performed in R, with significance of $p < 0.05$.

RESULTS: Of the 42 patients initially recruited, 22 were included in the final analysis. Most patients were male (63.6%), with a median age of 62 years (52.0 to 72.2). The most prevalent phototypes were III (40.9%) and V (31.8%), and most participants self-identified as white (68.2%). Regarding education level, there was a predominance of high school and elementary school (31.8% each). At initial melanoma diagnosis, 81.8% had localized disease; however, at the time of study inclusion, the majority were in stage IV (59.1%). Primary lesions were mostly acral (50.0%), with metastases most frequently in the lung (22.7%), CNS (13.6%), and liver (9.1%). All patients were treated with PD-1 inhibitors, and only one received combination therapy with a BRAF inhibitor. Treatment was discontinued in two cases (9.1%) due to non-dermatological adverse events. Regarding therapeutic response, 25% showed partial response, 60% maintained stable disease and 15% progressed.

CONCLUSION: This study provides preliminary data on cutaneous adverse events associated with ICIs in Brazilian melanoma patients, addressing a significant knowledge gap. The cohort predominantly consisted of advanced-stage patients with acral melanomas treated with PD-1 inhibitors, showing favorable disease control rates and no mortality during follow-up.

Keywords: immune checkpoint inhibitors; melanoma; cutaneous adverse events.

PAPER CODE: 89

The relevance of using handgrip strength as an indicator of muscle function in pediatric patients with hematologic neoplasms

ALEXO, I.C.S.1; SARAIVA, D.A.1; DE CARVALHO, B.P.2; MARTUCCI, R.B.2. 1. INSTITUTO NACIONAL DO CâNCER, RIO DE JANEIRO - RJ - BRASIL; 2. PROGRAMA DE PÓS-GRADUAÇÃO EM CLÍNICA MÉDICA, UNIVERSIDADE DO ESTADO DO RIO DE JANEIRO (UERJ) RJ, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Leukemias are the main neoplasms affecting children and adolescents. Childhood and adolescence are stages of high energy expenditure and intense metabolic demand, making hematologic malignancies in this age group a major factor for malnutrition and muscle loss. Sarcopenia, in addition to encompassing the reduction of muscle tissue quantity, is characterized by the loss of muscle function, which can lead to negative outcomes in the disease. Thus, muscle depletion affects anthropometric parameters related to muscle, such as mid-upper arm circumference (MUAC), mid-arm muscle circumference (MAMC), arm muscle area (AMA), tricipital skinfold (TSF), and body mass index-for-age (BMI/A), consequently altering handgrip strength (HGS).

OBJECTIVES: To analyze the association between handgrip strength (HGS) and other anthropometric assessment parameters, and to compare nutritional status classification by arm anthropometry and HGS.

METHOD: Prospective observational study with pediatric patients aged 7–18 years with hematologic neoplasms at INCA. Anthropometric measures included weight, height, mid upper arm circumference, mid-arm muscle circumference, arm muscle area, tricipital skinfold, calculated body mass index. Nutritional status was classified using WHO AnthroPlus. Data were analyzed in SPSS using Shapiro–Wilk, correlation, and chi-square tests. HGS percentiles followed Marrodán, with the right hand standardized for measurement.

RESULTS: Forty-two patients were included, median age 13.75 years (IQR: 11.8–16.77); 73.8% male. Lymphoma (57.1%) and leukemia (21.4%) were the most frequent neoplasms. According to BMI/A, 47.6% were eutrophic. Arm anthropometry indicated a higher frequency of nutritional deficit: 47.6% by AMA, 45.2% by MAMC, 33.3% by MUAC, and 7.1% by TSF. Median HGS was 19 kg/F, higher in males (24 kg/F) than females (14 kg/F). According to Marrodán, 57.1% were below adequacy. HGS correlated strongly with AMA and MAMC ($\rho = 0.757$; $p < 0.001$), moderately with MUAC ($\rho = 0.593$; $p < 0.001$), and weakly with BMI ($\rho = 0.482$; $p = 0.001$).

When correlating arm anthropometry with BMI, there was a very high positive correlation between BMI and MUAC (Spearman's $\rho = 0.945$; $p < 0.001$) and a high positive correlation between BMI and both AMA and MAMC (Spearman's $\rho = 0.852$; $p < 0.001$ for both). Chi-square tests showed no significant associations between HGS and other anthropometric parameters.

CONCLUSION: Nutritional status based on AMA, MAMC, and MUAC reflected the results from HGS classification according to Marrodán, indicating HGS as a good parameter for assessing muscle function and capacity. Moreover, HGS was more related to functional performance than to muscle mass, since adequate AMA, MAMC, and MUAC did not necessarily correspond to adequate HGS, as these parameters are associated with BMI and mainly measure muscle tissue, without expressing the muscle performance.

Keywords: Hematologic neoplasm; Pediatrics; Muscle Function.

PAPER CODE: 92

Functional profile of gynecological patients undergoing chemotherapy

MARCHON, R.M.; MAGALHÃES, A.C.D.; MORAIS, F.B.B.; MARCONI, E.M.; DE AGUIAR, S.S.; DE MELO, A.C.; BERGMANN, A.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Chemotherapy-induced peripheral neuropathy (CIPN) is a common toxicity that impairs quality of life and may limit oncological treatment, leading to dose reductions, delays, or discontinuation of chemotherapy (CT). Symptoms include pain, paresthesia, muscle weakness, and balance disturbances. Whole-body vibration exercises (WBVE) have demonstrated potential benefits as a non-pharmacological therapy for CIPN. This study investigates the effects of WBVE on the prevention of CIPN and its symptoms—paresthesia, balance, and functionality—in women with gynecological cancer.

OBJECTIVES: The main objective is to write the profile of patients at the beginning of chemotherapy.

METHOD: This RCT included patients in CT with carboplatin and paclitaxel (175 mg/m^2) in 6 cycles every 21 days. Prior to each cycle, patients underwent clinical evaluation with an oncologist. The control group (CG) received basic guidance for home exercises. The intervention group (IG), in addition to these instructions, participated in WBVE sessions during all hospital visits (12 sessions). They were seated with feet on the vibration platform (frequency 10–35 Hz, amplitude 2 mm, 10 minutes per session).

RESULTS: Primary Outcome: Assessed using the EORTC QLQ-CIPN20 questionnaire, validated for Brazilian populations. Additional assessments included the Neuropathy Impairment Score (NIS), superficial sensitivity test (Ipswich Touch Test), overall functionality and strength (dynamometry), Five Times Sit-to-Stand Test (FTSST), and balance (One-Leg Stand, OLS). Sociodemographic variables were collected as secondary outcomes. Partial Results: Eighty-four participants were recruited. Most were over 60 years old (58%), 44% identified as mixed race, 63% were unmarried, and 46% had less than eight years of education. Clinically, 62% were hypertensive and 43% were obese.

CONCLUSION: Continuation of the study may provide a deeper understanding of the health profile of these women and reinforce the importance of physiotherapy during chemotherapy, acting preventively and supporting the development of interventions such as WBVE aimed at reducing adverse effects.

Keywords: Chemotherapy-induced peripheral neuropathy; physical therapy; vibration.

PAPER CODE: 100

FUNCTIONAL PROFILE OF WOMEN INDICATED TO NEO-ADJUVANT CHEMOTHERAPY FOR BREAST CANCER TREATMENT AND INCLUDED IN A PREHABILITATION PROGRAM

SARAIVA, S.A.; SANT'ANNA JUNIOR, M.; TORRES, D.M.; DE AGUIAR, S.S.; COSTA, R.M.; FABRO, E.A.N.; BERGMANN, A.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Breast cancer (BC) has the highest incidence and mortality rates among women. BC patients face an increased risk of cardiovascular diseases due to age, chronic conditions, comorbidities, and oncologic treatment. Beyond the direct cardiotoxicity of chemotherapy, evidence suggest its negative impact on vascular endothelium and the muscular system. The decline in cardiorespiratory fitness observed during and after BC treatment is mainly attributed to insufficient physical activity levels, loss of muscle mass, and cardiotoxicity. Exercises since breast cancer diagnosis, known as prehabilitation, can minimize muscular deconditioning and the loss of exercise capacity, thereby preventing adverse side effects.

OBJECTIVES: To describe the functional profile of women indicated for neoadjuvant chemotherapy (NACT) for breast cancer who were enrolled in a prehabilitation program.

METHOD: Clinical trial that included women with breast cancer and indication for NACT, randomized into an intervention group (prehabilitation) or a control group (usual care). The functional profile was assessed by changes in the 6-Minute Step Test (6MST), handgrip strength (HGS), calf circumference (CC), and the Timed Up and Go test (TUG). The level of physical activity (PA) was measured using the International Physical Activity Questionnaire.

RESULTS: A total of 161 women were included (82 in the intervention group and 79 in the control group), with a mean age of 51.96 (± 12.14) years. Most participants self-identified as black or brown (70.2%), had advanced clinical staging (90.1%), and were classified as obese

by body mass index (55.9%). The 6MST showed a median of ± 112.5 repetitions, with 60.5% of patients demonstrating reduced cardiorespiratory capacity. Muscle assessment revealed a mean HGS of ± 27.1 kgf, with 87.5% of women classified as having low strength, and a median CC of ± 33.5 cm, with 47.8% showing muscle deficit. The median TUG was ± 9.38 seconds, with all participants demonstrating satisfactory physical performance. Overall, 33.5% of women had low PA levels, 36.6% moderate, and 29.8% high. No statistically significant differences were observed between groups.

CONCLUSION: Most women indicated for NACT were obese and had impaired functional status, demonstrated by reduced cardiorespiratory fitness and muscle strength, along with low-to-moderate physical activity levels prior to initiating oncologic. Therefore, implementing a prehabilitation program for this patient profile may help minimize the undesirable effects of systemic therapy on the functional status of women with breast cancer.

Keywords: Breast neoplasm; cardiorespiratory fitness; preoperative exercise.

PAPER CODE: 114

Developing a National Platform to Map the Genomic Landscape of Cancer in Brazil

CONTEVILLE, L.C.1; DE OLIVEIRA, G.A.1; PAREDES, M.E.1; PAIVA, V.B.1; ALTOE, M.2; OQUENDO, M.B.3; LEITE, M.S.1; DUARTE, J.L.4; SCHERER, N.D.M.1; PINTO, L.F.R.1; VIOLA, J.P.B.1; BORONI, M.1. 1. INSTITUTO NACIONAL DE CÂNCER (INCA), RIO DE JANEIRO - RJ - BRASIL; 2. BRISTOL MYERS SQUIBB (BMS), NEW YORK - ESTADOS UNIDOS DA AMERICA; 3. UNIVERSITY OF TÜBINGEN, TÜBINGEN - ALEMANHA; 4. HITSS BRASIL, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: The underrepresentation of Brazilian genomic diversity in public databases limits our understanding of how genetic variation shapes cancer in Brazil's highly admixed population. This distinctive genetic landscape underscores the need for national

initiatives that reflect the country's heterogeneity. The Atlas of Tumoral Profiles – Brazil (ATPBR) is a nationwide effort designed to establish a FAIR-compliant (Findable, Accessible, Interoperable, Reusable) data infrastructure for storing, integrating, and analyzing multiomics and clinical data from Brazilian tumor samples. By ensuring data quality, reproducibility, and robust governance, ATPBR paves the way for equitable precision oncology and the global inclusion of Brazilian genomic diversity in cancer research.

OBJECTIVES: To develop and implement a secure and interoperable platform for submitting, storing, and sharing multiomics data from cancers in the Brazilian population, aimed at mapping their molecular signatures.

METHOD: The platform's workflow begins with data ingestion, which involves registering comprehensive project, patient, and sample metadata alongside high-quality sequencing files. These data will be organized and stored in standardized directories under strict integrity and security protocols. Then, bioinformatic analyses to process the sequencing data will be performed using NF-core pipelines. Finally, the non-sensitive results will be publicly accessible via an interactive visualization portal.

RESULTS: To develop a robust infrastructure for the ATPBR platform, we have advanced through separate action plans to achieve independent project milestones. Completed steps include defining metadata variables for comprehensive data ingestion, designing barcodes to enable sample and data interoperability between the National Tumor Biobank and ATPBR datasets, and developing a prototype that integrates all stages of the submission workflow. Bioinformatics pipelines have been extensively tested for reliability and reproducibility of the analyses. Automated scripts are being optimized to streamline integration among data storage, analysis, access, and visualization. Preliminary results from multiomics analyses of cancer datasets have already been submitted to the interactive portal, which is currently being customized. The platform is being designed for scalability while maintaining strict security and data governance standards.

CONCLUSION: The ATPBR platform represents a strategic advancement in Brazilian oncology research, providing secure, standardized, and reusable multiomics datasets that capture the country's genomic diversity. By enabling data-driven discoveries, ATPBR strengthens translational oncology, promotes equitable access to precision medicine, and positions Brazil as a key contributor to global cancer genomics.

Keywords: Cancer Genomics; Multi-Omics Integration; Brazilian Population Diversity.

PAPER CODE: 120

Actinic Cheilitis and Lip Cancer at the Brazilian National Cancer Institute: Histopathological Review and Underreporting Warning

FURTADO, L.D.S.; CURVELO, J.A.D.R.; DA SILVA, B.N.M.; DE MELO, A.C.; GOLDEMBERG, D.C.. BRAZILIAN NATIONAL CANCER INSTITUTE, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Actinic cheilitis (AC) is a precancerous disorder that most commonly affects the lower lip. It is caused by chronic exposure to sunlight and is frequently overlooked, which can lead to late diagnosis and increase the risk of progression to invasive squamous cell carcinoma with the risk of developing metastases.

OBJECTIVES: This study aimed to perform histopathological evaluation of squamous cell carcinoma (SCC) cases on the lip in order to investigate the concomitance of AC.

METHOD: This retrospective descriptive study analyzed SCC cases of the lip diagnosed between 2000 and 2020. Recorded data were systematically collected, and a detailed histopathological analysis was performed on slides preserved in Pathology Division of INCA (Brazilian National Cancer Institute). The Criteria included epithelial dyskeratosis, hyperplasia, acanthosis or atrophy, dysplasia, inflammatory infiltrate, and solar elastosis. Findings were tabulated in Excel and analyzed using SPSS software.

RESULTS: A total of 682 cases of SCC of the lip were recorded between 2000 and 2020. Information in the medical records showed that only 7.9% of lip SCC cases were associated with actinic cheilitis. However, preliminary results from histopathological reviews revealed that approximately 61% of the slides were compatible with actinic cheilitis. To the current date, it has not been possible to correlate all the slides evaluated with demographic and clinical data.

CONCLUSION: The present study revealed that there is underreporting of cases of AC associated with SCC of the lip. The data presented reinforce the importance of histopathological evaluation in cases of AC. Low prevalence of AC in SCC of the lip might also be explained by late diagnosis with a higher proportion of tumors, sometimes with no connective tissue to be evaluated.

Keywords: Actinic cheilitis; Squamous cell carcinoma; Lip cancer.

PAPER CODE: 121

Childhood Thyroid Cancer Survivors - Impacts on Voice and Swallowing

DE ALMEIDA, L.N.D.S.P.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Pediatric thyroid cancer is the leading cause of pediatric endocrine cancer, accounting for more than 6% of all cancers in the pediatric population from 2012 to 2016. It is one of the most common secondary malignancies among childhood cancer survivors. Treatment for thyroid cancer in this population is usually surgical resection, total thyroidectomy, and modified neck dissection to remove regional lymph nodes. Recurrent laryngeal nerve injury is a very common sequela, but it can be devastating for children and can cause voice changes, aspiration, recurrent pneumonia, and dyspnea. This type of cancer often causes several speech-language pathological changes, requiring long-term evaluation.

OBJECTIVES: To evaluate the survival of patients with childhood thyroid cancer and its impact on aspects of

voice, swallowing and quality of life among survivors after cancer treatment.

METHOD: Longitudinal study approved by the INCA Research Ethics Committee 26331314.2.0000.5274. Inclusion criteria: patients over 18 years of age at the time of evaluation for this study, who had been treated for a thyroid tumor in childhood, and who were being followed at INCA, with no stage restriction. Sociodemographic and clinical data were collected from medical records. Assessments will include laryngoscopy, voice analysis, videofluoroscopy of swallowing, and quality of life protocols.

RESULTS: A total of 161 medical records were analyzed. ThyPRO-39 quality of life instrument showed that only 14.28% of patients had significant changes across all domains, except goiter. The most affected aspects were fatigue (57.14%), anxiety (57.14%), depression (42.86%), and emotional well-being (42.86%). In the SF-36, 28.56% showed impairments in social, emotional, and mental health domains. According to the Ferrans and Powers Quality of Life Index, no patients demonstrated poor quality of life. Moderate quality was reported in the following domains: health/functioning (42.86%), socioeconomic and family (28.57%), psychological/spiritual (57.12%). In the swallowing assessments, no changes were identified in the EAT-10, while in the MDADI, 28.57% reported difficulties regarding ingestion. . Vocal evaluations using the QVV and VHI showed that 28.57% had a mild vocal handicap, and 71.43% had normal voice quality. Laryngoscopic examination revealed vocal fold paralysis in 28.57%.

CONCLUSION: The findings highlight the need to monitor swallowing and voice changes and to assess the condition of this population over a long period of time. Objective examinations will start in the upcoming months until the entire population is complete. After the study, we hope to have a comprehensive assessment of all survivors of childhood thyroidectomy, aiming to improve their quality of life and the speech-language rehabilitation process.

Keywords: Childhood cancer; thyroid; survivors.

PAPER CODE: 129**Comparative study of quantifiable lesions and clinical impact of [18F] AIF-NOTA-Octreotide and [111In] In-DTPA-Pentetreotide in patients diagnosed with neuroendocrine tumors of grade 1 and 2.**

SOARES, F.D.S.; BULZICO, D.A.; PUJATTI, P.B.. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Neuroendocrine tumors (NETs) are well differentiated, slow-growing and overexpress the somatostatin receptor (SSTR). The SSTR can be used as a target in Nuclear Medicine diagnostic and therapy applications. NETs can have their SSTR expression assessed by radiolabelled somatostatin analogs; if overexpression is confirmed, the patient is eligible for peptide receptor radionuclide therapy. [In-111] In-DTPA-pentetreotide is still used in Brazil to perform SSTR status assessment despite its high cost and limited image quality. [F-18] AIF-NOTA-Octreotide is a promising radiopharmaceutical developed to improve the quality of SSTR imaging for a similar price. There are no studies comparing the diagnostic performance of these radiopharmaceuticals, hence the importance of this trial.

Objectives: To compare the diagnostic performance of [F-18] AIF-NOTA-Octreotide and [In-111] In-DTPA-pentetreotide, to validate [F-18] AIF-NOTA-Octreotide as an alternative diagnostic tool for SSTR imaging.

METHOD: This is a cross-sectional, prospective, blinded, controlled clinical study. Patients diagnosed with NETs referred to the Nuclear Medicine Service (NMS) for SSTR status assessment with [In-111] In-DTPA-Pentetreotide were invited to also undergo imaging with [F-18] AIF-NOTA-Octreotide. The two images were independently analyzed by two different nuclear medicine physicians blinded to the results. The physicians assessed number of lesions, Krenning score and clinical conduct derived from the image.

RESULTS: In a total of 517 organs/locations scanned, [F-18] AIF-NOTA-Octreotide detected more lesions in 62 and fewer lesions in 19 of them, in comparison to [In-111] In-DTPA-Pentetreotide. No statistical significance

was found between the number of lesions detected by the two radiopharmaceuticals in the pancreas and in the stomach. Liver, lungs, bones and lymph nodes showed a statistically significant increase in lesion detection using the F-18 labelled somatostatin analog. In the In-111 labelled analog images, 469 locations received Krenning scores of 0, 1 or 2, and 48 scores of 3 or 4; meanwhile the F-18 labelled analog images were scored 0, 1 or 2 in 439 locations and 3 or above in 78 of the organs/locations scanned. There was a statistically significant difference when considering the overall Krenning scores for the two images. The liver, lymph nodes and bone showed a statistically significant difference between the Krenning scores assigned to each image.

CONCLUSION: The [F-18] AIF-NOTA-Octreotide image resulted in higher lesion detection rate and higher lesion uptake (measured by Krenning score), especially for lesions found in liver, lymph nodes and bone. These preliminary results provide evidence for the suitability of [F-18] AIF-NOTA-Octreotide as a new radiopharmaceutical for SSTR-targeted imaging. This work also lays the foundation for further studies into the application of this new radiopharmaceutical as a candidate for PRRT-eligibility imaging.

Keywords: Somatostatin receptor expression assessment; Neuroendocrine tumors; Peptide receptor radionuclide therapy.

PAPER CODE: 130**FUNCTIONAL OUTCOMES AFTER ORGAN PRESERVATION TREATMENT FOR LARYNGEAL CANCER**

DOS SANTOS, N.P.B.; DE FREITAS, A.S.; DIAS, F.L.. INSTITUTO NACIONAL DE CÂNCER (INCA), RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Laryngeal carcinoma is one of the most prevalent neoplasms in the head and neck region, significantly impacting vital functions, including breathing, phonation, and swallowing. Radiotherapy represents a conservative treatment aimed at preserving the organ. However, post-treatment laryngeal function is often not fully recovered, leading to significant

sequelae. Dysphagia, one of the most severe and debilitating complications, can emerge as a late effect, substantially reducing the patient's quality of life. Such patients may develop malnutrition, require enteral nutrition, an increased risk of pulmonary aspiration and pneumonia, in addition to negative impacts on socialization and psychological well-being.

OBJECTIVES: To evaluate the survival and functional outcomes in patients undergoing radiochemotherapy for laryngeal carcinomas and identify patterns to more effective therapeutic interventions.

METHOD: A longitudinal study was conducted, approved by the Research Ethics Committee of the National Cancer Institute (approval no. 26331314.2.0000.5274). The study included patients over 18 years of age treated with radiotherapy, alone or in combination with chemotherapy, between 2015 and 2019, and without evidence of active disease. Swallowing functionality will be assessed by MDADI protocol and videofluoroscopy, allowing detailed analysis of oropharyngeal mechanics.

RESULTS: Of the 565 patients initially selected, only 153 were eligible to the study. Of the 153 patients collected, 123 deaths occurred, with 25 surviving patients. The average age of the patients in this study was 64 years, a predominance of males (80%), and a large proportion of smokers (92%) and alcoholics (80%). The prevalence of dysphagia among those patients was 63%, with 53% requiring temporary enteral nutrition and 19% progressing to permanent gastrostomy. Furthermore, 69% of patients underwent tracheostomy and 51% had significant dysphonia. Of the survivors, 61,5% of patients have dysphonia and 38,5% dysphagia.

CONCLUSION: Late functional analysis by videofluoroscopy should elucidate the patterns of oropharyngeal swallowing impairment in patients treated with conservative regimens. The findings of this study are expected to guide the development of evidence-based therapeutic protocols focused on swallowing rehabilitation and improving quality of life.

Keywords: Radiotherapy; Rehabilitation; Head and Neck Cancer.

PAPER CODE: 134

Sociodemographic and Clinical Profile of Patients Post-OPHL IIa at INCA: Foundation for Randomized Clinical Trial

BARBOSA, R.D.C.M.1; DE FREITAS, A.S.2; CERQUEIRA, R.B.A.1; MANCOPES, R.3; DIAS, F.L.1; STEELE, C.M.3. 1. INSTITUTO NACIONAL DE CANCER, RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL; 3. KITE RESEARCH INSTITUTE, ONTARIO - CANADA.

INTRODUCTION: Open Partial Horizontal Laryngectomy Type IIa (OPHL IIa), is a well-established surgical modality for laryngeal cancer treatment, aiming for functional preservation. However, postoperative dysphagia is a common sequela, compromising swallowing safety and efficiency. The supraglottic maneuver has shown potential in modulating swallowing physiology, yet its effectiveness in post-OPHL IIa patients remains understudied. National Cancer Institute (INCA) holds one of the largest global caseloads of patients undergoing this procedure, positioning it as a strategic center for evaluating rehabilitative interventions. This preliminary study aims to provide a solid foundation for an ongoing randomized clinical trial.

OBJECTIVES: Characterize post-OPHL IIa patients at INCA to provide baseline data for an RCT evaluating the supraglottic maneuver's effects on swallowing using the ASPEKT method.

METHOD: Retrospective and cross-sectional data were collected from 62 INCA medical records. Inclusion: adults (>18 years) without active cancer who underwent OPHL IIa (any stage). Exclusions: prior or post other head and neck surgeries or diagnosed neuropathy. Sociodemographic and clinical variables were extracted and descriptively analyzed.

RESULTS: In the full cohort of 62 patients post-OPHL IIa, demographics showed 91.9% (n=57) males with mean age 72 years (SD $\pm 10,7$, range 41-92); 43,5% (n=27) were white race; 58% (n=36) were married, and 19.3% (n=12) had incomplete primary education. Lifestyle factors included 74,1% (n=46) ex-smokers (mean 20 pack-

years) and 69,3% (n=43) alcohol consumers. Clinically, both arytenoids were preserved in 74,1% (n=45), Tumor staging was T1a in 23% (n=14), T2 in 32% (n=20), T3 in 37% (n=23), and T4 in 8% (n=5); all N0M0; 26% (n=16) received postoperative RT. Comorbidities: hypertension 19% (n=12), diabetes mellitus (DM) 6% (n=4), chronic obstructive pulmonary disease (DPOC) 42% (n=26). Functional outcomes: 98% (n=61) oral feeding, NG tube removed in 68% (n=42, mean 38 days), tracheostomy removed in 66% (n=41, mean 37 days); vocal complaints post-surgery in 35% (n=22), primarily hoarseness (29%, n=18). The analysis reveals higher comorbidity (COPD) rates in ex-smokers to guide trial design.

CONCLUSION: The characterization reveals a predominantly male, elderly profile, with a history of smoking and alcohol consumption. The high rate of arytenoid preservation and clear margins indicates the adequacy of the surgical technique. These data are crucial for contextualizing the ongoing randomized clinical trial, which will objectively evaluate the effects of the supraglottic maneuver on swallowing via the ASPEKT method, contributing to improving rehabilitation strategies for this specific population.

Keywords: Head and Neck Cancer; Dysphagia; Laryngectomy.

PAPER CODE: 135

ROBOTIC COLORECTAL SURGERY AT INCA: ANALYSIS OF PATIENTS WITH RECTAL ADENOCARCINOMA UNDERGOING NEOADJUVANCE SURGERY USING THE DAVINCI PLATFORM

NALIM, I.S.; DA SILVA, B.C.. INSTITUTO NACIONAL DE
CÂNCER, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Recent studies indicate that robotic surgery and neoadjuvant treatment can significantly improve clinical and surgical outcomes in patients with rectal cancer. Neoadjuvant treatment, performed before surgery, can reduce tumor size and increase the chances of successful intervention. Since 2012, more than 1,500

surgeries with the da Vinci system have been performed at INCA, resulting in better postoperative outcomes and quality of life.

OBJECTIVES: This study analyzes outcomes of non-metastatic rectal adenocarcinoma patients who had robotic surgery post-neoadjuvant therapy (2012–2023), correlating treatments, pathology, survival, and demographic.

METHOD: Conduct a retrospective study to analyze the medical records of patients with rectum adenocarcinoma who underwent neoadjuvant therapy and robotic surgery at INCA between 2012 and 2023, using data from a retrospective study approved by INCA's CEP (Registered Program for Clinical Research) 3186239. The patient list was obtained from INCA's DIPAT and compiled in RedCap. We will use Kaplan Meier curves and other statistical methods to present the analyzed results.

RESULTS: Data from the medical records of 600 eligible patients are being analyzed, with 100% of the demographic data and 80% of the clinical and surgical data collected. The results will be presented as soon as available

CONCLUSION: This study evaluates outcomes of robotic surgery for non-metastatic rectal adenocarcinoma after neoadjuvant therapy at INCA. Prior research shows fewer complications, faster recovery, and better quality of life with DaVinci. It also analyzes demographic and clinical correlations to understand factors influencing surgical results across diverse patient profiles treated by INCA.

Keywords: .

PAPER CODE: 147

PRODUCTION OF AMPLICON VIRAL VECTORS FOR GENE THERAPY: A NOVEL PURIFICATION SYSTEM BASED SITE-SPECIFIC RECOMBINATION AND MAGNETIC BEAD SEPARATION

PELLOSO, A.R.A.; DA COSTA, N.M.; MELENDEZ, M.E.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Amplicon vectors, derived from the human herpes simplex virus type 1, are extremely versatile and easy to produce. These viral vectors are used in various areas of molecular biology, such as gene transfer, monoclonal antibody synthesis, neuromotor disease studies, and vaccine design. However, these vectors currently have characteristics that limit their applicability in gene therapy protocols. Among these undesirable characteristics, we can highlight (i) contamination by “helper” viral particles and (ii) the presence of DNA of bacterial origin in its genome (originating from the plasmid amplicon), which can trigger immune responses and promote transgene silencing.

OBJECTIVES: The objective of this project is to develop a novel amplicon vector production system based on site-specific recombination, which allows purifying/separating amplicon vectors with magnetic beads.

METHOD: This proposed system can be considered as a floxed amplicon module (packaging sequences, viral replication origin, transgene and reporter gene), devoid of the bacterial origin sequences commonly present in amplicon plasmids, and cloned into the locus of one of the glycoproteins of the recombinant HSV-1 genome. The amplicons were produced in a transcomplementary cell line for essential viral genes, transiently transfected with the plasmid pBS.185, which expresses the Cre recombinase.

Results: This strategy resulted in an amplicon vector titer of 1.37×10^4 TU/mL. However, contamination with helper viral particles was detected, with a titer of 2.27×10^5 PFU/mL.

CONCLUSION: The amplicon vector was produced in a transcomplementary cell line that was transiently transfected. As a strategy to reduce contamination by helper viral particles, cell lines with stable expression of the Cre recombinase protein will be developed using a retroviral vector. The derived vectors are expected to have a gene insertion capacity of approximately 15 kb.

Keywords: Viral Vector; Gene Therapy; HSV-1 Amplicon.

PAPER CODE: 164

Accuracy of anal cytology and HPV genotyping in people living with HIV: comparison of liquid-based and conventional methods and implications for screening strategies

DIOGO, I.C.R.; BRUNO, M.D.M.; DE MELO, I.A.; EMERICK, C.; SILVA, J.A.D.D.C.E.; VALADÃO, M.; SIQUEIRA, J.D.; LEAL, F.E.; DE OLIVEIRA, É.M.; SOARES, M.A.; GOES, L.R.; ARAÚJO, R.O.D.C.. INSTITUTO NACIONAL DO CANCER, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Anal cancer represents 2% of gastrointestinal tract neoplasms, with increasing incidence, particularly in people living with HIV (PLWHIV), in whom persistent high-risk HPV infection substantially increases the risk of high-grade squamous intraepithelial lesions (HSIL). Anal cytology is an accessible screening method, but its performance varies depending on the diagnostic criteria, collection technique, and population profile.

OBJECTIVES: This study compared liquid-based and conventional anal cytology at $\geq$ ASCUS and $\geq$ ASC-H thresholds in PLWHIV cohorts (trans/MSM and WLWHIV), using HRA with biopsy as the HSIL gold standard.

METHOD: A total of 162 participants (32 trans/MSM and 130 WLWHIV) underwent anal cytology, HPV genotyping, HRA, and directed biopsies. High-risk HPV genotyping was performed using the multiplex HPV Flow Chip/XGEN Mobius kit. Statistical analyses were conducted with appropriate tests, with significance set at $p < 0.05$.

RESULTS: The overall prevalence of HSIL was 36.4% (43.8% in trans/MSM and 34.6% in WLWHIV). Using $\geq$ ASCUS, sensitivity was 66.7% in trans/MSM and 28.9% in WLWHIV. The $\geq$ ASC-H threshold increased specificity (80–82%) but reduced sensitivity, particularly in trans/MSM (8.3%). Among participants over 45 years, the sensitivity of liquid-based cytology with $\geq$ ASCUS nearly doubled (83.3% vs. 40.0%). HPV genotyping significantly enhanced detection, with sensitivity reaching 85.7% in trans/MSM and 44.4% in WLWHIV, representing a 150% relative increase in the latter. Overall, 71.9% of trans/MSM and 37.7% of WLWHIV had at least one high-risk HPV subtype detected. Multiple HPV co-infections were more frequent in trans/MSM (mean 7.3 subtypes) than in WLWHIV (3.9), with HPV 16, 18, 45, 52, and 68 predominating. Considering current vaccines, only 11%–13% of HSIL cases would be covered by the quadrivalent vaccine and 22%–26% by the nonavalent vaccine.

CONCLUSION: Liquid-based cytology combined with HPV genotyping demonstrated superior performance compared to conventional smears. Screening strategies should be adapted to cohort characteristics: sensitivity prioritized for trans/MSM, particularly older individuals, and specificity emphasized for WLWHIV. The high prevalence of high-risk HPV subtypes not covered by the quadrivalent vaccine underscores the need for broader prevention strategies in PLWHIV populations

Keywords: Anal Cancer; people living with HIV (PLWHIV); HPV.

PAPER CODE: 166

Applications of Artificial Intelligence in Voice Analysis for Early Detection of Laryngeal Cancer: A Scoping Review

SANTIAGO, D.R.; DIAS, F.L.; DE FREITAS, A.S.. INSTITUTO NACIONAL DE CÂNCER - INCA, RIO DE JANEIRO - RJ - BRASIL.

Introduction: Laryngeal cancer represents about 25% of malignant head and neck tumors, mainly affecting men with a history of smoking and excessive alcohol use. In Brazil, 4,478 deaths were reported in 2020,

and INCA estimates 7,790 new cases between 2023 and 2025. Persistent vocal alterations often represent the first clinical manifestation but are frequently underestimated, leading to delayed diagnosis and functional impairment. This is further exacerbated by disparities in access to specialized examinations, especially in remote regions. Innovative, accessible, and non-invasive screening strategies are essential. The application of Artificial Intelligence (AI) to voice analysis has emerged as a promising tool for identifying subtle acoustic patterns associated with laryngeal cancer.

OBJECTIVES: To map scientific evidence on Artificial Intelligence applied to voice analysis for early detection of laryngeal cancer, identify literature gaps, and guide future research.

METHOD: A scoping review was conducted using the PCC framework to map the use of Artificial Intelligence in voice analysis for early detection of laryngeal cancer. Searches were carried out in PubMed, Scopus, Web of Science, ScienceDirect, Journal of Voice, and SciELO, including studies published from 2010 to 2025. Descriptors covered laryngeal cancer, voice, artificial intelligence, and early detection. Clinical or experimental studies directly applying AI to human voice analysis were included.

RESULTS: The review identified a limited number of studies applying Artificial Intelligence to voice analysis for the early detection of laryngeal cancer. Most were conducted in Asian and European countries and exhibited methodological variability. The most frequently employed algorithms included convolutional neural networks, support vector machines, and hybrid deep learning models. Reported accuracy ranged from 75% to 90%, with higher sensitivity and specificity observed when combining vocal and clinical data. The review also revealed a scarcity of national studies and a lack of standardization in voice recording and analysis protocols.

CONCLUSION: The application of Artificial Intelligence to voice analysis appears promising for screening and early detection of laryngeal cancer, particularly when integrated with clinical variables. However, methodological inconsistencies and the lack of

national studies persist, underscoring the need for further research to refine and validate these methods, consolidating AI as a complementary tool in laryngeal cancer screening.

Keywords: Voice; Laryngeal Cancer; Artificial Intelligence.

PAPER CODE: 174

Therapeutic Pathway of Patients with Laryngeal Cancer

DOS SANTOS, T.E.. INSTITUTO NACIONAL DO CÂNCER, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Laryngeal cancer is part of the spectrum of head and neck tumors, associated with smoking and alcohol consumption, with a high incidence among men in Brazil. In the public system, access is regulated through platforms such as SISREG and SER, but operational limitations affect patient flow. National studies report regional heterogeneity and frequent delays between diagnosis and treatment for oral and oropharyngeal cancers, with many patients waiting more than 60 days. Despite Law No. 12,732/2012, which mandates treatment initiation within 60 days, delays are associated with worse outcomes. Identify the regulatory and care network steps that contribute to delays in the therapeutic pathway of patients with laryngeal cancer.

OBJECTIVES: To analyze the therapeutic pathway of laryngeal cancer patients at INCA, identifying key barriers in care levels and regulation that affect timely access to specialized treatment.

METHOD: A prospective, descriptive, qualitative study was conducted at INCA's Head and Neck Cancer Surgery Section (protocol 26331314.2.0000.5274). Patients ≥ 18 years with laryngeal cancer, treated between Feb/2022–Jul/2024, were included based on clinical eligibility and voluntary informed consent, including palliative cases. A structured questionnaire collected sociodemographic data, care pathway times, initial symptoms, and access barriers

RESULTS: A total of 110 patients were included during the study period, mostly male ($n = 92$; 83.6%), with a mean age of 64.4 years. Sixty patients responded to the questionnaire and were divided into two groups: those who returned and those who did not return to work after treatment. Low educational attainment was observed in both groups (61.7%). The time to seek medical care and the waiting time for the first appointment at a public health unit tended to correlate with tumor size ($p = 0.020$), as did the difficulty in reaching a reference center ($p = 0.006$). Dysphonia was the most prevalent initial symptom, reported by 80% of participants. Only a small proportion of patients were able to resume their professional activities after treatment ($n = 16$; 14.5%). The median time between diagnosis and treatment initiation was 62 days, while the mean time between diagnosis and completion of the therapeutic cycle was 356 days.

CONCLUSION: The analysis revealed structural deficiencies in primary care and regulation, causing significant delays in referral and treatment initiation. These delays compromise outcomes, affect quality of life, and hinder work reintegration. Findings highlight the urgent need to strengthen oncology care networks with effective prevention and integrated, agile regulation to ensure timely access and better clinical and functional results

Keywords: Laryngeal Cancer; Therapeutic Pathway; regulation system.

PAPER CODE: 184

Large-scale Manufacturing of CD19 CAR-T cells using a minicircle-based non-viral DNA vector carrying a Sleeping Beauty transposon platform

DOURADO, L.P.S.1; SILVA, L.R.B.2; DOS REIS, S.A.1; ABDO, L.1; ARAGÃO, E.1; BONAMINO, M.H.3. 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL; 3. INCA/FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Immunotherapy with CD19-directed chimeric antigen receptor T cells (CAR-T) has shown high response rates in B-cell malignancies. However, current CAR-T manufacturing relies on retroviral or lentiviral vectors, which are costly and complex, limiting its broader use. To overcome these barriers, we employed a non-viral minicircle (MC) plasmid system based on the Sleeping Beauty (SB) transposon to generate anti-CD19 CAR-T cells. MCs are derived from parental plasmids processed in bacteria expressing inducible recombinase, resulting in vectors containing only the therapeutic transgene under a specific promoter, free of prokaryotic sequences—enhancing safety and efficiency.

OBJECTIVES: The aim of this work was to produce anti-CD 19 CAR-T cells on a large-scale using a non-viral minicircle (MC) plasmid system based on the Sleeping Beauty (SB) transposon platform.

METHOD: Peripheral blood mononuclear cells (PBMCs) from healthy donors were isolated and enriched for T cells via CD3+ magnetic bead selection. Cells were activated for 48h and nucleofected using the 4D-Nucleofector System (Lonza), LV Unit, with the MC PT4 19BBz vector and SB100x transposase. Cultures were expanded using the G-Rex system, Wilson Wolf's gas-permeable membrane technology, in the presence of the cytokine IL-2.

RESULTS: In preliminary small-scale results, the system showed promising results; thus, we now present the efficacy and safety of the system also on a large scale. The results showed high viability (>90%) and CAR expression (>30%) after 6 days. From 5×10^7 T cells, we obtained over 1×10^8 CAR-T cells. In vivo studies in NSG mice bearing Nalm-6 leukemia demonstrated dose-dependent antitumor activity. Mice treated with 5×10^6 CAR-T cells showed noticeably improved survival compared to those that received 1×10^6 cells, and significantly greater survival compared to untreated controls ($p=0.01$).

CONCLUSION: In conclusion, this virus-free platform enabled efficient large-scale CAR-T cell production with robust antitumor activity, offering a scalable and cost-effective alternative to viral vector-based methods.

Keywords: CAR-T; Large-scale; Minicircle.

EPIDEMIOLOGICAL RESEARCH (ER)

PAPER CODE: 10

MISSING STAGING IN ESOPHAGEAL, GASTRIC, AND COLORECTAL CANCERS IN BRAZIL FROM 2010 TO 2019: ANALYSIS OF DATA FROM HOSPITAL-BASED CANCER REGISTRIES

COSTA, G.A.B.; CANCELA, M.D.C.. INSTITUTO NACIONAL DE CÂNCER - INCA (PPGO), RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Esophageal, gastric, and colorectal cancers have high lethality in Brazil, often diagnosed at advanced stages. Clinical staging is essential for therapeutic planning, prognosis assessment, and to evaluate health services access. In the context of Hospital-Based Cancer Registries (RHC), this variable is crucial to evaluate the quality of care and to support public policies. However, in Brazilian HBCR the completeness of this variable is inconsistent across Federative Units (FU).

OBJECTIVES: To analyze completeness of clinical staging in HBCR databases for esophageal, gastric, and colorectal cancers in Brazil, considering sociodemographic, clinical, and contextual factors.

METHOD: Cross-sectional study, on the outcome was unknown clinical stage at diagnosis. Data were collected from Brazilian HBCRs. Descriptive analyses were performed by FU and by macro-regions of Brazil. Pearson's chi-square, bivariate and multivariate Poisson regression with robust variance (5% significance) identified factors linked to missing staging. Cases from São Paulo (SP) were analyzed separately. Analyses used STATA/MP 16.0.

RESULTS: A total of 267,620 cases were included: 76,824 (28.7%) from SP and 190,796 (71.3%) from other states. The prevalence of unknown clinical stage was 10.2% in SP and 40.2% elsewhere. Excluding SP, gastric (PR 1.21; 95%CI 1.19-1.23) and esophageal tumors (PR 1.16; 95%CI

1.14-1.18) were more likely to present missing staging than colon cancer. Patients without prior diagnosis/treatment had higher prevalence of unknown stage (PR 1.28; 95%CI 1.25-1.31). The highest PR were observed in the North (PR 1.49; 95%CI 1.46-1.53), compared to the South. Prevalence increased with age: 70-79 years (PR 1.13; 95%CI 1.11-1.15), ≥80 years (PR 1.29; 95%CI 1.27-1.32) compared to those aged 18-49 years. Similarly in SP, older patients had highest prevalence (70-79: PR 1.12; 95%CI 1.04-1.20 / ≥80: PR 1.83; 95%CI 1.70-1.98). Patients with no/incomplete elementary education had higher prevalence compared to those with higher education (PR 1.18; 95%CI 1.08-1.30).

CONCLUSION: The completeness of clinical staging in the HBCR varied by FU, tumor type, age, and prior access to diagnosis or treatment, suggesting that regional and structural inequalities affect the completeness of clinical stage data in Brazil.

Keywords: completeness; clinical staging; hospital-based cancer registry.

PAPER CODE: 15

FACTORS ASSOCIATED WITH LOW HANDGRIP STRENGTH IN PATIENTS WITH INCURABLE CANCER IN PALLIATIVE CARE

SANTOS, L.P.1; PAIVA, B.S.R.2; ROSA, K.S.D.C.1; DE AGUIAR, G.B.1; DE ARAUJO, M.A.G.C.1; ALMEIDA, M.C.P.1; FERNANDES, R.C.1; DE OLIVEIRA, L.C.1. 1. NATIONAL CANCER INSTITUTE, RIO DE JANEIRO - RJ - BRASIL; 2. BARRETOS CANCER HOSPITAL, BARRETOS - SP - BRASIL.

INTRODUCTION: The literature lacks studies on factors associated with low handgrip strength (HGS) in patients with incurable cancer receiving palliative care (PC).

OBJECTIVES: To evaluate the factors associated with low HGS in patients with incurable cancer under PC.

METHOD: This cross-sectional study was conducted in the PC unit of the National Cancer Institute (INCA), including patients with incurable cancer. HGS was measured using a Jamar® hydraulic dynamometer. Clinical, sociodemographic, functional, and nutritional data potentially associated with HGS were collected.

Analyses, stratified by sex, included univariate and multivariate logistic regression, with odds ratios (OR) and 95% confidence intervals (95% CI) as estimators. Stata 15.1 was used.

RESULTS: A total of 424 patients were included, with a median age of 65 years, predominantly women (56.4%) and Black individuals (59%). The most prevalent tumors were gastrointestinal (24%) and head and neck (21%). Low HGS was defined as values below the 1st tertile: <12 kg for women and <20 kg for men. Higher risk of low HGS was observed among elderly women (OR 2.99; 95% CI 1.54-5.81; p<0.001) and those with reduced functional capacity - Karnofsky Performance Status (KPS <50%) (OR 6.60; 95% CI 3.57-12.19; p<0.001); and among elderly men (OR 4.68; 95% CI 1.78-12.26; p=0.002), those with reduced functional capacity (KPS <50%) (OR 6.12; 95% CI 2.99-12.52; p<0.001), and reduced calf circumference (CC <32 cm) (OR 2.73; 95% CI 1.37-5.48; p=0.004).

CONCLUSION: Age and KPS were factors associated with low HGS in both men and women with incurable cancer under palliative care. Given the predictive role of HGS, monitoring patients who are older, have lower KPS, and reduced CC may provide opportunities for preventive and individualized approaches in this highly vulnerable population.

Keywords: Incurable cancer; Palliative care; Handgrip strength.

PAPER CODE: 19

INCIDENCE TRENDS OF ORAL CAVITY AND OROPHARYNGEAL CANCERS IN BRAZIL: A DISAGGREGATED ANALYSIS OF TOPOGRAPHIES

DE ABREU, V.L.1; CANCELA, M.D.C.2. 1. UNIRIO, RIO DE JANEIRO - RJ - BRASIL; 2. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Head and neck tumors are among the most common cancers worldwide and consist of a combination of different subsites, as oral cavity (OCC) and oropharynx cancer (OPC). Tobacco and alcohol use are the main determinants for OCC; for OPC HPV infection is an additional risk factor. In Brazil, the National

Cancer Institute (INCA) estimates 15,100 new cases per year for the 2023–2025 triennium, including 10,900 in men and 4,200 in women. However, these figures aggregate tumors of the oral cavity, oropharynx, and salivary glands, which prevents site-specific analysis. Disaggregating these sites is essential to understand the real impact of these diseases in order to guide prevention and control strategies.

OBJECTIVES: To calculate and compare incidence trends of OCC and OPC in selected Brazilian Population-Based Cancer Registries (PBCR), analyzing differences by PBCR, sex, and topographic classification systems.

METHOD: This observational study used incidence data from PBCRs between 2000 and 2018. Eight PBCRs were selected after quality assessment. Different anatomical classifications (IARC, Conway, INHANCE, and SBCCP) for oral cavity and oropharynx were compared. Age-standardized incidence rates were calculated, stratified by sex, PBCR, and topography. Trend analyses were performed using the Joinpoint regression software, to obtain Annual Percent Changes (APC) and Average Annual Percent Changes (AAPC).

RESULTS: A total of 13,092 new cases were included from 2000 to 2018, distributed among the PBCRs of Manaus (7.7%), Belém (9.7%), João Pessoa (6%), Recife (12.8%), Belo Horizonte (26.5%), Curitiba (16.9%), Cuiabá (6.3%), and the Federal District (14.1%). The anatomical classification directly affected case distribution, with the proportion of OPC ranging from 25% (IARC) to 56% (INHANCE) of total cases. AAPC analysis revealed divergent incidence trends between OCC and OPC. Significant decreasing incidence trends of OCC were observed among men in Curitiba, Cuiabá, and the Federal District (AAPC ranging from -3% to -10%), regardless of classification system. On the other hand, for OPC incidence, significant increasing trends were observed in Recife (AAPC > +4%). Among women, a significant increase in OPC incidence was observed in Belo Horizonte. Across classification systems, the directions of trends were consistent, differing only in the magnitude of rates.

CONCLUSION: This study highlights differences in the distribution of incident cases from oral cavity and

oropharyngeal cancers by site, sex, and region in Brazil. The findings represent a step toward the dissociation of oral cavity and oropharyngeal topographies in official cancer statistics, in order to reflect their different risk factors and target prevention and public health policies.

Keywords: Oropharynge Cancer; Oral Cavity Cancer; Incidence trends.

PAPER CODE: 23

CLINICAL UTILITY OF A NUTRITIONAL SCREENING ALGORITHM IN PREDICTING THE DISCHARGE OF PATIENTS WITH INCURABLE CANCER IN PALLIATIVE CARE

DE ARAUJO, M.A.G.C.; DE AGUIAR, G.B.; ROSA, K.S.D.C.; SANTOS, L.P.; ALMEIDA, M.C.P.; FERNANDES, R.C.; DE OLIVEIRA, L.C.. INSTITUTO NACIONAL DE CÂNCER (INCA - UNIDADE IV), RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Nutritional screening is a simple, agile, and essential procedure for the early identification of malnourished individuals or those at risk of malnutrition, indicating the need for a more in-depth nutritional assessment. Nutritional screening algorithm for patients with incurable cancer receiving palliative care (NutriPal) showed an association with laboratory, nutritional, and predictive factors within 120 days. However, in the context of patients with a more limited prognosis, its clinical utility for predicting hospitalization outcomes remains to be clarified.

OBJECTIVES: To evaluate the clinical utility of NutriPal, in predicting the discharge of patients with incurable cancer admitted to the dedicated Palliative Care Unit (PCU).

METHOD: This study was approved by the Research Ethics Committee of National Cancer Institute (INCA) (CAAE: 66294422.9.0000.5274). It is a cohort study developed with patients assessed within 48 hours of their first admission to the PCU of INCA and followed until discharge or death. Nutritional risk was assessed using NutriPal. Analyses were performed using SPSS version 22.0, statistical significance was set at p-value >0.05.

RESULTS: 134 patients were evaluated during hospitalization, with a median age of 60 years, mostly female (n = 78; 58.2%), and the most prevalent tumor site was the gastrointestinal tract (n = 49; 36.6%). The majority had incomplete elementary education (n = 56; 41.8%), were married (n = 66; 49.3%), and had a Karnofsky Performance Status of 30% (n = 63; 47%). 79.1% (n = 106) of the patients underwent some previous antitumor treatment, with chemotherapy being the most frequent (n = 84; 62.7%). During hospitalization, 56% (n=75) of patients died; of these, 63.4% (n=85) were classified as NutriPal grades 3 or 4, indicating greater nutritional risk. However, no significant difference was observed in outcomes (discharge vs. death) across degrees of nutritional risk at admission (p-value =0.161).

Conclusion: Findings suggested that NutriPal did not predict patient discharge. Nonetheless, the limited sample size and subgroup stratification may have influenced the observed results.

Keywords: Neoplasms; Palliative care; Nutritional risk.

PAPER CODE: 25

ASSOCIATION BETWEEN THE C-REACTIVE PROTEIN-TO-ALBUMIN RATIO AND TOTAL SYMPTOM BURDEN IN PATIENTS WITH INCURABLE CANCER RECEIVING PALLIATIVE CARE

DE AGUIAR, G.B.; ROSA, K.S.D.C.; SANTOS, L.P.; DE ARAUJO, M.A.G.C.; ALMEIDA, M.C.P.; FERNANDES, R.C.; DE OLIVEIRA, L.C.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: The C-reactive protein-to-albumin ratio (CAR) is an easily obtainable inflammatory index that reflects systemic inflammation, disease severity, and cancer prognosis, and has been increasingly used in oncology. An exacerbated inflammatory process may be associated with a higher total symptom burden. However, scientific evidence on these associations in patients with incurable cancer receiving palliative care remains limited.

OBJECTIVES: To evaluate the association between CAR and the total symptom burden in patients with incurable cancer receiving palliative care.

METHOD: Cross-sectional study conducted in the Palliative Care Unit of INCA with patients with incurable cancer, from August 2023 to July 2025. Total symptom burden (dependent variable) was calculated by summing symptom intensities from Edmonton Symptom Assessment System. Serum albumin and C-reactive protein levels determined CAR (independent variable). Clinical and sociodemographic covariates were collected, and exploratory analyses were performed using SPSS 22.0.

RESULTS: A total of 741 patients were evaluated, predominantly female (n=430, 58.0%), aged ≥60 years (n=483, 65.2%), and mostly assessed in outpatient consultations (n=480, 78.3%). Regarding education, most had incomplete primary schooling (n=291, 39.3%). The most common primary tumor site was the gastrointestinal tract (n=195, 26.3%), followed by gynecologic (n=153, 20.6%) and head and neck cancers (n=115, 15.5%). The predominant Karnofsky Performance Status was 40–50% (n=340, 45.9%). The median CAR value was 1.55, and the median total symptom burden was 29.

CONCLUSION: Data collection was recently completed, and due to the need to assess consistency for database consolidation, only preliminary exploratory analyses have been conducted so far. We expect that the results of robust statistical analyses (regressions) will reveal associations between CAR and total symptom burden, suggesting that inflammatory markers may help identify patients with greater clinical vulnerability and guide symptom management strategies.

Keywords: Incurable Cancer; Symptom Burden; Inflammatory Markers.

PAPER CODE: 30**Association between Nutritional and Functional Status and Treatment-Related Adverse Events in Breast Cancer Patients Receiving Neoadjuvant Chemotherapy.**

MOTTA, R.S.T.; FERNANDES, M.C.C.; VAQUEIRO, V.D.S.; SANT'ANA, M.E.D.S.; COSTA, A.F.; DE OLIVEIRA, L.C.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Breast cancer (BC) is the most prevalent neoplasm among women in Brazil and worldwide. Neoadjuvant chemotherapy (NAC), a systemic treatment administered prior to surgical procedures, aims to reduce tumor size, allowing for more conservative surgeries. However, chemotherapy may trigger adverse events such as nausea, vomiting, diarrhea, constipation, fatigue, and mucositis, which can compromise nutritional status. Furthermore, many women are already overweight or sarcopenically obese at diagnosis, factors that worsen prognosis, complicate surgical recovery, and reduce treatment tolerance. Therefore, periodic nutritional assessment becomes essential to identify risks and provide adequate nutritional support.

OBJECTIVES: To evaluate indicators of nutritional and functional status in breast cancer patients undergoing neoadjuvant chemotherapy and their association with treatment-related adverse events.

METHOD: This is a prospective cohort study involving women with BC indicated for NAC (4 cycles of doxorubicin and cyclophosphamide, followed by 4 cycles of docetaxel, with or without trastuzumab). The initial interview is done before the first chemotherapy cycle to gather clinical, sociodemographic, nutritional, and functional data. Adverse events are checked 7 days after each cycle. Nutritional reassessments occur before the 5th cycle and up to 30 days after the 8th.

RESULTS: To date, the cohort includes 184 patients. Of these, data from 37 patients have been entered into the database and are considered in this analysis. The mean age was 50.41 years $\pm$ 11.94. Most patients presented with clinical staging IIB (40.5%) and invasive ductal carcinoma as the histological type (89.2%). At

baseline, 64.9% of patients presented with excess body weight. The most common adverse events reported were fatigue (85,9%), pain (76,6%), frequent urination (75,7%), nausea (75,2%), and xerostomia (71,6%) during the four initial cycles, whereas pain (93%), xerostomia (88,3%), dysgeusia (87,6%), abdominal pain (76,4%) and frequent urination (75,7%) predominated in the later four cycles. Among the 37 evaluated patients, 25 completed the standard chemotherapy treatment, 11 had their protocols modified, and 1 abandoned treatment. The reasons for protocol modifications included neutropenia, poor tolerance to docetaxel by asthenia and lower limb pain and the detection of metastasis.

CONCLUSION: Preliminary results reveal a high prevalence of excess body weight at the start of NAC in breast cancer patients, as well as an elevated frequency of adverse events throughout the treatment, including fatigue, pain, and gastrointestinal symptoms. Continued follow-up of this cohort will enable a more robust analysis of the relationship between nutritional status, functional capacity, and adverse events, providing a basis for more effective and individualized nutritional interventions.

Keywords: Nutritional status; adverse events; chemotherapy.

PAPER CODE: 34**Nutritional status assessment of breast cancer patients before neoadjuvant chemotherapy**

DE SANT'ANA, M.E.D.S.; MOTTA, R.S.T.; FERNANDES, M.C.C.; VAQUEIRO, V.D.S.; DE OLIVEIRA, L.C.. HOSPITAL DO CÂNCER (INCA), RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: The nutritional status of breast cancer patients is often compromised at diagnosis, impacting treatment efficacy and prognosis. Obesity, particularly sarcopenic obesity, is associated with worse outcomes, such as increased risk of infections, heightened toxicity, and reduced tolerance to chemotherapy. Adverse effects of chemotherapy, including nausea, vomiting, fatigue, and taste alterations, can further exacerbate nutritional status, impairing food intake

and metabolism, and negatively affecting recovery and quality of life. Early nutritional assessments are essential to identify at-risk patients and enable interventions that improve prognosis.

OBJECTIVES: To evaluate the nutritional status of breast cancer patients prior to neoadjuvant chemotherapy.

METHOD: This cross-sectional study is part of an ongoing prospective cohort conducted at Hospital do Câncer III since October 2024, involving women diagnosed with breast cancer and indicated for neoadjuvant chemotherapy according to institutional protocols. Baseline data from the study, collected before chemotherapy initiation, include sociodemographic information, lifestyle habits, clinical data, anthropometric assessments, and body composition measurements obtained via bioelectrical impedance analysis.

RESULTS: To date, 184 patients have been recruited, of whom 37 have had their data entered and were included in the exploratory analysis. The patients had a mean age of 50.41 years (± 11.94), with the majority presenting initial clinical staging IIB (40.5%), invasive ductal carcinoma histology (89.2%), and luminal B molecular subtype (56.8%). Anthropometric evaluation revealed a mean body mass index (BMI) of 28.1 kg/m² (± 6.8), calf circumference of 31.62 cm (± 5.74), triceps skinfold thickness of 27.72 mm (± 7.44), arm muscle circumference of 22.86 cm (± 2.42), and corrected arm muscle area of 35.72 cm² (± 9.21). BMI classification showed prevalences of 5.4% (n=2) underweight, 29.7% (n=11) eutrophic, 32.4% (n=12) overweight, and 32.4% (n=12) obesity. Bioimpedance analysis indicated a mean fat mass of 42.1% (± 7.02) and mean skeletal muscle mass of 29.77 kg (± 3.8).

CONCLUSION: The partial results presented thus far indicate that the nutritional status of breast cancer patients prior to neoadjuvant chemotherapy is compromised by excess body weight and fat mass. Personalized nutritional interventions are recommended to mitigate risks and improve clinical outcomes. The ongoing study will provide longitudinal data to validate these findings.

Keywords: Nutritional status; breast cancer; neoadjuvant chemotherapy.

PAPER CODE: 38

Interchangeability of Reference Trastuzumab and Biosimilars: Five-Year Real-World Outcomes in Early HER2-Positive Breast Cancer

FERREIRA, L.A.A.; DA SILVA, M.J.S.. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: The interchangeability between originator trastuzumab and its biosimilars in the treatment of breast cancer (BC) represents a significant challenge in clinical practice. While the use of biosimilars is a key strategy to increase access and reduce healthcare costs, uncertainties remain regarding the impact of this practice on effectiveness and safety outcomes in real-world settings. Generating evidence in this context is essential to guide clinical and regulatory decisions within the Brazilian Unified Health System (SUS).

OBJECTIVES: To evaluate the effectiveness and safety outcomes of trastuzumab interchangeability in the initial treatment (neoadjuvant and adjuvant) of women with HER2-positive BC in a real-world setting.

METHOD: Retrospective cohort of women with HER2-positive breast cancer treated with chemotherapy and trastuzumab (2018–2022) at a major oncology hospital in Brazil. Data from medical records will include sociodemographic, clinical, therapeutic, survival, and safety variables. Exposure is trastuzumab interchangeability. Effectiveness includes pCR, EFS, and OS (logistic and Cox models). Safety is based on adverse events and their severity.

RESULTS: The study is currently in the data collection and consolidation phase. It is expected to generate robust evidence on the effectiveness and safety of interchangeability, supporting clinical and regulatory decision-making.

CONCLUSION: No conclusions are available yet, as the study is still in its early development stage.

Keywords: Breast Neoplasms; Biosimilar Pharmaceuticals; Interchange of Drugs.

PAPER CODE: 57**Potential Effects of Age and Tumor Genetic Profile on Clinical Outcomes in Women Diagnosed with Triple-Negative Breast Cancer Treated with Doxorubicin, Cyclophosphamide, and Taxane Regimen**

PIRES, M.C.D.S.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Breast cancer is the most common type among women and represents a challenge to global public health. The triple-negative (TNBC) subtype stands out for its aggressiveness, poorer prognosis, limited therapeutic options, and predominance in younger women. Age is a factor that can influence treatment and clinical outcomes; older patients may face additional challenges related to access and treatment tolerance compared to younger patients. However, studies show that age-related differences should be analyzed in the context of genomic characteristics to guide therapeutic decision-making. Therefore, understanding the effects of age and genetic profile is relevant, especially given the scarcity of national data on the treatment of these patients within the Brazilian Unified Health System

OBJECTIVES: To analyze the influence of age and genetic profile on clinical outcomes in women with TNBC treated with doxorubicin, cyclophosphamide, and taxane (AC-T) with adjuvant, neoadjuvant, or curative intent

METHOD: Retrospective cohort involving 450 women with triple-negative breast cancer (2016–2020). Sociodemographic, clinical, and therapeutic data will be collected and analyzed using frequencies and measures of central tendency. Time from treatment initiation to therapeutic failure, overall survival, and progression-free survival will be assessed by Kaplan-Meier and Log-Rank methods. Germline variants will be identified through whole-exome sequencing, and classified according to pathogenicity.

RESULTS: O estudo encontra-se atualmente em fase de coleta e consolidação de dados. Espera-se que os resultados esclareçam a influência da idade nos desfechos clínicos em mulheres com câncer de mama triplo-negativo tratadas com o regime AC-T na

instituição e ajudem a reduzir a escassez de dados sobre os padrões de tratamento em mulheres idosas, visando otimizar a terapia e melhorar a qualidade de vida dessa população de pacientes.

CONCLUSION: -

Keywords: Triple Negative Breast Neoplasms; Age Groups; Genetic Profile.

PAPER CODE: 63**ASSOCIATION BETWEEN SEVERE FATIGUE AND ADHERENCE TO A HOME-BASED EXERCISE PROGRAM DURING NEOADJUVANT CHEMOTHERAPY IN WOMEN WITH BREAST CANCER**

FERREIRA, G.L.D.1; SARAIVA, S.A.1; SANT'ANNA JUNIOR, M.2; DE AGUIAR, S.S.1; COSTA, R.M.1; FABRO, É.A.N.1; BERGMANN, A.1; TORRES, D.M.1. 1. NATIONAL CANCER INSTITUTE - INCA, RIO DE JANEIRO - RJ - BRASIL; 2. FEDERAL INSTITUTE OF RIO DE JANEIRO - IFRJ, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Breast cancer (BC) and its treatment can impact women's physical, psychosocial, and emotional health, which may be associated with fatigue. The fatigue can manifest as both physical and mental symptoms. Exercise-based interventions (EX) are effective in managing this symptom during treatment, improving quality of life and perception of physical function. Despite clear evidence of exercise benefits, many survivors fail to engage at it in sufficient levels, reporting a significant decline in activity. Moreover, among patients undergoing cancer treatment, adherence to exercise may be influenced by treatment-related side effects. Studies have shown that those who consistently adhered to physical exercise programs, experienced greater reductions in cancer-related fatigue.

OBJECTIVES: To evaluate the association between severe fatigue and adherence to home-based exercises performed during neoadjuvant chemotherapy in women with BC enrolled in a surgical prehabilitation (PH) program.

METHOD: A clinical trial was conducted with women undergoing surgical treatment for breast cancer. The program included home-based EX 3 days/week, 45 min/day. High adherence was defined as $\geq 70\%$, moderate $< 70\%$ and $> 40\%$, or low $\leq 40\%$. Severe fatigue was assessed using the Functional Assessment of Chronic Illness Therapy-Fatigue, FACIT-F. The association between severe fatigue and EX adherence was analyzed using logistic regression with odds ratios (OR), 95% confidence intervals (CI), and p-values ≤ 0.05 .

RESULTS: To date, 42 patients have been evaluated. The mean age was 57 (± 10.89) years; 88.10% presented with advanced clinical staging and underwent an average of 5.57 (± 3.07) cycles of neoadjuvant chemotherapy (NAC). At the end of NAC, 33.30% (n = 14) of patients presented with severe fatigue, 42.90% (n = 18) showed low adherence to home-based EX, 35.70% (n = 15) moderate adherence, and 21.40% (n = 9) high adherence. No association was observed between severe fatigue and low adherence (OR = 2.23, 95% CI 0.36–13.96) or moderate adherence (OR = 1.75, 95% CI 0.26–11.74), when compared to those with high adherence to EX.

CONCLUSION: Based on these preliminary results, no association was found between severe fatigue and adherence to the surgical PH program during NAC.

Keywords: Fatigue; Treatment adherence and compliance; Breast neoplasms.

PAPER CODE: 66

Adequacy of Caloric and Protein Intake and Its Correlation with Quadriceps Muscle Thickness in Pediatric Cancer Patients in Intensive Care

DE OLIVEIRA, P.G.D.S.1; MELO, F.D.S.1; TRAMONTANO, C.A.1; MURAD, L.D.1; SARAIVA, D.A.2; DE OLIVEIRA, L.C.3. 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. INCA, NITEROI - RJ - BRASIL; 3. INCA, MAGÉ - RJ - BRASIL.

INTRODUCTION: Pediatric cancer patients in intensive care represent a high-risk group for caloric-protein malnutrition, which is associated with a reduction in

muscle mass during hospitalization. Ultrasonography (US) is a tool that has been increasingly used to quantify muscle segments, offering advantages such as low cost, bedside assessment capability, and absence of radiation exposure. The Quadriceps Muscle Thickness (QMT) is one of the measures that can be monitored throughout hospitalization by US. Nutritional therapy (NT) is defined as a set of procedures aimed to restore nutritional status by promoting the nourishment of organs and tissues and providing essential nutrients for muscle synthesis or recovery.

Objectives: To evaluate the adequacy of caloric and protein intake and its correlation with QMT in pediatric cancer patients in intensive care.

METHOD: This is an ongoing prospective cohort study conducted in the Pediatric Intensive Care Unit (PICU) of Hospital do Câncer I, since October 2024 and patients were followed from D0 to D14 of hospitalization. Data on NT (caloric intake, and protein intake) were extracted from medical records. QMT was assessed using US. The relationship between the adequacy of caloric-protein intake and QMT was analyzed using Pearson's correlation. p-values < 0.05 were considered statistically significant.

RESULTS: To date, six patients have completed follow-up in the cohort, 83% of whom were male, with a mean age of 12 (± 4.6) years. According to the Body Mass Index-for-Age (BMI/A) indicator, 66% of the children presented obesity and 34% were classified as normal weight at the time of admission. The mean length of hospital stay was 30 (± 21) days, primarily due to clinical complications associated with oncologic treatment (66%). Solid tumors were the predominant diagnosis during hospitalization (83%). A reduction in QMT was observed between D0 [1.91 (± 0.92) cm] and D14 [1.22 (± 0.49) cm] in all patients (p = 0.03). None of the patients fully received the prescribed total energy or protein. The intake adequacy percentage was 53% for calories and 55% for proteins. There was a positive correlation between the adequacy of caloric intake (r = 0.709; p = 0.05) and protein intake (r = 0.82; p = 0.02) with QMT values throughout hospitalization.

CONCLUSION: Over the 14-day follow-up period, there was a significant reduction in QMT, indicating that these patients may be susceptible to musculoskeletal and functional complications during the post-hospitalization period. When NT is adequately implemented with respect to its caloric and protein targets, it may be associated with the preservation of muscle compartments and a reduction in complications related to muscle mass loss in pediatric patients who have been under intensive care.

Keywords: Pediatric; Nutritional Therapy; Muscle Mass.

PAPER CODE: 67

Vitamin D Nutritional Status in Pediatric Cancer Patients under Intensive Care

TRAMONTANO, C.A.1; MELO, F.D.S.1; DE OLIVEIRA, P.G.D.S.1; MURAD, L.D.1; SARAIVA, D.A.2; DE OLIVEIRA, L.C.3. 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. INCA, NITEROI - RJ - BRASIL; 3. INCA, MAGÉ - RJ - BRASIL.

INTRODUCTION: Pediatric cancer patients under intensive care represent a high-risk group for degrees of malnutrition, including protein–energy or micronutrient deficiencies. Vitamin D (1,25-dihydroxyvitamin D) is an essential micronutrient with significant impacts on child growth and development. Its function is mainly associated with bone mineralization and density, as well as the regulation of calcium absorption, which is fundamental to physiological processes and electrolyte balance.

OBJECTIVES: To assess the nutritional status of vitamin D in pediatric cancer patients under intensive care.

METHOD: This is an ongoing cross-sectional study including patients admitted since October 2024 to the Pediatric Intensive Care Unit (PICU) of the National Cancer Institute I - INCA. Sociodemographic data, anthropometric assessments, and serum vitamin D values were collected from electronic medical records within 48 hours after admission. Serum vitamin D levels were classified according to the criteria established by the Brazilian Society of Pediatrics.

RESULTS: A total of 34 patients with a mean age of 9.8 (± 4.8) years were included in the study to date, most of whom were male (57%), diagnosed with solid tumors (71%), and admitted for postoperative monitoring (47%). According to the Body Mass Index-for-Age (BMI/A) indicator, normal weight (42.1%) was the most frequent nutritional diagnosis. The mean serum vitamin D level was 15.9 (± 7.2) ng/mL. Regarding the classification of serum vitamin D levels, deficiency was observed in 77.8% of patients, insufficiency in 16.7%, and adequacy in only 5.5% at the beginning of intensive care.

CONCLUSION: Pediatric oncology patients presented low serum vitamin D levels at the time of admission in the PICU. The diagnosis of vitamin D deficiency observed in most patients may contribute to the development of deficiency-related conditions in a population already weakened by factors associated with oncologic treatment. Further studies are needed to investigate the clinical effects of this deficiency in this population.

Keywords: Pediatric; Vitamin D; Intensive Care.

PAPER CODE: 91

CHARACTERIZATION OF THE CLINICAL AND EPIDEMIOLOGICAL PROFILE OF HIV-POSITIVE PATIENTS TREATED AT INCA

FERREIRA, M.P.; SOARES, M.A.; BERGMANN, A.; DE MELO, A.C.; SOARES, E.A.J.M.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Individuals living with HIV have a higher risk of developing cancer compared to the general population. The most frequent cancers among HIV-positive individuals are AIDS-defining cancers, which are related to low immunity. Following the introduction of antiretroviral therapy, HIV-positive individuals are living longer, and as a result, other types of cancer, such as non-AIDS-defining cancers, have increased in this population.

OBJECTIVES: This project aims to characterize the clinical profile of a cohort of 743 HIV-positive patients treated at INCA between 2000 and 2014.

METHOD: Retrospective cohort study that included patients over 18 years with a confirmed diagnosis of both cancer and HIV. Information regarding HIV infection was collected from physical medical records, the SISCEL system. Cancer treatment data had been collected during a doctoral study. A data collection instrument was constructed with the following information: date of HIV diagnosis, use of antiretroviral treatment, date of antiretroviral treatment initiation, CD4+ count, CD8+ count, and viral load.

RESULTS: Data from 743 patients were collected. Following data collection, the information was digitized into an Excel spreadsheet. These data are currently being cross-referenced with the cancer data for subsequent analysis. To date, we have observed a median age of 43 years; 51% of the patients were male and 45% were White. Most patients had more than 8 years of formal education and were without a partner. Regarding clinical data related to HIV infection, we observed that the median CD4 count and viral load at the time of cancer diagnosis were 334 cells/mm³ and 57,551 copies/mL, respectively. Regarding the follow-up time and history of CD4 and viral load data, 224 patients had 1 to 3 years of follow-up, 105 patients had 4 to 7 years of follow-up, and 167 patients had more than 8 years of HIV infection follow-up. According to the antiretroviral treatment data, 67% of patients were on treatment.

CONCLUSION: HIV-positive patients were diagnosed with cancer at a relatively younger age compared to the general population and also presented with low CD4 counts and high viral load around the time of cancer diagnosis. Viral load data are being compiled for subsequent analysis of immunological success in this population.

Keywords: Epidemiology; Cancer; HIV.

PAPER CODE: 105

Information and Misinformation Regarding Skin Cancer Across Short- Form Video Environments of Social Media Platforms

RODRIGUES, B.R.1; DE LIMA, F.L.T.1; NEVES, G.G.2;
QUAGLIONI, L.R.M.2; SILVA, T.E.2; COSTA, M.R.L.2. 1.
INSTITUTO NACIONAL DE CÂNCER (INCA), RIO DE JANEIRO
- RJ - BRASIL; 2. UNIVERSIDADE UNIGRANRIO, RIO DE
JANEIRO - RJ - BRASIL.

INTRODUCTION: Skin cancer is one of the most common types of malignant tumors, with over 220,000 estimated cases in Brazil for 2025. While age, sex, and genetics are major endogenous risk factors, ultraviolet (UV) exposure is the most relevant environmental factor. The increasing use of social media has reinvented how people access health information. With over 1.5 billion users globally in 2024, TikTok has inspired other platforms like Instagram Reels, Facebook Reels, and YouTube Shorts to incorporate short-form videos into their platforms. Although international studies have analyzed skin cancer information online, no national research has examined how this topic circulates through short-video environments in Brazil.

OBJECTIVES: This study aimed to explore how short-form social media videos disseminate information and misinformation about skin cancer.

METHOD: An exploratory, observational, infodemiological, qualitative approach was used to analyze 40 public short videos on TikTok, Instagram/Facebook Reels, and YouTube Shorts. The sample was selected from 120 screened videos based on inclusion criteria (public access videos in Portuguese with direct relation to skin cancer published within the last three years). The ten most-viewed per platform were examined using multimodal content analysis to identify communication patterns.

RESULTS: Video durations ranged from 5 seconds to 10 minutes, with views from 17,631 to 26.4 million. Main topics were prevention (47.5%), diagnosis (70%), and treatment (42.5%). Most content was produced by specialists or institutions, mainly white female presenters, who used various strategies to attract attention and engagement. While most specialists kept a professional tone, some chose to approach the topic informally. Authors also used real cases to increase emotional appeal, and some conveyed an alarming

tone by adding emotional soundtracks or shocking images. Editing techniques were also used to maintain attention, along with invitations for interaction with the accounts. Misinformation appeared in seven videos, mainly on Instagram Reels (3). Totalling over 6.35 million views, videos contained false claims, including denying sun exposure as a risk factor, portraying sunscreen as harmful, and unsafe practices like using coconut oil for sun protection or tanning during peak UV hours.

CONCLUSION: The findings reveal a predominance of specialist-led content and the strategic use of emotional and informal approaches to boost engagement. However, widespread misinformation, mainly on Instagram Reels, underscores the need for digital literacy across short-form video platforms and urges evidence-based communication strategies in social media environments.

Keywords: Disease Prevention; kin Neoplasms; Social Media.

PAPER CODE: 109

EPIDEMIOLOGICAL ASSESSMENT OF ACTINIC QUEILITIS IN CASES OF LIP SQUAMOUS CELL CARCINOMA AT INCA

ALVES, T.F.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Actinic cheilitis (AC) is a former potential malignant disorder characterized as a disease that affects the lip vermillion, clinically it may exhibit areas of ulceration, crusting, dryness and effacement of the vermillion border of the lip. It has a higher prevalence among white men over 40 years old with a history of chronic sun exposure, smoking consumption and alcohol abuse. According to the literature, 95% of cases of squamous cell carcinoma (SCC) of the lip vermillion originate from previous AC and have a worse prognosis than cutaneous SCC.

OBJECTIVES: The study to discuss the clinical and sociodemographic aspects of lip SCC, and its association with a previous AC diagnosis.

METHOD: This is a retrospective study that evaluated the patients diagnosed with lip SCC registered at INCA between 2000–2020 with International Classification of Diseases codes 00.0 to 00.2 and 00.6 to 00.9, whose medical records also reported the presence of AC. Clinical and sociodemographic data were collected from medical records and entered into a RedCap® database and analyzed.

RESULTS: A total of 682 cases of lip SCC, 54 (7.9%) were associated with AC, and therefore selected for the study. The majority of the cases occur in male (74.1%). Regarding race, 81.5% were White, 16.7% Mixed-race, and 1.9% Black. Concerning risk factors, the rate of active smokers was lower than active alcohol users, with 20.4% current smokers and 48.1% former ones. Active alcohol users were 31.5% while 25.9% were former users. Cases of occupational sun exposure were reported in only 3.7% of the records. Regarding educational level, 50.0% had incomplete elementary education, whereas only 3.7% had completed high school. The evaluation of clinical characteristics showed that the lower lip was the most affected (88.9%). Ulcerated lesions were the most frequent (77.8%), followed by infiltrative (35.2%), painful (37.0%). Moderately differentiated SCC was the most prevalent (46.3%), followed by well-differentiated (37.0%). Recurrence occurred in 16.7% and 22.4% of the patients deceased.

CONCLUSION: Based on these results, and mainly in the lack of information about the presence of AC on lip SCC cases, this study concludes that the development of effective public health policies aiming to improve the training of healthcare professionals is expected. Mainly assisting in prevention, provision of lip sunscreen, proper management, and accurate diagnosis of AC, which, according to some studies, is often neglected, thereby favoring progression to invasive lip SCC.

Keywords: Actinic cheilitis; Squamous cell carcinoma; Lip vermillion;.

PAPER CODE: 115

Incidence of Acute Graft-versus-Host Disease in Hematopoietic Cell Transplant Recipients

LEÃO, A.M.L.1; DA COSTA-JUNIOR, L.C.2; TAVARES, R.D.C.B.D.S.2; LERNER, D.2; PEREIRA, S.C.M.2; SANTOS, P.C.J.D.L.3; MOREIRA, M.C.R.2; IZU, M.2; FERNANDEZ, T.D.S.2. 1. INSTITUTO NACIONAL DE CÂNCER (INCA), RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO NACIONAL DE CÂNCER-INCA, RIO DE JANEIRO - RJ - BRASIL; 3. FACULDADE DE MEDICINA DA UNIVERSIDADE DE SÃO PAULO (FMUSP), SÃO PAULO - SP - BRASIL.

INTRODUCTION: Acute Graft-versus-Host Disease (aGvHD) is an immune-driven complication of allogeneic hematopoietic cell transplantation (HCT), caused by donor T cells attacking host epithelial tissues, mainly the gut, liver, and skin. Even with preventive immunosuppression, aGvHD remains common and clinically relevant, especially grades II–IV, which need treatment and predict poorer outcomes. Around 35–50% of affected patients develop steroid-resistant forms, associated with high morbidity and mortality. Understanding its incidence, severity, and risk factors is key to improving prevention, identifying patients at risk, and supporting policies that expand access to second-line therapies.

OBJECTIVES: To analyze the frequency, degree, and contributing factors of acute graft-versus-host disease in patients undergoing hematopoietic cell transplantation.

METHOD: This retrospective, single-center study encompassed individuals who underwent allogeneic HCT between 2002–2023. Acute GvHD severity was classified as grades I–IV. Collected variables comprised demographic data, underlying disease, stem cell source (bone marrow or peripheral blood), donor category (MRD, MUD, MMUD, haploidentical), conditioning regimen, and calcineurin inhibitor (cyclosporine or tacrolimus). Bivariate analyses and univariate logistic regression were conducted using SPSS v21.

RESULTS: Among 634 individuals who underwent allogeneic hematopoietic cell transplantation, 360

(60.1%) developed acute GvHD: 66 (18.3%) grade I, 191 (53.1%) grade II, 65 (18.1%) grade III, and 38 (10.6%) grade IV. The overall incidence of grade II–IV and grade III–IV disease was 49.1% and 17.2%, respectively. Independent predictors of grade II–IV aGvHD included adult age (≥ 18 years; OR 1.41, $p=0.049$), peripheral blood graft source (OR 1.62, $p=0.007$), mismatched unrelated donor (OR 2.56, $p=0.015$), White race (OR 1.40, $p=0.040$), and cyclosporine use (OR 1.76, $p=0.033$). White race also correlated with a higher risk of any-grade aGvHD (OR 1.76, $p=0.001$). Patients with grade II–IV disease were older (mean 31.9 vs. 28.6 years; $p=0.012$), while those with grade III–IV received higher cyclosporine doses during the initial 21 days (191 vs. 174 mg/day; $p=0.016$).

CONCLUSION: The high occurrence of aGvHD, especially grades II–IV, highlights its clinical importance post-HCT. Adult age (≥ 18), peripheral blood grafts, MMUD, White race, and cyclosporine prophylaxis increased the risk of grade II–IV aGvHD. By detailing its incidence, severity, and risk factors, this study provides key epidemiological insights to guide future research on treatment outcomes and to support preventive strategies and public health planning, including in systems like Brazil's SUS.

Keywords: Acute Graft-versus-Host Disease; Allogeneic transplant; Epidemiology.

PAPER CODE: 122

EPIDEMIOLOGICAL PROFILE OF PATIENTS WITH METASTATIC NON-SMALL CELL LUNG CANCER USING ERLOTINIB AND GEFITINIB

PESTANA, I.D.P.. INCA PPGO, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Lung cancer has the highest mortality rate and is the second most common cancer worldwide. Erlotinib and gefitinib are epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) that were incorporated into the Brazilian Unified Health System (SUS) in 2013. Although this incorporation represents an important milestone in the access to EGFR-TKIs, there remains a lack of real-world evidence in Brazil.

OBJECTIVES: To analyze the sociodemographic, clinical and safety profile of patients with non-small cell lung cancer using erlotinib and gefitinib.

METHOD: Patients who initiated treatment with the investigated therapies between January 1, 2014, and December 31, 2018, at a cancer hospital in Rio de Janeiro were included in the study. Sociodemographic, clinical, and adverse event data were collected from physical and electronic medical records. The data were compiled into a study-specific database, and statistical analysis was performed using SPSS and Microsoft Excel.

RESULTS: Data from 78 patients were analyzed in two groups: first-line TKI users (G1) and subsequent-line users (G2). Mean age was 60 years (G1 SD $\pm$ 14.6; G2 SD $\pm$ 10.6). Females predominated in both groups (G1 46.2%; G2 43.6%). Most were non-smokers (G1 67.6%; G2 64.1%) and non-alcoholics (G1 59.5%; G2 51.3%). Adenocarcinoma was predominant (G1 92.3%; G2 97.4%), mostly stage IV (G1 97.4%; G2 82.1%). Performance status was reported in 51.3% (G1) and 97.4% (G2). Overall survival for the total population (n = 70; 8 events) was 36 months; median OS = 17 months (95% CI 14.668–19.332). Progression-free survival (n = 78) at 36 months: median PFS = 8 months (95% CI 6.558–9.442). EGFR mutations: exon 18 7.7%, 19 59%, 20 6.4%, 21 26.9%.

CONCLUSION: The population profile allowed us to identify clinical characteristics and outcomes that differed from those reported in randomized clinical trials and real-life studies conducted in other countries.

Keywords: adenocarcinoma; Tyrosine kinase inhibitor; egfr mutation positive.

PAPER CODE: 128

Prospective analysis of financial toxicity assessed by the COST in breast cancer patients treated at a national Cancer reference institution

NUNES, N.C.C.; DA SILVA, J.L.; DE MELO, A.C.. PPGO, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Financial toxicity (FT) represents the negative economic impact experienced by patients due to cancer diagnosis and treatment. It goes beyond direct medical costs, including non-medical expenses and income loss from work interruption. FT can compromise quality of life, reduce treatment adherence, and even affect survival. Studies associate FT with younger age, female sex, lower income, and advanced stage. The Comprehensive Score for Financial Toxicity (COST) provides a standardized way to measure this aspect of patient experience. Breast cancer (BC), which primarily affects women, poses a high risk of FT due to extended treatment regimens and work-related challenges. However, data on FT within the Brazilian population is still limited.

OBJECTIVES: To evaluate FT using COST before and after 6 months of systemic therapy in BC patients treated at Hospital do Câncer 3 (HCIII) and correlate FT with clinical and sociodemographic variables.

METHOD: A prospective cohort study will be conducted involving treatment-naïve women $\geq$ 18 years with invasive BC who are eligible for systemic chemotherapy at HCIII during 2025–2026. Participants with prior cancer (excluding non-melanoma skin cancer) will be excluded. FT will be measured using the COST at baseline and 6 months. Data on clinical and sociodemographic, toxicities (CTCAE v5.0), treatment interruptions, recurrence, progression, and death will be recorded in RedCap and analyzed in R.

RESULTS: A total of 220 patients are expected to be enrolled, based on a sample size calculation, assuming a 20% mean decrease in COST scores after 6 months of systemic therapy, with $\alpha=0.05$, $\beta=0.20$, and an effect size of 0.2. This corresponds to 199 evaluable patients, which increases to approximately 220 when considering a 20% loss to follow-up. The expected reduction reflects worsening FT related to systemic treatment. Associations between FT and sociodemographic, and economic variables will be explored, including out-of-pocket expenses (transportation, exams, medications), housing conditions, employment status of patients and their relatives, and social benefits (previously existing or newly acquired), as well as correlations with grade ≥ 3

toxicities, early treatment interruption, and oncological outcomes (disease-free and progression-free survival for early and metastatic disease, respectively).

CONCLUSION: This study will provide data on FT at diagnosis and the financial impact of systemic therapy among Brazilian women with BC treated in INCA using a validated instrument. The prospective and longitudinal design will help identify factors associated with worsening FT and its relationship with toxicity, treatment interruption, and survival, offering insight into potential barriers to comprehensive cancer care.

Keywords: Financial toxicity; Breast Cancer; Patient-reported outcomes.

PAPER CODE: 141

Total Laryngectomy: Survival Outcomes and Prognostic Factors in a 15- Year Cohort

ALEXANDRINO, B.D.O.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Among head and neck malignancies, laryngeal cancer is one of the most common, predominantly affecting men over 50 years of age, with squamous cell carcinoma being the most frequent histological type. For Brazil, the estimated number of new cases for the 2023–2025 triennium is 7,790, with 6,570 cases in men and 1,220 in women. Approximately 65% of tumors involve the glottis, 35% the supraglottic region, while primary subglottic tumors are rare (<1%). Treatment may involve surgery, radiotherapy, or chemotherapy. However, patients admitted to INCA often present with advanced disease and poor clinical status, precluding eligibility for organ preservation protocols.

OBJECTIVES: The aim of this study was to analyze the prevalence and factors associated with 5-year overall survival in patients undergoing total laryngectomy.

METHOD: We conducted a retrospective study including all patients who underwent total laryngectomy at INCA between January 2007 and December 2022. In addition, a prospective quality-of-

life analysis was performed using the EORTC QLQ-C30 and QLQ-H&N35 instruments. Univariate analyses were used to identify predictors of overall, disease-specific, and progression-free survival.

RESULTS: Most patients were male (88.9%), older than 60 years (58.7%), alcohol users (78.8%), and smokers (91.4%). The majority presented with stage IV disease (70.4%), and squamous cell carcinoma was the predominant histology (97.1%). Independent predictors of overall survival included age, N2–N3 lymph node status, stage IV, perineural invasion, adjuvant radiotherapy, recurrence, distant metastasis, and residual tumor. Predictors of disease-specific survival were N2– N3 lymph node status, perineural invasion, adjuvant radiotherapy, recurrence, distant metastasis, and residual tumor. Predictors of progression-free survival included N2–N3 lymph node status, stage IV, perineural invasion, and adjuvant radiotherapy. Quality-of-life analysis revealed high functional capacity and global health status. Emotional functioning improved and insomnia and dyspnea decreased over time post-surgery.

CONCLUSION: This study highlights a patient profile characterized by late diagnosis, advanced stage, and a high prevalence of risk factors, resulting in modest survival rates. Clinical-pathological variables such as advanced nodal status, stage IV disease, perineural invasion, distant metastasis, and residual tumor were strongly associated with worse survival.

Keywords: total laryngectomy; survival; quality of life.

PAPER CODE: 142

STUDY OF CLINICAL, PATHOLOGICAL, RADIOLOGICAL CHARACTERISTICS, TREATMENT AND SURVIVAL OF PEDIATRIC PATIENTS WITH LOW-GRADE GLIOMA AT THE NATIONAL CANCER INSTITUTE

DOS SANTOS, G.P.B.; FERMAN, S.E.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Central nervous system (CNS) tumors are the most common solid neoplasms in childhood. Low-grade gliomas are the most common subtype, with heterogeneous behavior and location. Their management involves surgical resection, with the possible need for adjuvant chemotherapy and/or radiotherapy. In recent years, the differences between these tumor types have been increasingly studied, and new therapies, primarily related to molecular targeted therapy, have emerged to improve survival and quality of life for these patients.

OBJECTIVES: To study the demographic, clinical, histopathological, radiological profile and treatments performed, correlating with the prognosis.

METHOD: Retrospective study with analysis of the medical records of patients with low-grade glioma admitted to the pediatric oncology department between January 1, 2006, and December 31, 2020 and followed until December 31, 2023. Cases with nonspecific report had pathological review. The sociodemographic, clinical, tumor location and pathological profiles of the patients are described using descriptive measures. Cumulative survival probabilities were estimated using the Kaplan-Meier statistical method.

RESULTS: A total of 217 patients were included in the study. The most common age group of presentation was between ≥ 2 and < 5 years old. The sex ratio was similar between boys and girls (ratio 1.1:1). Regarding location, the posterior fossa was the most prevalent with 70 cases (32.8%), followed by the telencephalon with 47 cases (21.6%). The most prevalent pathological subtype was from the circumscribed astrocytomas family (71%), of which the most frequent was pilocytic astrocytoma, followed by optic pathway glioma. The second most prevalent pathological subtype was from the neuronal and glioneuronal family (11%), with ganglioglioma being the most prevalent. There were 20 deaths (9.2%) during the period, with the most frequent causes being infection (35%) and disease progression (25%).

CONCLUSION: These findings are consistent with the known epidemiology of low grade gliomas in childhood, with pilocytic astrocytoma as the main representative of

low-grade gliomas. The posterior fossa is also the main site of presentation for these tumors. The management of these patients was associated with favorable survival outcomes.

Keywords: Low Grade Glioma; Pediatric Oncology; Central Nervous System Tumors.

PAPER CODE: 145

EPIDEMIOLOGICAL PROFILE OF ORAL AND LIP MUCOSAL MELANOMA AT THE BRAZILIAN NATIONAL CANCER INSTITUTE

ELLER, E.M.; BARROSO, R.D.S.A.; DA SILVA, B.N.M.; CURVELO, J.A.D.R.; DE MELO, A.C.; GOLDEMBERG, D.C..
BRAZILIAN NATIONAL CANCER INSTITUTE, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Oral mucosal melanoma is a rare neoplasm with aggressive clinicopathological behavior. It commonly affects male individuals and its 5-year survival rate remains around 15%. This specific neoplasm represents 2% of all mucocutaneous melanomas and 0.5% of all oral malignancies. Clinically, oral melanoma usually presents itself as a sessile, irregular, black nodular mass, sometimes mixed with blue-black tones. In early stages this neoplasm tends to be painless, compromising diagnosis and impoverishing prognosis in most of the cases. Oral melanoma is not associated with sun exposure like skin melanoma making its etiology unclear, but probably associated with alcoholism and smoking, genetic predisposition, local trauma and exposure to formaldehyde.

OBJECTIVES: To analyze the cases of oral and lip melanoma diagnosed at INCA between 2000 and 2020, including epidemiological features, sociodemographic profile, and survival assessment of these patients.

METHOD: The demographic variables were sex, race/skin color, education level, date of diagnosis, topography, histological type, and five-year overall survival rate. Data were analyzed with clinical stage (AJCC 8th), time between histopathological diagnosis

and treatment, type and status of first treatment, and SEER/NCI classification. For survival analysis, start of treatment, surgery date, cause of death, and last follow-up or death were recorded.

RESULTS: A total of 19 medical records were analyzed, forming a sample of 63.2% women and 36.8% men. Sociodemographically, a history of tobacco use was identified in 52.6% of patients, including current and former smokers, while 47.4% denied it. Regarding alcohol consumption, 31.6% of patients reported current or past use, whereas 68.4% denied it. Histological variants included nodular (15,8%), mucosal (15,8%), desmoplastic (5,3%), and cases classified as melanoma, not otherwise specified (31,6%). Clinically, the majority of patients presented with advanced disease. Concerning the initial therapeutic approach, most patients underwent a combination of surgery and radiotherapy (68,4%). In addition, recurrences were documented in at least six patients, at different locations, representing (41,2%) of the sample, and the overall 5 year survival was 18,7%.

CONCLUSION: This study showed that oral and lip mucosal melanoma were predominantly diagnosed at advanced stages. The five year overall rate of the sample was higher than reported in the literature, pointing to the need to develop effective strategies that favor early diagnosis and consequently improving prognosis, such as biopsy recommendation of suspicious oral pigmented lesions, not presenting as nodular and hemorrhagic masses, such as in late stages. Rapid evolution of the lesions is also a challenge.

Keywords: Oral mucosal melanoma; Malignant melanoma; Lip melanoma.

PAPER CODE: 168

Evaluation of Vocal Rehabilitation Methods in Total Laryngectomy Patients: Effectiveness and Challenges within the Brazilian Health System

RODRIGUES, C.F.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Laryngeal cancer is among the most prevalent head and neck malignancies, significantly affecting both mortality and patients' quality of life. According to the Brazilian National Cancer Institute (INCA), approximately 7,790 new cases are expected annually from 2023 to 2025, predominantly in men. Total laryngectomy is the standard treatment for advanced (T3-T4) tumors or when conservative approaches fail. This procedure has major repercussions, as the loss of natural voice leads to functional, social, and psychological challenges, requiring rehabilitation through an electronic larynx, esophageal speech, or tracheoesophageal prosthesis.

OBJECTIVES: The aim of this study was to evaluate the effectiveness, complications, and overall impact of different vocal rehabilitation methods employed in total laryngectomy patients over a 16-year period.

METHOD: This is a retrospective observational study based on the analysis of medical records and clinical data from 618 patients who underwent total laryngectomy over a 16-year period (from 2007 to 2022). All patients included in the study underwent some form of vocal rehabilitation, either through the use of an electronic larynx, esophageal speech, or a tracheoesophageal prosthesis.

RESULTS: The present study is currently in the data collection phase, focusing on medical records; therefore, the analysis of results has not yet been completed. However, the results section will aim to compare the functional outcomes of the three main methods of vocal rehabilitation in terms of speech intelligibility, voice quality, and patient satisfaction.

Additionally, the study will assess the incidence of complications associated with each method, such as infections, device failure, or the need for additional interventions; correlate the rehabilitation methods with clinical and demographic variables in order to determine which factors influence the selection and success of each one; and analyze the impact of rehabilitation methods on patient survival, evaluating both short-term results and long-term outcomes. Finally, the study seeks to contribute evidence-based data to guide the selection of vocal rehabilitation methods in total laryngectomy patients.

CONCLUSION: This study aims not only to fill an important gap in the literature on vocal rehabilitation among total laryngectomy patients in Brazil but also to positively influence public health policies by promoting improved rehabilitation practices and enhancing patients' quality of life. Furthermore, it seeks to provide robust data to support the incorporation of new technologies into the Brazilian Unified Health System (SUS).

Keywords: total laryngectomy; vocal rehabilitation; rehabilitation practices.

PAPER CODE: 176

EPIDEMIOLOGICAL PROFILE OF GINGIVAL CANCER AT THE BRAZILIAN NATIONAL CANCER INSTITUTE

NETTO, P.P.S.; CANCELA, M.D.C.; GOLDEMBERG, D.C.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: In 2022, head and neck cancer reached the third highest incidence rate worldwide. Among its types, gum cancer is a rare disease, with a higher incidence in Asian countries. Squamous cell carcinoma is the most common type in this region and has a poorly understood etiology. Its diagnosis is often delayed, resulting in a poor prognosis and low survival rates due to advanced staging, requiring radical treatments. Considering the above, understanding this pathology is essential to improve treatment standards and early diagnosis.

OBJECTIVES: The present study aimed to understand the demographic characteristics of patients with gum cancer, their habits, tumor staging and histology.

METHOD: A retrospective cross-sectional study was conducted using the INCA HBCR, with patients over 18 years of age between 2010 and 2022. Demographic and clinical data were analyzed to conduct studies around the ICD 10-C03 topography. The RStudio software was used to extract data from the consulted database and for statistical tests.

RESULTS: Through a preliminary analysis performed in RStudio, demographic and clinical data were obtained

from 213 patients between 2010 and 2022. A 62% male predilection was observed. White skin color was more prevalent (54%) among patients. Age showed a higher prevalence in patients between 50 and 69 years old (54%), followed by patients over 69 years old (33%). Habits such as smoking (42% smokers) and alcohol consumption (44% alcoholics) were analyzed. The most frequent topography was the upper gingiva (C03.1, 46%), followed by unspecified gingiva (C03.9, 32%) and lower gingiva (C03.0, 20%). The histological type was squamous cell carcinoma (SCC) in appeared 83% of cases. Most cases were diagnosed in advanced stages (III and IV, 65%), with 22% of missing data. The chi-square test was conducted, with no significant association between demographic data and staging.

CONCLUSION: Based on the data obtained, a predominance of advanced staging was observed, which reinforces the identification of associated factors that facilitate early diagnosis. A large amount of missing data was noted, which limits a complete analysis. Data recovery and integration across physical and virtual medical records are necessary to make the associations more consistent.

Keywords: tumor staging; epidemiology; gingival cancer.

PAPER CODE: 178

DETERMINANTS OF TIME FROM DIAGNOSIS TO TREATMENT INITIATION IN PEDIATRIC PATIENTS WITH OSTEOSARCOMA AND EWING SARCOMA: A NATIONAL ANALYSIS OF CANCER HOSPITAL RECORDS

DA PAIXÃO, W.H.P.; CANCELA, M.D.C.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Childhood and adolescent cancer (ages 0–19), although rare, represents a significant public health challenge, primarily affecting blood cells and supportive tissues, as in bone and soft tissue sarcomas. Children and adolescents often face substantial delays between histopathological diagnosis and treatment initiation, partly due to diagnostic complexity and limited availability of specialized services. Reducing

this interval is essential, as it can accelerate access to oncologic care, optimize clinical outcomes, and minimize the physical and psychological impacts of the disease in this particularly vulnerable population.

OBJECTIVES: To describe the characteristics of osteosarcomas and Ewing sarcomas in children and adolescents, analyze the time between diagnosis and treatment, and identify factors contributing to delays.

METHOD: Exploratory, descriptive ecological study with a quantitative approach, based on secondary data from the Cancer Hospital Records Integrator. Sociodemographic, clinical, and service access variables were considered, with the outcome being the time between diagnosis and treatment initiation, categorized as ≤ 60 or ≥ 60 days. Analyses included descriptive statistics and Poisson regression with sensitivity analyses using STATA 15.

RESULTS: A total of 2,989 cases were included, mostly male (58.8%), with 80% aged 11–19 years. Multivariate analysis showed that residing in the North, Northeast, or Southeast regions, having a previous diagnosis without treatment or with prior treatment, and receiving care outside the state of residence significantly increased the likelihood of treatment initiation after 60 days (IRRs 1.32–13.59). Oncopediatrics accreditation was not associated with delays. Non-white patients represented 40.6%, with 36.7% missing skin color information. Most cases were from the Southeast (43.8%) and referred via the SUS (51.9%). Osteosarcomas were most prevalent (69.6%), and 67% arrived without prior diagnosis or treatment. Median time to treatment varied little by sex, age, or skin color, but was higher in the North and among patients treated outside their state of residence.

CONCLUSION: Preliminary results suggest that health service organization, regional distribution of oncology centers, and complexity of the care pathway influence treatment initiation time. Despite the predominance of adolescents, osteosarcomas, and SUS referrals, factors such as region of residence and prior diagnosis or treatment may impact delays. Findings are preliminary, as the study is still ongoing.

Keywords: sarcomas; childhood and adolescent neoplasms; health services.

PAPER CODE: 182

NutriPal (Nutricional screening algorithm): association with nutritional status, quality of Life, and survival, and development of personalized recommendations

ROSA, K.S.D.C.1; PAIVA, B.S.R.2; SANTOS, L.P.3; DE AGUIAR, G.B.4; DE ARAUJO, M.A.G.C.3; FERNANDES, R.C.3; ALMEIDA, M.C.P.3; DE OLIVEIRA, L.C.3. 1. PROGRAMA DE PÓS-GRADUAÇÃO EM ONCOLOGIA, INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL; 2. HOSPITAL DE CÂNCER DE BARRETOS, BARRETOS - SP - BRASIL; 3. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL; 4. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - MS - BRASIL.

INTRODUCTION: Nutritional screening is the first step toward providing high-quality nutritional care for patients with cancer. However, in the context of palliative care, scientific evidence to guide nutritional care planning for this group remains limited. To address this gap, the NutriPal: Nutritional screening algorithm for patients with incurable cancer receiving palliative care was recently developed. The higher the degrees of NutriPal, the worse the nutritional risk, comparing nutritional measures, laboratory data, and overall survival (OS). In this context, it is essential that such tools demonstrate associations with nutritional status, OS, and quality of life (QoL). Moreover, to be considered comprehensive, they should provide nutritional recommendations.

OBJECTIVES: The study is divided into two axes: 1) to investigate the association of NutriPal with nutritional assessment, QoL, and OS; 2) to develop and validate nutritional recommendations for NutriPal.

METHOD: Axis1: Prospective cohort study including patients with cancer in palliative care. Nutritional risk was assessed using NutriPal, alongside evaluations of cancer cachexia (CC), calf circumference (CCF), QoL, and OS up to 120 days. Axis 2: The initial version of the nutritional recommendations was developed based on a literature review and brainstorming sessions, followed by validation through the Delphi method and pre-testing.

RESULTS: In axis 1, 417 patients were included; most (47.72%) were classified as NutriPal degree 1, followed by degrees 2 (24.22%), 3 (17.51%), and 4 (10.55%). Significant differences were observed among NutriPal degrees regarding primary tumor site ($p=0.049$), care setting (outpatient vs. inpatient; $p<0.001$), and Karnofsky Performance Status ($p<0.001$). Association and survival analyses are ongoing. In axis 2, four Delphi rounds and two pre-tests were required to achieve consensus. Major adjustments were made after the first Delphi round and initial pre-test, although no items were added or removed. Fifteen NutriPal nutritional recommendations were established across six domains: re-administration, symptom control, nutrient supply, nutritional assessment, nutritional support, and comfort.

CONCLUSION: Evaluating nutritional risk using NutriPal in association with nutritional status, QoL, and OS is essential. Analyses are being developed to evaluate such associations. Furthermore, the development and validation of NutriPal nutritional recommendations made the tool complete, as it allows the establishment of risk and provides nutritional care guidance for each degree of nutritional risk.

Keywords: Incurable cancer; Palliative Care; Nutritional screening.

PAPER CODE: 183

Assessment of the Unmet Needs of Cancer Survivors: Support for Proposing Care Plans for Counter-Referral to Primary Health Care

FIGUEIRA, P.H.M.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Cancer treatment has a profound impact on the lives of patients and their caregivers. An integrated approach to this individual is essential to ensure that all needs arising from cancer and its treatment can be met.

OBJECTIVES: To understand the unmet socioeconomic and health needs, as well as adherence to recommended cancer prevention and control strategies for survivors

METHOD: This is a mixed-method study, with a mixed-method approach, to be developed in three stages: content development, content validation, and modular care plan development.

RESULTS: The database search yielded 184 manuscripts, which are currently undergoing eligibility assessment.

Conclusion: The study is still ongoing

Keywords: Unmet Needs; Cancer Survivors; Primary Health Care.

TRANSLATIONAL RESEARCH AND DIAGNOSTIC APPLICATION (TR)

PAPER CODE: 4

ANCESTRY-ASSOCIATED EPIGENETIC PROGNOSTIC BIOMARKERS IN BREAST CANCER OF BLACK WOMEN

CHRISTIANES, A.L.D.S.1; LOPES, M.D.S.A.1; DE PAULA, D.J.G.1; VIEIRA, J.G.1; SIMÃO, T.D.A.2; CARDOSO, C.C.3; DELGADO-CRUZATA, L.4; LIMA, S.C.S.1. 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. UERJ, RIO DE JANEIRO - RJ - BRASIL; 3. UFRJ, RIO DE JANEIRO - RJ - BRASIL; 4. CUNY, NEW YORK - ESTADOS UNIDOS DA AMERICA.

INTRODUCTION: Breast cancer is the most common and the deadliest cancer type among women. Its incidence and mortality rates vary according to racial group. White women present higher incidence but lower mortality compared to black women, which has been associated with social vulnerability caused by structural racism. However, recent evidence suggests that biological mechanisms might also be involved in these disparities, since Black women show a higher frequency of the most aggressive subtype, triple-negative breast cancer (TNBC).

OBJECTIVES: This study evaluated prognostic epigenetic biomarkers in Brazilian Black women diagnosed with breast cancer.

METHOD: A total of 48 Brazilian Black women enrolled at the Brazilian National Cancer Institute (INCA) were included in this study. Ancestry was inferred using Infinium Global Screening Array (Illumina) and DNA methylation profiled with the Illumina MethylationEPIC BeadChip v1.0 (Illumina) in matched tumor and adjacent non-tumor tissue (NTAT). Differentially methylated positions (DMPs) (adjusted p -value <0.0001 , $\Delta\beta>|0.2|$) were defined by comparing tumor and NTAT tissue.

RESULTS: A total of 56,679 DMPs were identified. Most DMPs were hypomethylated in tumors (72.7%), which were enriched in intergenic and open sea regions, suggesting global hypomethylation. Hypermethylated DMPs were enriched in CpG islands and promoters, possibly silencing tumor suppressor genes. Gene set enrichment analysis using KEGG database identified "Pathways in Cancer", "cAMP", and "Ras" among the most frequently altered. Tumor clustering by their 1,000 most variable CpG sites revealed a group with higher median methylation levels (0.8 vs 0.55, p -value $=2.1\times10^{-5}$), associated with worse survival (log-rank p -value $=0.0024$, HR $=5.52$), unrelated to staging ($p=0.1157$). This group was enriched for TNBC (p -value $=0.00034$) and African Ancestry (p -value $=0.03005$). When excluding TNBC, association of DNA hypermethylation cluster with poorer survival remained (log rank p -value $=0.017$), but not with African Ancestry (p -value $=0.3239$).

Conclusion: These findings suggest that DNA methylation changes contribute to breast carcinogenesis by affecting key cell signaling pathways and can predict prognosis regardless of subtype. A higher proportion of genetic African ancestry, even among self-declared Black women, was associated with a more aggressive DNA methylation profile, suggesting a biological mechanism behind racial disparities in breast cancer.

Keywords: Breast Cancer; Black Women; DNA methylation.

PAPER CODE: 11

MUTATIONAL PROFILE AS A GENOMIC MARKER OF PROGNOSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

DOS SANTOS, M.P.M.P.; FERREIRA, G.M.; ABDELHAY, E.S.F.W.. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Chronic lymphocytic leukemia (CLL) is the most prevalent leukemia in Western adults, characterized by clonal proliferation of dysfunctional mature CD5+, CD23+, CD19+, CD20- and FMC7- B cells in the bone marrow, peripheral blood, and secondary lymphoid organs. Patients can remain asymptomatic for years, while in other cases, symptoms manifest rapidly, leading to disease progression and transformation. In this context, prognostic markers play an important role in clinical decision-making and patient management. The mutational status of the immunoglobulin heavy chain variable region (IGHV) gene is a well-known genomic marker in CLL patients. Mutations in TP53, SF3B1, NOTCH1, MYD88, BIRC3, and ATM have also become recognized as possible indicators of disease progression and poor prognosis.

OBJECTIVES: This study aims to correlate the IGHV mutational status and mutations in TP53, SF3B1, NOTCH1, MYD88, BIRC3, and ATM with CLL progression in patients from INCA (Brazilian National Cancer Institute).

METHOD: This study analyzed 72 CLL patients from INCA (approved by the Ethics Committee) whose DNA underwent IGHV gene Sanger sequencing to determine mutational status, which were classified as CLL – M (mutated IGHV) or CLL – UM (unmutated IGHV). Next-generation sequencing (NGS) for a panel of the 6 genes was performed to identify pathogenic variants (P); probably pathogenic variants (pP) and variants of uncertain significance (VUS). The findings were then correlated with CLL evolution and prognosis.

RESULTS: 66.7% of the cohort was male, mean age 66.5 years $\pm$ 9.36. Sanger sequencing was performed in 65 patients, where 36.92% was CLL – M and 63.08% CLL – UM. In the CLL – M group, 58.3% of patients were not

receiving treatment, while in the CLL – UM group, 65.9% were undergoing treatment. The gene that presented more VUS was NOTCH1 (n=37), followed by ATM (n=14), SF3B1 and BIRC3 (n=7 each), and MYD88 (n=4). TP53 had the most pP variants (53.8%), followed by ATM and SF3B1. Seven P variants were identified: ATM (n=4) and TP53 (n=3). In CLL – M, SF3B1 pP and MYD88 VUS were exclusive. SF3B1 pP was exclusive in pre-treatment. ATM pP/P was found in treatment patients, and TP53 pP/P in transformation. No ATM P/pP variants appeared in transformation. In CLL – UM, all groups showed TP53 pP/P variants, while ATM pP/P appeared in treatment and transformation. Unlike pre-treatment CLL – M, pre-treatment CLL – UM had only one TP53 pP/P variant.

CONCLUSION: CLL – M patients showed better clinical outcomes compared to the CLL – UM group. The two groups presented distinct variants in different phases of the disease; with MYD88 VUS and SF3B1 pP variants exclusive in CLL – M group. However, due to the low number of variants detected, it was not possible to establish statistical differences. The results so far allowed characterization of the mutational profile of patients, contributing to understanding the genetic profile of Brazilian CLL patients.

Keywords: Chronic Lymphocytic Leukemia; Mutational profile; prognosis.

PAPER CODE: 20

The Role of the PRC2 Complex in T-cell Acute Lymphoblastic Leukemia

FAGUNDES, M.C.N.1; BORBA, L.M.1; LEMOS, R.M.D.B.1; COSTA, E.S.2; FERRAZ, T.3; SCHRAMM, M.T.4; LINS, M.M.5; BARBOSA, T.D.C.1; LOPES, B.D.A.6; BLUNCK, C.B.1; MACIEL, A.L.T.7; DE SÁ, M.E.C.1. 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. IPPMG-UFRJ, RIO DE JANEIRO - RJ - BRASIL; 3. HEMORIO, RIO DE JANEIRO - RJ - BRASIL; 4. PRONTOBABY / INCA, RIO DE JANEIRO - RJ - BRASIL; 5. IMIP, RECIFE - PE - BRASIL; 6. INCA / UERJ, RIO DE JANEIRO - RJ - BRASIL; 7. UFPR, CURITIBA - PR - BRASIL.

INTRODUCTION: T-cell acute lymphoblastic leukemia (T-ALL) is a rare and aggressive ALL subtype, accounting

for ~15% of pediatric and 25% of adult cases. Early T-cell precursor ALL (ETP-ALL), that affects immature lymphoid cells, represents 10–15% of T-ALL and presents mutations in epigenetic regulators such as EZH2, the catalytic subunit of the Polycomb Repressive Complex 2 (PRC2). PRC2 catalyzes H3K27me₃, a repressive mark that is essential for hematopoietic stem cell maintenance. Genetic and epigenetic alterations in PRC2 components can reduce this mark and are associated with treatment resistance and poor prognosis in T-ALL.

OBJECTIVES: Thus, evaluating PRC2 alterations and expression of its components and target genes is very important to understand T-cell acute lymphoblastic leukemia development.

METHOD: Two independent T-ALL cohorts are being investigated: (1) Data from the TARGET initiative to assess mutations and mRNA expression in PRC2 (EZH2, SUZ12, EED) and target gene FLT3, as well as CDKN2A/B deletions by RNA-seq and exome; (2) Patient samples from our lab provided by partner institutions, to evaluate PRC2 mutations (Sanger sequencing), FLT3 (fragment analysis), gene expression (RT-qPCR), and CDKN2A/B deletions (MLPA).

RESULTS: From TARGET, 264 pediatric T-ALL samples were analyzed to investigate PRC2 mutations in ETP and non-ETP ALL (n = 23 and 241). Mutations in PRC2 genes (SUZ12, EZH2, and/or EED) were identified in 8 ETP and 24 non-ETP samples. The most frequent genetic alteration in non-ETP ALL was CDKN2A/B deletions (84.6%), while FLT3 overexpression predominated in ETP ALL (73.9%). FLT3 mutations occurred in 17.4% of ETP and 2.1% of non-ETP. Patients with SUZ12 mutations showed reduced gene expression compared to wild-type, whereas FLT3-mutated patients exhibited increased expression in T-ALL samples. Our lab included 148 T-ALL patients, mainly pediatric (86.5%), male (69.6%), with high leukocyte counts (93.9%). This profile matches TARGET data. Of 36 samples analyzed by fragment analysis, all were wild-type for FLT3 ITD. For CDKN2A/B deletions, we analyzed 117 samples: 74 showed deletions (44 biallelic, 30 monoallelic), and 43 were wild-type. mRNA and mutation analyses are ongoing.

CONCLUSION: Our findings highlight PRC2 genetic alterations between ETP ALL and nonETP-ALL, as well as FLT3 expression, in T-ALL pathogenesis. Moreover, the analysis of mutations and gene expression in samples from our lab will help clarify T-ALL biology and provide novel targeted therapeutic strategies.

Keywords: T-cell acute lymphoblastic leukemia (T-ALL); Polycomb Repressive Complex 2 (PRC2); Molecular profiling.

PAPER CODE: 26

Validating a manufacturing process for CAR-T cells accessible to the Brazilian healthcare system for treating pediatric hematologic cancers - CHOP - INCA partnership

DOS REIS, S.A.1; DE GODOY, D.T.1; DOURADO, L.P.S.1; ABDO, L.1; ARAGÃO, E.1; NEUMANN, A.1; BRAGA, F.H.P.1; KADAUKE, S.2; GRUPP, S.2; BONAMINO, M.H.1. 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. CHOP, PHILADELPHIA - ESTADOS UNIDOS DA AMERICA.

INTRODUCTION: Cellular immunotherapy has been transformed by the development of T lymphocytes genetically modified to express chimeric antigen receptors (CARs), enabling targeted recognition and elimination of tumor-associated antigens. Although CAR-T therapies are approved and commercially available in high-income countries, their implementation in Brazil remains limited due to high costs and infrastructure constraints. To address this challenge, the Brazilian National Cancer Institute (INCA), in collaboration with the Children's Hospital of Philadelphia (CHOP), launched a technology transfer initiative to adapt CAR-T manufacturing for the treatment of pediatric hematologic malignancies within the Brazilian healthcare system.

OBJECTIVES: The strategy is to use the laboratory infrastructure to comply with good manufacturing practices (GMP), using the CliniMACS Prodigy platform for cellular processing.

METHOD: The validation process employed leukapheresis material from a pediatric patient with acute lymphoblastic leukemia. T cells were purified, expanded, and transduced with a lentiviral vector encoding the CD19-BBz CAR. On the final day of culture, the CAR-T cell product was cryopreserved and subjected to quality control testing.

RESULTS: Initial results demonstrated that the product met all predefined release criteria, including sterility, viability, transduction efficiency, absence of mycoplasma and replication-competent lentivirus, and a vector copy number of less than 8 per cell.

CONCLUSION: This work validates the feasibility of GMP-compliant CAR-T manufacturing in Brazil, representing a key step toward expanding national access to advanced immunotherapies through cost-effective and scalable solutions.

Keywords: CAR-T cells; Immunotherapy; GMP manufacturing.

PAPER CODE: 27

Characterization of KRAS mutations and HER2 expression in Mucinous Ovarian Carcinoma

SOUZA, M.E.C.; ROMA, N.R.; RODRIGUES, E.M.; GUIMARÃES, I.D.S.; DE MELO, A.C.. INSTITUTO NACIONAL DE CâNCER (INCA), RJ - RJ - BRASIL.

INTRODUCTION: Ovarian cancer is the eighth most common malignancy worldwide and one of the most lethal cancers among women. Mucinous ovarian carcinoma (MOC), although rare, is difficult to diagnose due to its similarity to metastatic tumors and responds poorly to standard chemotherapy. Patients with advanced or recurrent MOC have an unfavorable prognosis and limited therapeutic options. Molecular alterations such as KRAS mutations and ERBB2(HER2) amplification are frequent in this subtype and are linked to tumor growth and treatment resistance. Identifying these alterations is essential for the development of targeted therapies. There is a lack of data on MOC in Brazil, and this study aims to characterize the frequency and molecular profile of these alterations in a Brazilian cohort.

OBJECTIVES: The study aims to investigate KRAS mutations and HER2 alterations in MOC to improve its molecular characterization and identify potential therapeutic targets.

METHOD: Tumor and non-tumor regions will be microdissected from FFPE blocks for DNA extraction. KRAS mutations will be investigated by PCR followed by Sanger sequencing. HER2 expression will be evaluated by immunohistochemistry and confirmed by FISH in equivocal cases. Statistical analyses will be performed using R software, applying association tests and survival models with a significant level of $p < 0.05$.

RESULTS: Of 105 cases identified by CID C56, 38 patients with MOC were included after exclusions and diagnostic reviews. Cases with a history of prior neoplasia, association with other subtypes, or evidence of metastatic lesions, mainly from the appendix and colon, were excluded. Clinical data collection, tumor area demarcation for microdissection, and preparation of slides for immunohistochemical analysis are currently in progress.

CONCLUSION: The preliminary findings highlight not only the low frequency of MOC but also the difficulty in diagnosing based only on histological evaluation. This study aims to contribute to a better molecular understanding of this ovarian cancer histotype.

Keywords: Mucinous ovarian carcinoma; KRAS; HER2 .

PAPER CODE: 28

Characterization of genetic predisposition to hereditary breast cancer in young women using RNA sequencing

AZEVEDO, L.B.D.B.E.; GOMES JUNIOR, R.G.; DOS SANTOS, A.C.E.; MOREIRA, M.Â.M.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Breast cancer (BC) in young women are more frequently associated with hereditary cases, and identification germline pathogenic variants in genes associated with BC predisposition are crucial

for clinical management of these cases. However, DNA germline analyses frequently identify variants of uncertain significance (VUS) in genes associated with breast cancer predisposition, many of which affect splicing leading to aberrant transcripts. Around 15–25% of pathogenic variants in hereditary cancer impact mRNA processing. In this context RNA sequencing (RNA-seq) emerges as a powerful tool for the detection of spliceogenic events and expression changes, supporting VUS reclassification and improving genetic testing.

OBJECTIVES: This study aims to identify and quantify aberrant splicing isoforms in BC predisposition genes using RNA-seq in women under 36 without known pathogenic variants in eight BC-related genes.

METHOD: Target NGS-sequencing are being carried out to exclude patients with pathogenic variants in BRCA1, BRCA2, ATM, BRIP1, CHEK2, PALB2, RAD51C, and TP53. PBMCs isolated from the selected patients are being submitted to short-term cell-culture, adding puromycin during the last 4h, followed by RNA isolation and build of RNA-Seq NGS libraries using depletion of rRNA strategy. Transcriptome analyses will be carried out by assembly with a reference and de novo assembly.

RESULTS: Partial Results: To date, DNA from 59 of 67 patients has already been sequenced for the eight genes, and PBMCs have been isolated from 67 patients. Thirteen patients were excluded due to the presence of pathogenic variants. Thirty samples were submitted to short-term cell cultured and the RNA extraction performed for 14 of them.

CONCLUSION: Perspectives: Next steps include: (i) Sanger sequencing of regions with insufficient coverage to definitively exclude the presence of pathogenic variants; (ii) NGS sequencing of DNA of the remaining 8 patients; (iii) library preparation of isolated RNA; and (iv) RNA-Seq of the selected patients will be carried out to characterize the splicing products of the genes of interest.

Keywords: Splicing; hereditary cancer; gene expression.

PAPER CODE: 42

Non-Viral Generation of CD19 CAR-T Cells via a Minicircle-Based Sleeping Beauty Transposon System

NEUMANN, A.1; SILVA, L.R.B.2; DOURADO, L.P.S.1; DOS REIS, S.A.1; ABDO, L.1; ARAGÃO, E.1; CORREIA, E.M.1; DE GODOY, D.T.1; BONAMINO, M.H.1. 1. INSTITUTO NACIONAL DO CâNCER - INCA, RIO DE JANEIRO - RJ - BRASIL; 2. FUNDAÇÃO OSWALDO CRUZ - FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Chimeric Antigen Receptor T cell (CAR-T) immunotherapy targeting CD19 has achieved remarkable outcomes in patients with B-cell malignancies. However, its conventional production relies on viral vectors, making the process complex, expensive, and difficult to scale. To overcome these limitations, our group implemented a non-viral platform Sleeping Beauty (SB) transposon system for the generation of anti-CD19 CAR-T cells using a minicircle-based (MC) structure. MCs are simplified DNA vectors produced by engineered bacteria expressing an inducible recombinase, resulting in molecules that contain only the gene of interest, free of bacterial backbone sequences, thereby enhancing the safety and efficiency of gene delivery.

OBJECTIVES: In this study, we compared the generation of anti-CD19 CAR-T cells using the SB system delivered via either minicircle (MC) or conventional (full) plasmid (FP).

METHOD: T lymphocytes from healthy donors were isolated, activated with anti-CD3 beads, nucleofected with the PT4-19BBz vector (MC or FP), and expanded in G-Rex plates.

RESULTS: MC-derived cells exhibited high viability ($78.35 \pm 8.73\%$) and robust CAR expression ($35.6 \pm 5.95\%$) after 8 and 12 days of culture. From an initial population of 5×10^6 T lymphocytes, we obtained $1.7 \pm 0.8 \times 10^6$ CAR-positive cells, indicating greater expansion compared to FP platform. In vivo assays demonstrated that MC CAR-T cells displayed antitumor efficacy comparable to FP CAR-T cells.

CONCLUSION: These results indicate that the Sleeping Beauty minicircle-based platform provides a safe, efficient, and cost-effective alternative for generating CAR-T cells, with strong potential for large-scale and point-of-care clinical applications.

Keywords: CAR-T; leukemia; immunotherapy.

PAPER CODE: 49

Construction and evaluation of an HSV-1-based oncolytic virus expressing the pro-apoptotic protein sfTRAIL, for the treatment of head and neck tumors

PERINA, L.B.; REALE, A.; CALISTRI, A.; DA COSTA, N.O.M.; MELENDEZ, M.E.. DEPARTMENT OF MOLECULAR MEDICINE, UNIVERSITY OF PADUA, PADUA - ITALIA.

INTRODUCTION: Head and neck squamous cell carcinoma (HNSCC) is a malignant tumor originating from epidermoid cells, arising from the mucosal surfaces of the upper aerodigestive tract, salivary glands and paranasal sinuses. It is estimated that more than 900,000 people are diagnosed with HNSCC each year worldwide, representing the sixth most common cancer incidence. Although there is already extensive molecular and clinical knowledge about HNSCC, an effective therapy that increases the life expectancy of these patients, who have a 5-year survival rate of 35 to 70%, is still unknown.

OBJECTIVES: The objective was to construct oncolytic viruses (Ovs) derived from the herpes simplex virus (HSV-1) capable of expressing the soluble form of the TNF-related apoptosis-inducing ligand (sfTRAIL).

METHOD: Two homologous recombination strategies were used for OV construction to insert TRAIL into different expression loci of the HSV-1 genome. Recombinant clones were validated by restriction analysis, PCR, and Sanger sequencing. Viral transfection and amplification were performed in Vero cells. Viral titers were determined by plaque assay and plaque diameters were measured. TRAIL expression was confirmed by Western blot, and HNSCC cell lines were evaluated for cell viability using the MTS assay.

RESULTS: We generated two recombinant oncolytic HSV-1 constructs by targeting different genomic loci. Restriction enzyme digestion and sequencing demonstrated the success of the cloning methodology for the insertion of TRAIL protein. We also validated all the transfection and virus amplification by fluorescence along with the positive control HSV-1 EGFP and follow to detect the expression of the TRAIL protein by Western Blot. Microscopy images showed that the virus expressing TRAIL, generated by replacing one of the viral genes as a form of attenuation, exhibited larger plaque diameters compared to the unmodified virus or the virus expressing EGFP (HSV-1 WT and HSV-1 EGFP). The viruses were able to reduce cell viability in the FaDu, UD-SCC-2, VU-SCC-120, and VU-SCC-147 cell lines; however, only in the latter three was increased sensitivity observed upon infection with TRAIL-expressing viruses.

CONCLUSION: The development and characterization of the oncolytic viruses were successfully completed. Functional assays in HNSCC cell lines are ongoing. Different effects on cell viability were observed between viruses with or without TRAIL expression. Future assays, including Western blot and flow cytometry for apoptosis and caspase-8 activity, will clarify the role of TRAIL and oncolysis in inducing tumor cell death.

Keywords: Oncolytic Virus; Head and Neck tumors; TRAIL protein.

PAPER CODE: 53

N-MYC Amplification Detected by FISH: Prognostic Impact on Survival in Pediatric Neuroblastoma at a Single Cancer Reference Center

BASTOS, N.C.1; COELHO, S.D.O.1; DA ROCHA, A.C.A.1; LIMA, L.D.J.2; MOREIRA, V.G.1; FERMAN, S.E.1; FERNANDEZ, T.D.S.1. 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. UFRJ, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Neuroblastoma (NB) is the most common extracranial solid tumor in childhood. NB is characterized by significant clinical heterogeneity. While some cases experience complete regression, others

exhibit metastasis, rapid progression, and resistance to therapy despite multimodal treatment approaches. Key prognostic factors in NB include tumor location, metastasis at diagnosis, patient age, tumor histology, and N-Myc amplification (NMA). NMA is considered a pivotal change in the oncogenic process of NB and is strongly associated with poorer outcomes.

OBJECTIVES: Analyse the NMA and its association with clinical and demographic characteristics of NB pediatric patients, and also the overall survival.

METHOD: Between 2010 and 2023, 190 NB pediatric patients diagnosed and treated at the Pediatric Oncology Department of the National Cancer Institute (INCA), Brazil, were included. Fluorescence in Situ Hybridization (FISH) analysis was performed in formalin-fixed and paraffin-embedded samples to evaluate NMA. Clinical and demographic characteristics were obtained from medical records, and statistical methods were applied to analyze the prognostic impact of NMA on treatment response and survival.

RESULTS: The mean age at diagnosis was 3 years, with no significant sex differences. The main primary tumor site was the abdominal area, most frequently the adrenal gland. Fifty-one percent of patients had metastasis at the time of diagnosis (primarily involving the bone). These patients demonstrated worse overall survival compared to those without metastasis. The median overall survival was nearly nine years, though 46% of patients died from the disease. N-Myc amplification was assessed by FISH in 127 cases. NMA was detected in 25% of NB pediatric patients, and it was positively correlated with metastasis at diagnosis. The statistical analysis revealed a significant association between NMA and disease progression (OR = 7.5). Furthermore, patients with NMA had a lower probability of surgical intervention, likely contributing to their significantly lower overall survival rate compared to those without NMA.

CONCLUSION: These findings underscore the clinical impact of NMA evaluation in NB patients, confirming its value as an essential prognostic tool in childhood NB.

Keywords: Neuroblastoma; N-Myc amplification; FISH.

PAPER CODE: 54

OCCUPATIONAL PESTICIDE EXPOSURE AND ITS IMPACT ON TELOMERE LENGTH IN RURAL WORKERS

PEREIRA, C.D.; DA POÇA, K.S.; DA SILVA, P.V.B.; OTERO, U.B.; MELLO, M.S.D.C.; SOARES-LIMA, S.C.. INSTITUTO NACIONAL DE CâNCER, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: The occupational use of pesticides, especially among rural workers (RW), has been associated with many adverse health outcomes. Among these, an important change can be seen in the telomere length (TL), a critical structure to preserve the stability of the genome during cell division. Shortened telomeres are recognized as biomarkers of aging and genomic instability, processes that contribute to the development of chronic diseases, including cancer. The literature shows that occupational exposure to pesticides may increase the risk of cancer and one of the mechanisms studied is the interference with telomere dynamics. While telomere shortening favors chromosomal instability, telomerase reactivation is a central pathway enabling uncontrolled proliferation of malignant cells.

OBJECTIVES: The objective of this study was to evaluate the telomere length in lymphocytes of rural workers and residents of the rural and urban area of the municipality of Casimiro de Abreu (RJ).

METHOD: The cohort consisted of 78 occupationally exposed RW, 25 rural residents (RR), and 33 urban residents (UR). TL was estimated by quantitative PCR using HGB gene as two-copies reference gene and the ΔC_t method.

RESULTS: Median ages did not differ between groups (RW: 54 [27–79]; RR: 49 [23–75]; UR: 47 [18–71]). Men represented 62.66% of RW, 36% of RR, and 58.06% of UR. There was no difference in relative TL between the groups, but an inverse correlation with age was observed in the RR group ($r = -0.4196$; $p = 0.0368$). Among the RW, some individuals showed higher TL ($T/S > 40$). No associations with the time living in the rural region and the smoking status were observed, but pesticides applicators (AP) showed higher TL than non-

applicators (NAP) (AP: 19.84 [0.95–176.9]; NAP: 9.24 [2.01–79.33]; $p = 0.0018$). Among the AP, 40% had elevated TL (> 40), compared to 6.35% among NAP, with a 9.83 times greater chance of increased values ($p = 0.002$).

CONCLUSION: Therefore, TL can be affected by pesticide exposure and it is important to consider factors such as intensity, frequency and type of exposure in the dynamics of this change. The consequences in the organism resulting from the use of these harmful substances highlight the need for health surveillance and protection strategies for exposed workers and populations.

Keywords: telomere length; pesticides; occupational exposure.

PAPER CODE: 59

Epigenetic Reprogramming in Myelodysplastic Syndrome: The Role of Histone Modulation

LOVATEL, V.L.; DOS SANTOS, E.C.; FERNANDEZ, T.D.S.. INSTITUTO NACIONAL DE CâNCER - INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Histone deacetylases (HDACs) are crucial epigenetic regulators involved in chromatin remodeling and are essential for hematopoiesis. They maintain self-renewal, guide lineage commitment, and regulate cell differentiation and proliferation. The HDAC family is divided into: Class I (HDAC1–3/8), Class II (HDAC 4–7/9/10), Class III, known as sirtuin (SIRT1–7), and Class IV (HDAC11). These enzymes influence chromatin structure and gene expression by modifying the acetylation on lysine residues of histone proteins. However, the role of HDACs in Myelodysplastic Syndrome (MDS) remains poorly understood. MDS is a heterogeneous clonal neoplasm, characterized by ineffective hematopoiesis, bone marrow dysplasias, peripheral blood cytopenias, and risk of progression to acute myeloid leukemia (AML).

OBJECTIVES: To characterize the HDACs expression profile in pediatric and adult patients with MDS, and its association with clinical characteristics, karyotypes, and progression to AML.

METHOD: HDAC transcript expression profiles were evaluated using microarray data from nine MDS studies from the Gene Expression Omnibus database. Differential HDAC family expression was analyzed by comparing MDS patients to healthy bone marrow controls and analyzing subtypes, karyotypes, and progression to AML. Protein-protein interaction networks associated with HDACs were constructed using BioGRID and STRING. A significance threshold of $p > 0.05$ was considered for all analyses.

RESULTS: Transcriptome analysis revealed heterogeneous differential expression of HDACs 1–11, with most SIRT3, 4, and 7 being overexpressed. Overexpression of HDAC11 and SIRT3, 4, and 7 was independent of MDS clinical features compared to controls. In contrast, HDAC3, 9, and 10 were downregulated in MDS patients, with HDAC3 downregulation being more pronounced in advanced MDS, particularly in cases with cytogenetic abnormalities and MDS evolution to AML. Gene network analysis identified 847 targets for HDAC3, 5, 7, 9, 11, and SIRT3, 4, 7. Of these, 427 genes were differentially expressed in MDS patients, with 209 downregulated and 218 upregulated. Functional and network analyses revealed strong associations with oncogenic pathways, with significant enrichment in sustained proliferative signaling, resistance to apoptosis, immune evasion, and replicative immortality, as categorized by cancer hallmarks. Additionally, genes associated with leukemic transformation were also significantly enriched.

CONCLUSION: These findings underscore the molecular background and its complexity in MDS, highlighting specific epigenetic players HDAC (HDAC3, 5, 7, 9, and 11) and SIRT members (SIRT3, 4, and 7) as potential biomarkers or therapeutic targets. The preliminary data demonstrated that the differential expression patterns and their association with disease progression and oncogenic pathways suggest a pivotal role for histone deacetylation in MDS pathogenesis and leukemic transformation.

Keywords: Epigenome; histone deacetylases; myelodysplastic syndrome.

PAPER CODE: 60

Revisiting PAX5: Molecular Insights into Childhood B-Cell Precursor Acute Lymphoblastic Leukemia

DA SILVA, P.V.B.. NATIONAL CANCER INSTITUTE - INCA, RIO DE JANEIRO - RJ - BRASIL.

Introduction: B-cell precursor acute lymphoblastic leukemia (B-ALL) is the most common pediatric hematologic malignancy, accounting for about 85% of cases. Its development involves genetic and cytogenetic alterations that affect B-cell differentiation, maturation, and proliferation, compromising the adaptive immune response. Among the most frequently altered genes, especially in the B-other subtype, PAX5 stands out as a key transcription factor for B-lineage commitment and identity maintenance. Although affected by diverse genetic and epigenetic mechanisms, PAX5 alterations remain underexplored, particularly in Brazil. Characterizing these changes is essential to advance biological understanding, refine prognostic stratification, and support the development of more precise, individualized therapies.

OBJECTIVES: This study aims to characterize the molecular profile of PAX5 in pediatric B-ALL, exploring genetic, epigenetic, and mRNA expression alterations to clarify its role in disease development.

METHOD: A retrospective analysis was performed on 231 pediatric B-ALL cases (0–18 years) diagnosed from 2015 to 2019. Copy number alterations (CNAs) were assessed using Multiplex Ligation-dependent Probe Amplification (MLPA probemix P335). Genetic variants and PAX5 promoter DNA methylation were analyzed by pyrosequencing (PyroMark Q96). Gene expression was quantified by RT-qPCR using two Taqman probes, targeting the initial (probe 1) and the terminal (probe 2) regions of the gene.

RESULTS: The study included 231 pediatric cases: 54.5% male, 53.7% non-white, 52.4% aged 0–4 years, and 53.7% B-Others subtype. Only 3.5% had a family history of hematologic malignancies. PAX5 CNAs occurred in 27% of the cases, with a significant higher frequency in the B-Others group. Deletions were more common (18%),

being mainly partial and monoallelic, and affecting exon 8. Amplifications were observed in 9% of the cases and were distributed along the gene. Gene expression analysis showed a higher expression of the initial region of the gene, especially in the Classical subtype and in KMT2A-r and ETV6::RUNX1 subtypes. CNAs did not significantly affect expression. Methylation levels were low (<10%) in both subtypes and did not correlate with expression. The NM_016734(PAX5):c.239C>G (p.Pro80Arg) (P80R) variant was detected in one male patient (0.43%), 12 years old, B-Others subtype, carrying a PAX5 monoallelic deletion, 76% of blasts and a variant allele frequency of 73%.

CONCLUSION: In summary, PAX5 CNAs were the most frequent alteration, mainly monoallelic deletions in exon 8 affecting the transactivation domain. DNA methylation was low, suggesting a limited role in gene dysregulation. PAX5 expression was heterogeneous, more variable in the initial region of gene, with higher levels in subtypes where the gene is not expected to play a role. The somatic P80R variant was rare.

Keywords: B-cell precursor acute lymphoblastic leukemia; PAX5; copy number alterations.

PAPER CODE: 65

MOLECULAR INVESTIGATION OF COLORECTAL TUMORS DIAGNOSED IN YOUNG ADULTS

PEREIRA, J.P.1; PEREIRA, A.S.1; MARSILLAC, S.M.1; DAMASCENA, A.C.O.1; BASTOS, N.C.2; DE PAULA, D.J.G.1; GRASEL, R.S.1; DOS SANTOS, A.C.E.1; MOREIRA, M.Â.M.1; DE ALBUQUERQUE, C.G.P.3; TORRES, L.C.4; ASHTON-PROLLA, P.5; ANTONIAZZI, A.P.6; CARRARO, D.M.7; PALMERO, E.I.1. 1. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO NACIONAL DO CÂNCER, RIO DE JANEIRO - RJ - BRASIL; 3. INSTITUTO DE CÂNCER DO CEARÁ, FORTALEZA - CE - BRASIL; 4. INSTITUTO DE MEDICINA INTEGRAL PROFESSOR FERNANDO FIGUEIRA, RECIFE - PE - BRASIL; 5. HOSPITAL DE CLÍNICAS DE PORTO ALEGRE, PORTO ALEGRE - RS - BRASIL; 6. HOSPITAL DE AMOR DE BARRETOS, BARRETOS - SP - BRASIL; 7. AC CAMARGO CANCER CENTER, SÃO PAULO - SP - BRASIL.

INTRODUCTION: O câncer colorretal (CCR) é um dos tumores sólidos mais comuns e ocupa o terceiro lugar entre os cânceres mais incidentes em todo o mundo. Em contraste com o CCR em adultos mais velhos, o câncer colorretal de início precoce (CCR-OE), definido como diagnóstico aos 50 anos ou menos, tem apresentado incidência crescente na última década. Embora alterações na linha germinativa contribuam para o desenvolvimento precoce da doença, a maioria dos casos de CCR-OE não é atribuída a síndromes hereditárias de predisposição ao câncer. Uma investigação abrangente dos fatores associados a esses casos é necessária para aprimorar a compreensão e pode ajudar a elucidar os mecanismos moleculares subjacentes à tumorigênese precoce, bem como orientar estratégias terapêuticas.

OBJECTIVES: Este estudo tem como objetivo identificar a base molecular dos tumores colorretais diagnosticados em indivíduos jovens e caracterizá-los em termos de características clinicopatológicas, sociodemográficas e de exposição.

METHOD: Serão incluídos 500 indivíduos diagnosticados com CCR com idade ≤ 50 anos, recrutados em centros de referência em oncologia (SUS) de diferentes regiões do Brasil. Dados clinicopatológicos, sociodemográficos e de exposição ambiental serão armazenados no RedCap. O DNA tumoral será analisado utilizando o painel TruSight Oncology 500 (TSO500) para identificar variantes de nucleotídeo único (SNVs), variações no número de cópias (CNVs), instabilidade de microssatélites (MSI) e carga mutacional tumoral (TMB).

RESULTS: Até o momento, 324 indivíduos foram incluídos, dos quais 148 já possuem dados coletados, e 70,3% são do sexo feminino. A maioria dos casos foi diagnosticada com câncer no cólon (66,5%), seguido pelo reto (31,9%), ceco (0,8%) e flexura hepática (0,8%). A média de idade no diagnóstico foi de 40,8 anos (variação de 18 a 50), com mediana de 43 anos, variando de acordo com o local do tumor: 42,4 anos para o reto, 41,3 anos para o cólon sigmoide e 40,1 anos para o cólon direito, esquerdo e transversal. Em relação ao grau histológico, 82,6% dos indivíduos foram classificados como de baixo grau (bem a moderadamente diferenciados). O DNA tumoral de 49 pacientes foi extraído e quantificado. Além disso, amostras de tecido

tumoral já foram coletadas dos seguintes centros: INCA (183), HCPA (87), HA-Barretos (48), ICC (5) e HCP (1).

CONCLUSION: Por fim, o estudo demonstrou predomínio de casos em mulheres, sendo o cólon o sítio tumoral mais frequente. O seccionamento das amostras coletadas está em andamento para a extração do DNA tumoral, seguida da caracterização das variantes identificadas por meio do painel somático. A análise por meio deste painel permitirá a identificação de alterações moleculares que podem aprimorar a compreensão dos mecanismos associados ao desenvolvimento do câncer colorretal de início precoce.

Keywords: colorectal cancer; early-onset colorectal cancer; somatic analysis.

PAPER CODE: 69

EVALUATION OF ASSOCIATION BETWEEN EZH2 AND FLT3 IN T CELL ACUTE LYMPHOBLASTIC LEUKEMIA

BORBA, L.M.1; LEMOS, R.M.D.B.1; COSTA, E.S.2; FERRAZ, T.3; SCHRAMM, M.T.4; LINS, M.M.5; BARBOSA, T.D.C.1; LOPES, B.D.A.6; BLUNCK, C.B.1; MACIEL, A.L.T.7; DE SÁ, M.E.C.1; FAGUNDES, M.C.N.1. 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. IPPMG-UFRJ, RIO DE JANEIRO - RJ - BRASIL; 3. HEMORIO, RIO DE JANEIRO - RJ - BRASIL; 4. PRONTOBABY/INCA, RIO DE JANEIRO - RJ - BRASIL; 5. IMIP, RECIFE - PE - BRASIL; 6. INCA/UERJ, RIO DE JANEIRO - RJ - BRASIL; 7. UFPR, CURITIBA - PR - BRASIL.

INTRODUCTION: T-cell acute lymphoblastic leukemia (T-ALL) affects T-lymphoid cells and accounts for 10–15% of pediatric and 25% of adult leukemia cases. The early T-cell precursor subtype (ETP-ALL) is aggressive with poor prognosis. Mutations in the Polycomb Repressive Complex 2 (PRC2), particularly EZH2, disrupt epigenetic regulation, reducing H3K27me3 and activating pathways like JAK/STAT that promote leukemia. EZH2 mutations also appear linked to FLT3 overexpression (a tyrosine kinase receptor essential for hematopoiesis), commonly mutated in ETP-ALL, suggesting FLT3 as a PRC2 target gene.

OBJECTIVES: This study investigates the relationship between EZH2 mutations and FLT3 expression in T-ALL.

METHOD: Two independent T-ALL cohorts were analyzed: (1) publicly available data from the TARGET initiative and (2) patient samples from our laboratory. We characterized all cases for EZH2 and FLT3 mutations, their mRNA expression, and CDKN2A/B deletions. RNA sequencing and exome sequencing data from the TARGET cohort were analyzed, while PCR followed by Sanger sequencing and real-time quantitative PCR (RT-qPCR) were performed on our laboratory cohort.

RESULTS: In the TARGET cohort (n=260), median age was 9 years, mostly males (76.5%), with high WBC counts (50.3%). 6.9% of patients had CNS involvement and 5.4% died. EZH2 mutations were detected in 14 patients (5.4%), FLT3 mutations in 3 (1.2%), and FLT3 overexpression in 64 (24.6%). Notably, 1.5% showed EZH2 mutations also exhibited FLT3 overexpression. Mutations in NOTCH1 and FBXW7 occurred in 73.1% and 23.8%. In our cohort (n=130), the median age was 9, mostly males (69.2%), with high leukocyte counts (98.3%). CNS involvement was found in 7.2% and 22.2% died. CDKN2A/B biallelic deletions occurred in 42 patients (37.2%), monoallelic deletions in 30 (26.5%) and wild-type status in 40 (37.4%). CDKN2A biallelic deletions were found in 55 (48.7%) and CDKN2B in 36 (31.9%). Monoallelic gains in CDKN2A and CDKN2B were detected in 2 (1.5%). FLT3-ITD analysis (n=36) was wild-type status in all cases. Ongoing analyses of mutations and expression status are being performed in our laboratory.

CONCLUSION: These preliminary results suggest a possible association between EZH2 mutations and FLT3 overexpression in driving T-ALL progression. Furthermore, the frequency of NOTCH1 mutations and CDKN2A/B deletions are common events in T-ALL, underscoring the molecular complexity of this disease and indicating that multiple genetic alterations may act synergistically to promote the aggressiveness.

Keywords: T-cell acute lymphoblastic leukemia (T-ALL); Polycomb Repressive Complex 2 (PRC2); FLT3.

PAPER CODE: 70

Analysis of Genetic Variants in TPMT, NUDT15, and ITPA and Their Association with Thiopurine Toxicity in Children with Acute Lymphoblastic Leukemia

DAROS, A.N.1; LINS, M.M.2; JÚNIOR, A.D.A.M.C.2; GOMES, R.G.2; IKOMA-COLTURATO, M.R.V.3; CALANCA, N.3; ROSA, V.M.4; DE SÁ, M.E.C.5; LOPES, B.D.A.5. 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. PEDIATRIC ONCOLOGY UNIT, INSTITUTO DE MEDICINA INTEGRAL PROF FERNANDO FIGUEIRA, RECIFE - PE - BRASIL; 3. CENTER FOR ADVANCED RESEARCH IN PRECISION MEDICINE, AMARAL CARVALHO HOSPITAL, JAÚ - SP - BRASIL; 4. PEDIATRIC ONCOLOGY UNIT, HOSPITAL ARAÚJO JORGE, GOIÂNIA - GO - BRASIL; 5. GENETICS OF ACUTE LEUKEMIA LABORATORY - GENLAB, INSTITUTO NACIONAL DE CÂNCER, INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Acute lymphoblastic leukemia (ALL) is characterized by the accelerated proliferation of blasts in the bone marrow, which reduces the production of normal blood cells and leads to the accumulation of immature cells. Chemotherapy is essential to achieve remission and cure in patients with ALL. It includes thiopurines, however some patients may experience toxic effects as a consequence of variations in thiopurine metabolism, which is mediated by the enzymes TPMT, NUDT15, and ITPA. In Brazil, there is a scarcity of studies demonstrating the clinical association between drug-induced toxicity during ALL treatment and genetic variants in genes related to thiopurine metabolism.

OBJECTIVES: Evaluate thiopurine-related toxicities associated with pharmacogenetic variants of TPMT (rs1142345, rs1800460, rs1800462), NUDT15 (rs116855232), and ITPA (rs7270101) genes in children with ALL.

METHOD: We included pediatric patients diagnosed with ALL and treated under the RELL and GBTLI 2021 protocols. Genotyping of TPMT, NUDT15, and ITPA was performed by RT-qPCR using allelic discrimination assays. Statistical analyses, including odds ratio (OR) and significance tests, were performed to assess the association between genotypes and toxicity events. We also investigated the expression of the ITPA gene using

RNA-seq data to explore its relationship with the ITPA rs7270101.

RESULTS: Among 289 patients, 84.4% had B-ALL and 14.6% T-ALL, with 40.8% females and 59.2% males. Variant allele frequencies were 4.9% (TPMT rs1142345), 2.1% (rs1800460), 1.2% (rs1800462), 0.7% (NUDT15 rs116855232), and 12.7% (ITPA rs7270101). Febrile neutropenia (FN) was among the most frequent (29.1%) toxicities. Although no associations were found for TPMT or NUDT15, ITPA variant genotypes (A/C + C/C) were significantly associated with higher FN risk (OR=3.03; 95%CI: 1.76–5.21; p=0.018) and neutropenia (OR=1.93; 95%CI: 1.02–3.65; p=0.03). Carriers of the C allele showed nearly threefold higher FN risk. Expression analysis revealed differential ITPA transcript levels among its genotypes (p = 0.008), which may be a consequence of this splicing site variant. These findings highlight ITPA rs7270101 as a potential predictor of thiopurine toxicity in the Brazilian population.

CONCLUSION: The significant association between the ITPA gene and the occurrence of neutropenia and FN is novel, highlighting its potential role as a predictive marker of therapeutic toxicity. Gene expression analyses suggest that rs7270101 may influence the splicing of ITPA transcripts, indicating a possible functional loss-of-domain effect. These findings support the use of pharmacogenetics in therapy individualization and the reduction of adverse events during treatment.

Keywords: ALL; thiopurine; toxicity.

PAPER CODE: 71

Evaluation of the role of lncRNAs in the deregulation of CRLF2 in patients with precursor B-cell acute lymphoblastic leukemia

LUCAS, M.D.V.1; MACIEL, A.L.T.2; POUBEL, C.D.A.P.1; BASTOS, A.C.F.3; BLUNCK, C.B.1; FAGUNDES, M.C.N.1; LOPES, B.D.A.1; BORONI, M.1; DE SÁ, M.E.C.1; BARBOSA, T.D.C.1. 1. INSTITUTO NACIONAL DE CÂNCER - INCA, RIO DE JANEIRO - RJ - BRASIL; 2. FEDERAL UNIVERSITY OF PARANÁ (UFPR), CURITIBA - PR - BRASIL; 3. NATIONAL CENTER FOR RESEARCH IN ENERGY AND MATERIALS (CNPEM), SÃO PAULO - SP - BRASIL.

INTRODUCTION: Acute lymphoblastic leukemia of B-cell precursor lineage (B-ALL) shows high genetic heterogeneity with frequent alterations in CRLF2. However, in approximately 50% of CRLF2 overexpressed cases (CRLF2-high), the underlying regulatory mechanism remains unknown. Since CRLF2 activates the JAK/STAT pathway, its dysregulation contributes to leukemogenesis and poor prognosis in B-ALL. Previous study indicates a potential mechanistic role of lncRNAs differentially expressed (DE) in CRLF2-high patients in the dysregulation of CRLF2.

OBJECTIVES: This study investigates how LINC00161, PWRN1 and RPL34-AS1 contribute to CRLF2 dysregulation in B-ALL, exploring their potential regulatory mechanisms.

METHOD: We analyzed 126 B-ALL diagnostic cases from the TARGET cohort, characterized by WGS and RNA-Seq data. Correlation analysis of LINC00161, PWRN1, RPL34-AS1, their predicted mRNAs targets and CRLF2 expression was performed in R Studio. The functional impact of these lncRNAs will be tested by silencing in MHH-CALL4 and MUTZ5 cell lines, followed by qPCR, and viability, proliferation and apoptosis assays. B-ALL samples by FISH and RNA-Seq will be used to validate the CRLF2-lncRNAs interactions.

RESULTS: Correlation analysis revealed that the positive association between lncRNA and CRLF2 expression levels is subtype-dependent. Specifically, RPL34-AS1 and PWRN1 showed significant positive correlations with CRLF2 expression in BCR::ABL1, TCF3::PBX1 and KMT2A-r subtypes. In contrast, LINC00161 was positively correlated with CRLF2 only in TCF3::PBX1 and KMT2A-r subtypes. Interestingly, among patients without known primary molecular alterations (B-others), only RPL34-AS1 was correlated with CRLF2 ($R=0.52$; $p=0.00041$). These correlations were not observed in ETV6::RUNX1, Ph-like and hyperdiploid subgroups. Additionally, we investigated previously reported interactions between these lncRNAs and cancer-related mRNA targets. Among them, only the interaction between LINC00161 and MIR21 was confirmed in Ph-like, hyperdiploid and TCF3::PBX1 patient subgroups.

CONCLUSION: Preliminary findings suggest a potential mechanistic role for LINC00161, PWRN1 and RPL34-AS1 in the dysregulation of CRLF2 expression in B-ALL. Functional validation may uncover novel molecular mechanisms, enhancing our understanding of B-ALL pathogenesis and paving the way for the clinical application of lncRNAs in diagnosis and therapy. This study underscores the importance of integrating functional and clinical analyses to advance diagnostic and therapeutic strategies in leukemia.

Keywords: B-ALL; lncRNAs; regulation mechanism.

PAPER CODE: 72

INVESTIGATION OF IL7R DYSREGULATION IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

LEMOs, R.M.D.B.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematopoietic disorder triggered by the acquisition of molecular alterations that affect thymocytes during its development. The activation of IL7R, either due to the presence of gain-of-function mutations (~10% of cases) or its upregulation, is a molecular event associated with T-ALL relapse. In particular, the overexpression of IL-7R α activates the JAK/STAT, PI3K/Akt/mTOR, and NOTCH pathways in mice, even in the absence of IL7R mutations.

OBJECTIVES: Here, we aimed to evaluate the role of IL7R activation in patients diagnosed with T-ALL.

METHOD: We analyzed two independent T-ALL cases series: 1) data available online (TARGET); 2) samples received in our laboratory. All cases were characterized according to the presence of IL7R mutations/SNP and transcriptional levels of the genes of interest. To this end, we evaluated previously processed RNA sequencing and exome sequencing data in the TARGET set, and PCR followed by Sanger sequencing and qPCR in the second set. Patients with IL7R overexpression were classified based on fourth quartile.

RESULTS: Activating IL7R mutations were found in 6.8% of TARGET patients and were associated with IL7R higher transcriptional levels. We compared subgroups according to the expression of genes involved in cellular pathways related to lymphopoiesis: IL7R mutated vs. wild-type and, excluding all patients with IL7R mutations, IL7R overexpressed vs. low-expressed. IL7R mutations were associated with increased transcription of JAK-STAT pathway genes, while IL7R overexpression increases the transcription levels of genes involved on JAK-STAT, PI3K/mTOR and Ras/MAPK pathways. Of these, we highlight MYC, NRAS, PTPN2, and CD4. In the second set, 74 samples have been included, 58 pediatric and 16 adult cases. 2 presented activating mutations in IL7R and 33.8% presented SNP rs6897932 in exon 6. We did not observe an impact of the presence of mutations or SNPs on IL7R expression.

CONCLUSION: Corroborating with the previous data performed in mice model, here we demonstrated that in human T-ALL, the IL7R overexpression in the absence of activating mutation might lead to the activation of cellular pathways involved in lymphocytes development and leukemogenesis.

Keywords: T-ALL; IL7R; Mutations.

PAPER CODE: 73

Evaluation of TWIST1 and TERT Proteins Expression and Telomere Length as Prognostic Biomarkers in Pediatric Ewing Sarcoma

SILVESTRE, R.T.1; LAVIGNE, T.S.2; DE LIMA, G.F.2; MOREIRA, V.G.2; BASTOS, N.C.2; FERMAN, S.E.2; FERNANDEZ, T.D.S.2. 1. INSTITUTO NACIONAL DE CANCER - INCA, RIO DE JANEIRO - RJ - BRASIL; 2. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Ewing sarcoma (ES) is a malignant small round cell tumor, mainly defined by the EWSR1::FLI1 fusion present in 85% of cases with an estimated incidence of about 2.1 per million children globally. The 5-year disease-free survival rate for localized ES is approximately 60-70%. However, due to its aggressive behavior, nearly one-third of patients develop

metastatic, recurrent, or refractory disease, significantly reducing survival. In metastatic ES, overexpression of the transcription factor TWIST1 is strongly associated with enhanced invasion, poor prognosis, and shorter survival. Furthermore, ES genomic instability and tumor biology have been linked to TERT expression and telomere dynamics, as alterations in these mechanisms drive tumor aggressiveness and unfavorable outcomes.

OBJECTIVES: To evaluate TWIST1 and TERT expression and telomere length in pediatric Ewing sarcoma, aiming to identify their role as prognostic biomarkers and in understanding tumor biology.

METHOD: Immunohistochemical analysis for TWIST1 and TERT proteins was performed on paraffin-embedded tumor samples from pediatric patients with Ewing sarcoma enrolled at INCA between 2012 and 2021. DNA extraction was performed using the ReliaPrep FFPE gDNA Miniprep System kit (Promega) following the manufacturer's protocol. Relative telomere length was performed by qPCR, calculating the T/S ratio (T: telomeric repeat region; S: single-copy gene, human β -globin) using primers for small fragments.

RESULTS: Thus far, 20 samples from pediatric patients with Ewing sarcoma have been analyzed, of which 12 (60%) were female and 8 (40%) were male, with a mean age of 12.3 years (SD = 3.7 years). Metastatic disease was observed in 50% of cases, and the oncogenic fusion EWSR1::FLI1 was confirmed in 45%. The immunohistochemical analysis of TERT revealed two patterns of expression, positive in 50% of samples and absent in 45% and one was not analyzed due to insufficient tumor tissue. In turn the TWIST1 expression analysis was positive in all samples: 75% strong, 15% moderate, one weak, and one not evaluated. Relative telomere length was assessed in 10 samples (8 female, 2 male; mean age 11.6 years), with a mean T/S ratio of 0.85. Among these, 45% had metastasis, in 70% EWSR1::FLI1 fusion was detected, 70% showed negative TERT expression, and 80% had strong TWIST1 expression in tumor tissue.

CONCLUSION: Our preliminary results demonstrate variations in the expression profile of TWIST1 and TERT in Ewing sarcoma, and the presence of the EWSR1::FLI1

fusion and shorter telomeres in some samples indicate a relationship between telomere dynamics and tumor aggressiveness. We plan to expand our analysis to include new retrospective cases to identify prognostic factors and, in prospective studies, identify the EWSR1 fusion through liquid biopsy to aid in diagnosis and monitoring therapeutic response.

Keywords: Pediatric Ewing's sarcoma; TWIST1; TERT.

PAPER CODE: 76

RNA genetic testing as a tool to improve hereditary breast cancer diagnosis

GOMES, R.; AZEVEDO, L.B.D.B.E.; TELES, K.D.A.; DOS SANTOS, R.O.J.; DOS SANTOS, A.C.E.; MOREIRA, M.A.M.. INSTITUTO NACIONAL DE CANCER, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Breast cancer (BC) is associated with pathogenic germline variants in approximately 15% of cases. Identifying these variants can improve treatment outcomes for patients and their families while reducing healthcare costs related to diagnosis and therapy. Although DNA sequencing (DNA-seq) has advanced genetic diagnosis in the last years, roughly 70% of individuals tested remain without a conclusive result. RNA sequencing (RNA-seq) can help address this gap by revealing additional molecular mechanisms that contribute to inherited cancer risk. Recent studies have shown that integrating RNA sequencing significantly increases the diagnostic yield of genetic testing, thereby enhancing clinical management for patients and their relatives.

OBJECTIVES: To identify molecular mechanisms of inherited breast cancer risk via RNA-seq of puromycin-treated lymphocytes from patients negative on DNA panel testing.

METHOD: Ninety-six BC patients (48 women $\geq$ 30 years at diagnosis and 48 men) will be selected among individuals previously tested negative for pathogenic variants in 8 hereditary BC genes. PBMCs will be isolated and cultured for 120h, treated with puromycin for the

last 4h, and processed for RNA isolation using TRIzol. RNA libraries will be prepared with TruSeq Stranded Total RNA kit (RiboZero Plus) and sequenced on the NovaSeq X Plus platform. RNA-seq data will be analyzed using nextflow-based pipeline.

RESULTS: A total of 162 patients met the inclusion criteria, had their DNA isolated, and underwent targeted gene panel sequencing, which includes eight genes associated with hereditary breast cancer (ATM, CHEK2, BARD1, BRCA1, BRCA2, PALB2, RAD51C, and TP53). To date, 50 patients have been excluded due to the presence of pathogenic variants, 33 will be resequenced because of insufficient coverage ($>30\%$) across target regions, and 79 will undergo Sanger sequencing to fill low-coverage regions of the target genes. Lymphocyte isolation has been completed for 89 out of 112 eligible patients; among these, 65 had their lymphocytes cultured and treated with puromycin and are currently awaiting confirmation of DNA mutational status before proceeding with RNA preparation and sequencing

CONCLUSION: DNA panel sequencing and the collection of patients' PBMCs have proven to be challenging. Over the next three months, we expect to complete DNA sequencing and PBMC collection in order to initiate RNA library preparation and data analysis.

Keywords: splicing; hereditary cancer; spliceogenic variants.

PAPER CODE: 77

Development and Validation of a qPCR Method for Quality and Safety Assessment of Non-Viral CAR-T Cell Products

DE GODOY, D.T.; DOS REIS, S.A.; NEUMANN, A.; ABDO, L.; ARAGÃO, E.; DOURADO, L.P.S.; BONAMINO, M.H.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Cellular immunotherapy with Chimeric Antigen Receptor (CAR) T cells represents a major advance in cancer treatment. However, the currently approved therapies rely on viral vectors for gene transfer, which increase costs and complexity.

Our group developed a non-viral strategy using the Sleeping Beauty (SB) transposon system, based on plasmid electroporation to generate CAR-T cells in a safer and more economical way. For clinical application, regulatory agencies require rigorous quality control, including determination of the vector copy number (VCN) integrated into the genome, which is directly related to product safety. Quantitative PCR (qPCR) is a reliable method for this purpose, allowing accurate quantification of integrated sequences.

OBJECTIVES: To develop and validate a qPCR-based assay for vector copy number determination as part of the quality control of non-viral anti-CD19 CAR-T cell products generated with the Sleeping Beauty system.

METHOD: CAR-T cells were generated by electroporation with the PT4-19BBz transposon and SB100X transposase plasmids. Genomic DNA was extracted for qPCR amplification targeting the FMC63 scFv transgene. Specific primers and probes were designed, and amplification efficiency, linearity, and correlation coefficient (r^2) were determined. To distinguish integrated from non-integrated plasmids, treatment with DpnI enzyme was evaluated. Results were analyzed to establish method accuracy and reproducibility.

RESULTS: The qPCR assay successfully detected the FMC63 scFv sequence in genomic DNA from CAR-T cells generated with the Sleeping Beauty system. Standard curves showed linearity with correlation coefficients around $r^2 = 0.98$, indicating good quantification performance. Amplification efficiency was consistent across replicates. Preliminary results demonstrated the method's ability to detect transgene copy number. However, residual non-integrated plasmid DNA may lead to overestimation. To overcome this limitation, enzymatic digestion with DpnI was implemented, effectively eliminating non-integrated plasmid. This refinement will enable accurate quantification of integrated vector copies. The validated assay will ensure reliable VCN measurement as a key safety parameter in the quality control of CAR-T products.

CONCLUSION: We developed a qPCR-based assay capable of detecting and quantifying integrated

transgene copies in non-viral CAR-T cells. Preliminary data demonstrated robust performance and linearity. Ongoing validation with DpnI digestion will ensure accurate vector copy number determination, meeting regulatory requirements. This approach provides an essential and cost-effective tool for quality and safety assessment of CAR-T products manufactured using the Sleeping Beauty system.

Keywords: CAR-T cells; Vector copy number; Quality control.

PAPER CODE: 82

STUDY OF THE ROLE OF POLYCOMB PROTEIN - EZH2 AND ITS ASSOCIATION WITH THE PRESENCE OF EWSR1 REARRANGEMENT IN PEDIATRIC EWING SARCOMA

LAVIGNE, T.S.1; MOREIRA, V.G.1; BASTOS, N.C.1; DE LIMA, G.F.2; DE FARIAS, C.B.3; FERNAN, S.E.2; FERNANDEZ, T.D.S.2. 1. INSTITUTO NACIONAL DE CÂNCER - INCA, RIO DE JANEIRO - RJ - BRASIL; 2. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL; 3. INSTITUTO DO CÂNCER INFANTIL, LABORATÓRIO DE PESQUISAS EM CÂNCER, PORTO ALEGRE, RS, PORTO ALEGRE - RS - BRASIL.

INTRODUCTION: Ewing's Sarcoma (ES) is a rare, aggressive tumor that primarily affects bones. It is the second most common bone tumor in children. This tumor is characterized primarily by the presence of a cytogenetic alteration, t(11;22)(q24;q12), resulting in a gene fusion EWSR1::FLI1, and in a chimeric protein, EWS-FLI1, which is directly involved in transcriptional regulation, tumor cell immortalization, and chemotherapy resistance. EZH2 activity is dysregulated in cancer, contributing to tumorigenesis, and it represents an important target for anti-cancer therapy. Epigenetic dysregulation has been studied in cases of ES, and the Polycomb protein EZH2 has been indicated as a possible prognostic biomarker.

OBJECTIVES: To analyze the EZH2 expression profile, its association with EWSR1 rearrangement, and its impact as a prognostic biomarker in pediatric patients with ES.

METHOD: Seventy-eight pediatric patients (0-18 years) diagnosed with ES between 2012 and 2022 were studied. Fluorescence in situ hybridization (FISH) was used to analyze EWSR1 rearrangement, and immunohistochemistry (IHC) was used to evaluate EZH2 expression in SE paraffin samples. The clinical and demographic characteristics of the patients were obtained through an analysis of medical records.

RESULTS: Of the total patients studied, 55% were male and 45% female. Most patients, 79% were between 12 and 18 years old. The median age of all patients was 14 years. Regarding race, 47% self-reported white, 37% mixed race, and 12% black. Metastases were observed in 45% of patients at diagnosis and in 54% after treatment. Tumors occurred in bones (56%) and soft tissues (44%). FISH was performed in 51 patients, detecting the rearrangement in 82% of patients. EZH2 expression analysis was performed in 73 pediatric patients with ES, of which 55% showed high expression, 26% intermediate expression, 4% low expression, and 15% no expression. An association was observed between high and intermediate EZH2 expression and the presence of EWSR1 rearrangement. Patients who presented metastases at diagnosis had the following results when associated with EZH2 expression: 55% of patients had high expression, 24% had intermediate expression, 3% had low expression, and 18% had no expression.

CONCLUSION: Our results showed that EWSR1 rearrangement and EZH2 expression profile are important biomarkers for the diagnosis and evaluation of the clinical evolution of pediatric patients with ES, respectively. Evaluation of the EZH2 expression profile by IHC may be a novel molecular test in clinical practice. Understanding the complexity of the disease is important for the development of new studies on novel treatment strategies.

Keywords: Ewing's Sarcoma; EWSR1; Polycomb EZH2 protein.

PAPER CODE: 83

Advancing CAR-T Cell Engineering: Hybrid Transposons as Cost-Effective non-Viral Alternatives

ABDO, L.1; ARAGÃO, E.1; NEUMANN, A.1; CORREIA, E.M.1; RIBEIRO, L.1; BONAMINO, M.H.2. 1. BRAZILIAN NATIONAL CANCER INSTITUTE (INCA), RIO DE JANEIRO - RJ - BRASIL; 2. BRAZILIAN NATIONAL CANCER INSTITUTE (INCA); OSWALDO CRUZ FOUNDATION (FIOCRUZ), RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: CAR-T cell therapy is a major advance in treating B cell tumors, but its high cost—partly due to viral vectors—limits access, highlighting the need for alternative gene modifying tools. In this study, we used transposon-based tools called Sleeping Beauty (SB) and piggyBac (PB). Also, we designed hybrid vectors combining components from both systems to evaluate new constructs developed in our laboratory: SBnew and PBnew. These systems, SB and PB, were tested for their efficiency in inserting the CAR gene into T cells

OBJECTIVES: To compare SB and PB transposon systems and their hybrid versions to develop a more efficient and affordable non-viral method for CAR-T cell production.

METHOD: SB used PT2, PT3, and PT4 transposons with the SB100x transposase, while PB transposon with the PBbase transposase. T cells were isolated, electroporated with plasmid DNA, activated, and expanded. In vitro and in vivo experiments were performed to evaluate CAR expression, memory phenotype, exhaustion markers, tumor burden, and survival rates in mouse models.

RESULTS: SB groups showed a consistent increase in CAR expression over 12 days, while PB had high initial expression that declined over time. CAR-T cells modified using both transposon systems improved survival in mice, with PB demonstrating the lowest tumor burden and highest survival rates. We therefore tested the newly developed constructs to better understand the factors behind PB's higher efficiency, given that the same CAR dose was administered. In vivo testing of CAR-T cells modified with SBnew and PBnew—a hybrid version—showed that SBnew performed better than the original

SB, while PBnew did not show improvements compared to PB. We conclude that transposon-based vectors can efficiently mediate CAR gene insertion, with PB proving more effective in reducing tumor burden and improving survival.

CONCLUSION: Moreover, our results suggest that specific regions from the PB construct may enhance the efficiency of the SB system in CAR-T cells context. This advancement could significantly broaden patient access to CAR-T cell therapy, as these tools are more cost-effective and less labor-intensive than viral approaches.

Keywords: CAR-T cells; immunotherapy; Transposon system.

PAPER CODE: 87

Nationwide prevalence analysis of the Brazilian founder mutation R337H in the TP53 gene

ZANELLA, K.M.1; CABRAL, B.C.A.1; GONÇALVES, C.N.1; PEREIRA, A.S.1; DE MARSILLAC, S.M.1; PEREIRA, J.P.1; RIBAS, F.S.B.D.S.2; MOREIRA, S.J.M.2; DE CARVALHO, E.M.2; DE PAULA, D.J.G.3; PINTO, L.F.R.4; MOREIRA, M.Â.M.1; ACHATZ, M.I.W.5; PALMERO, E.I.1. 1. TUMOR GENETICS AND VIROLOGY PROGRAM, NATIONAL CANCER INSTITUTE (INCA), RIO DE JANEIRO - RJ - BRASIL; 2. DIAGNOSTIC SUPPORT UNIT OF RIO DE JANEIRO (UNADIG - FIOCRUZ), RIO DE JANEIRO - RJ - BRASIL; 3. NATIONAL TUMOR AND DNA BANK OF THE NATIONAL CANCER INSTITUTE (BNT-INCA), RIO DE JANEIRO - RJ - BRASIL; 4. MOLECULAR CARCINOGENESIS PROGRAM, NATIONAL CANCER INSTITUTE (INCA), RIO DE JANEIRO - RJ - BRASIL; 5. DEPARTMENT OF ONCOLOGY, CANCER GENETICS UNIT, HOSPITAL SIRIO-LIBANES, SÃO PAULO - SP - BRASIL.

INTRODUCTION: Cancer is a public health problem in Brazil, and approximately 10–20% of cases are associated with hereditary cancer predisposition syndromes (HCPS). Li-Fraumeni Syndrome (LFS) is an HCPS that confers a lifetime cumulative risk of up to 90% for multiple tumors, including breast, sarcomas, adrenocortical, and brain cancers. In the South and Southeast regions of Brazil, the prevalence of LFS is

exceptionally high compared to the global average (1:300 vs. 1:5,000–1:20,000), due to the Brazilian founder variant c.1010G>C (p.Arg337His/R337H) in the TP53 gene, known as the “guardian of the genome.” This variant increases cancer predisposition and poses a public health challenge due to its high regional frequency. However, its prevalence in other regions of the country remains unknown.

Objectives: This study aims to determine the nationwide prevalence of the pathogenic germline variant R337H in the TP53 gene.

METHOD: A total of 25,000 anonymized newborn screening samples donated by the National Neonatal Screening Program are being analyzed, equally distributed across the five Brazilian regions. DNA from these dried blood samples is extracted using the MagMAX DNA Multi-Sample 2.0 kit on the KingFisher Apex system (Thermo Fisher). Genotyping is performed by real-time PCR (TaqMan® SNP Genotyping) with wild-type and mutant controls. Positive cases are confirmed by Sanger sequencing of exon 10 of the TP53 gene.

RESULTS: To date, 10,000 samples have been analyzed: 5,000 from the North (Belém–PA) and 5,000 from the Northeast (Fortaleza–CE/Teresina–PI). In the North, the R337H variant was identified in heterozygosity in one sample (prevalence 0.02% – 1:5,000), similar to that described for LFS in the European population. In the Northeast, all samples were homozygous for the wild-type allele. In the Central-West (Cuiabá–MT), 3,962 samples were extracted, 368 genotyped, with no variant detected so far. Samples from the South and Southeast will be processed in the last quarter of 2025. The R337H variant is associated with the “Trapeiro hypothesis,” which suggests its origin and spread along the São Paulo–Rio Grande do Sul axis. The finding of this study, regarding the variant outside the South–Southeast axis, is highly relevant, as it shows its circulation beyond this axis, expands understanding of its geographic spread, and reinforces the importance of surveillance in less-studied regions.

CONCLUSION: Given that the R337H variant is strongly associated with rare neoplasms with high morbidity

and early onset, such as sarcomas, adrenocortical carcinoma, and choroid plexus carcinoma, its detection outside the South–Southeast axis (0.02%) is crucial, as it reflects internal migration in the Brazilian population and shows the need for public surveillance policies and personalized prevention and risk-reduction strategies adequate to the Brazilian reality.

Keywords: R337H; TP53 gene ;Li-Fraumeni syndrome.

PAPER CODE: 95

Development of colorimetric assay for detecting TMPRSS2-ERG gene fusion and PCA3 RNAs for prostate cancer diagnosis

DE BRITO, N.F.; PINTO, L.F.R.; MELENDEZ, M.E.. INSTITUTO NACIONAL DE CÂNCER (INCA), RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Prostate cancer (PCa) screening enables early detection while the disease is still curable, but current methods—serum PSA testing and digital rectal examination—have major drawbacks. PSA can rise in benign conditions, causing unnecessary biopsies, higher costs, and patient distress. Because PCa is clinically heterogeneous, precision medicine is needed for better risk stratification. Biomarkers such as TMPRSS2-ERG and PCA3 show higher specificity, reflecting early tumorigenic events. Their presence in urine supports non-invasive testing, yet current detection techniques like NGS and FISH are complex and unsuitable for routine screening.

OBJECTIVES: This project aims to develop a portable and low-cost colorimetric biosensor for detecting TMPRSS2-ERG and PCA3 RNAs in urine, simplifying PCa diagnosis.

Method: The assay is based on sandwich hybridization using specific capture and detection probes. TMPRSS2-ERG and PCA3 serve as molecular biomarkers, with the biotin/streptavidin-peroxidase system enabling colorimetric detection.

RESULTS: Biomarker detection was successfully demonstrated at different concentrations, with a significant correlation between concentration and absorbance readings. Standard curves with synthetic oligonucleotides exhibited a sigmoidal profile, similar to dose-response curves. The system allows for TMPRSS2-ERG quantification, which, although not essential for current diagnosis, may provide valuable insights in future studies. Tests with synthetic samples showed a linear detection range of 0.10–30 nM, and urine did not interfere with the analysis when spiked samples were used. Optimization of test conditions is underway before validation with patient samples.

CONCLUSION: This colorimetric biosensor has the potential to be integrated into early diagnosis and residual disease monitoring in the Unified Health System (SUS), improving patient management.

Keywords: prostate cancer; TMPRSS2-ERG; PCA3.

PAPER CODE: 96

CLINICAL AND MOLECULAR STUDY OF THE RS3824662 POLYMORPHISM IN GATA3 IN THE DEVELOPMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA IN ADOLESCENTS AND YOUNG ADULTS

DOS SANTOS, R.L.; AMARAL, T.F.; LUCAS, M.D.V.; BLUNCK, C.B.; LOPES, B.D.A.; DE SÁ, M.E.C.; BARBOSA, T.D.C.. INSTITUTO NACIONAL DE CÂNCER - INCA,, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: The rs3824662 single nucleotide polymorphism (SNP) in the GATA3 gene is a known risk factor for developing B-cell precursor acute lymphoblastic leukemia (B-ALL). The high prevalence of adverse genetic alterations, together with the absence of specific treatment protocols for adolescents and young adults (AYA), has negatively impacted the prognosis in this age group. Moreover, studies evaluating this SNP in GATA3 among AYA patients are scarce in the literature

OBJECTIVES: In this context, this study aims to evaluate the clinical and molecular characteristics related to the rs3824662 polymorphism in AYA B-ALL.

METHOD: DNA was extracted using the DNA Mini Blood Kit. The rs3824662 SNP was genotyped by TaqMan assay and validated by direct sequencing. The SNP was correlated with clinical and molecular variables, including qPCR-detected primary alterations (ETV6::RUNX1, TCF3::PBX1, BCR::ABL1, KMT2A::AFF1), BCR::ABL1-like and high hyperdiploidy by FISH, and additional alterations detected using SALSA MLPA P335-C1. Overall survival analysis were performed using survival and survminer package in R Studio.

RESULTS: Among the 104 patients analyzed, the median age was 19.5 years, 60% presented low white blood cell (WBC) counts, and the most frequent molecular subgroups were B-other (36.1%) and BCR::ABL1 (30.1%). GATA3 SNP genotypes were distributed as 40.4% wild-type (CC), 41.3% heterozygous (CA), and 18.3% mutant homozygous (AA). Low WBC predominated among heterozygous patients (66.7%, $P=0.004$). BCR::ABL1 and B-other subtypes were the most frequent in patients carrying the risk allele ($P<0.05$). BCR::ABL1-like subtype was more prevalent among mutant allele carriers (70% vs. 28.6%), who also showed frequent deletions in IKZF1, CDKN2A/B, and PAX5, but no deletions in the PAR1 region were observed. Although OS did not differ significantly across genotypes, independently of the genetic model tested, there was a trend toward poorer outcomes in AA patients. Curiously, the predominance of low WBC count in CA patients classifying these patients as good responders to treatment.

CONCLUSION: Our study reinforces the role of the rs3824662 polymorphism in GATA3 in AYA B-ALL, showing a high prevalence of the risk allele (A) in this cohort. The most prevalent subtypes were BCR::ABL1-like, BCR::ABL1, and B-other, regardless of the GATA3 SNP genotype, reflecting the cohort's genomic heterogeneity. The apparently favorable outcome in AC patients likely results from other prognostic factors, underscoring the need for larger cohorts to confirm the impact of this genotype on survival.

Keywords: GATA3; AYA B-ALL; overall survival.

PAPER CODE: 97

METHYLOME OF PEDIATRIC AND ADULT PATIENTS WITH MYELODYSPLASTIC SYNDROME AND ITS ASSOCIATION WITH CYTOGENETIC AND CLINICAL CHARACTERISTICS

DA SILVA, B.F.; CHRISTIANES, A.L.D.S.; LIMA, S.C.S.; FERNANDEZ, T.D.S.. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Epigenetic reprogramming has emerged as an enabling hallmark of cancer, making epigenetic alterations potential sources for diagnostic and prognostic biomarkers, as well as therapeutic targets. In hematopoietic stem cells, epigenetic mechanisms such as DNA methylation are essential for self-renewal and differentiation. Thus, myelodysplastic syndrome (MDS), a hematologic neoplasm characterized by bone marrow dysplasias and risk of progression to acute myeloid leukemia (AML), is considered a prototypical epigenetic disease. To date, only a few studies have analyzed the MDS methylome, and these have focused only on adult patients. However, pediatric MDS has distinct clinical, cytogenetic, and molecular characteristics underscoring the need for research that includes both age groups.

OBJECTIVES: Characterize and compare the methylome of pediatric and adult MDS patients, analyzing its association with karyotype and progression to AML, seeking diagnostic and prognostic biomarkers.

METHOD: Bone marrow (BM) cells were obtained from 80 pediatric and 80 adult MDS patients (2000–2025). Classification followed the WHO criteria (2022). Cytogenetic analysis was done by G-banding and fluorescence in situ hybridization. Methylome analysis was performed using the EPIC V2 Methylation array on 6 pediatric and 6 adult patients and 2 pediatric and 2 adult controls (BM healthy donors). Validation by pyrosequencing will be done in all 160 patients and in 25 pediatric and 25 adult controls.

RESULTS: The mean age at diagnosis was 8 years in pediatric patients and 53 years in adult patients. Cytogenetic analysis revealed an abnormal karyotype

in 42.5% (34/80) of pediatric patients and 36.3% (29/80) of adult patients. Evolution to AML occurred in 22.5% (18/80) of patients of both age groups. The global methylation analysis, after normalization and probe filtering, retained 12 samples (4 pediatric and 4 adult patients, 4 controls). Methylation analysis using M-values identified 24,740 differentially methylated probes (DMPs) with a $|\log_{10}FC| > 1.2$ between all patients and controls. Hierarchical clustering with these DMPs was able to separate patients from controls. Analyses of each age group were conducted independently. A comparison between the two groups revealed that pediatric patients had a total of 59,987 DMPs, of which 49,078 were unique to this age group. In contrast, adults had a total of 42,374 DMPs, with 31,465 DMPs being unique to adults. Both age groups shared 10,909 DMPs.

CONCLUSION: Our preliminary results suggest that MDS patients have an altered methylation profile compared to healthy controls. Pediatric patients have a higher number of DMPs than adults; however, there is an overlap of DMPs between them. We intend to continue this study in order to identify the altered signaling pathways. Also, analyze the associations between epigenetic alterations, the cytogenetic pattern, and clinical data, seeking to identify epigenetic markers for diagnosis and prognosis.

Keywords: Síndrome mielodisplásica; Metilação do DNA; Evolução leucêmica.

PAPER CODE: 99

Genetic variants potentially associated with congenital erythrocytosis

JUNG, L.V.B.M.; BULCHI, J.; GUTYAMA, L.M.; ZALCBERG, I.; DOS SANTOS, A.C.E.; MONTE-MÓR, B.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Erythrocytosis is an absolute increase in red blood cell mass, reflected by elevated hemoglobin and hematocrit, which may increase thromboembolic risk. It can be primary, caused by an intrinsic defect in the red cell compartment, or secondary, due to raised erythropoietin levels. Most cases are acquired, including polycythemia vera-PV

caused by JAK2V617F somatic mutation. Rare congenital cases are associated with germline mutations in oxygen sensing and transport pathways. Higher incidence of HFE mutations in erythrocytosis is also described. Next generation sequencing (NGS) is a key tool in the genetic investigation of idiopathic erythrocytosis (IE). Our group has previously applied a custom NGS panel to study 20 IE cases and found known and novel genetic variants in VHL, HBB and BHLHE41.

OBJECTIVES: The aim of this study is to analyze genetic variants potentially implicated in erythrocytosis, validating the rare mutations identified by NGS and screening for HFE mutations in Brazilian patients.

METHOD: The study was approved by INCA's Ethics Committee (CAAE: 60711722.0.0000.5274). Peripheral blood samples from patients aged 18 years or older presenting erythrocytosis were referred to the Molecular Biology Laboratory, INCA. DNA was extracted using Maxwell automated system. RNA was extracted using Trizol, followed by cDNA synthesis. Target gene regions were amplified by PCR/RT-PCR, using specific primers. Amplicons were analyzed by electrophoresis, purified, and subjected to Sanger sequencing.

RESULTS: We analyzed 4 genetic variants in erythrocytosis. A pathogenic VHL p.Arg176Gly mutation was confirmed as germline by Sanger sequencing of blood and buccal DNA in a male IE patient; his asymptomatic son also carried the variant. As VHL mutations in IE are usually homozygous or compound heterozygous, we hypothesize that additional non-coding VHL variants could play a role. A pathogenic HBB p.Glu102Gly mutation was confirmed in a female patient with familial erythrocytosis. HBB encodes the beta subunit of hemoglobin; the high-oxygen-affinity variant causes erythrocytosis. A novel BHLHE41 splice-site variant c.346+1G>C was also validated. This gene encodes a transcription factor involved in HIF-1 proteasomal degradation. Although pathogenic mutations are not known in IE, this variant warrants further investigation. Finally, 21 of 50 IE patients (42%) had HFE mutations, in line with international data. C282Y was detected in 3 heterozygous patients, and H63 in 18 (16 heterozygous, 2 homozygous).

CONCLUSION: This study confirmed known pathogenic and novel mutations associated with erythrocytosis. As a perspective, we aim to investigate additional genetic variants in the patient carrying the VHL mutation, possibly through large scale genomic analysis. Regarding the BHLHE41 variant, we are currently studying its gene expression through qualitative RT-PCR. Lastly, we will extend the analysis of HFE mutations in IE and PV patients exploring its impact in the clinical presentation of these cases.

Keywords: hematological disease; genetic variants; Erythrocytosis.

PAPER CODE: 101

MOLECULAR CLASSIFICATION OF DUCTAL ADENOCARCINOMA OF THE PANCREAS FOR PROGNOSTIC STRATIFICATION IN TISSUE SAMPLES FROM ULTRASOUND GUIDED BIOPSIES IN INCA PATIENTS

DO NASCIMENTO, C.M.; DE FARIAS, J.P.F.; NICOLAU, P.; DA SILVA, S.G.; PINTO, L.F.R.. NATIONAL CANCER INSTITUTE RIO DE JANEIRO BRAZIL, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Pancreatic ductal adenocarcinoma (PDAC) is an aggressive disease. The PDAC diagnosis is based on imaging and endoscopic ultrasound-guided biopsy (EUS), and often occurs in advanced stages, resulting in a rapid clinical decline. Consequently, most patients receive palliative chemotherapy (CTx), mainly FOLFIRINOX (FFX) or Gemcitabine (GEM) regimes. Recent molecular classification has defined two PDAC subtypes, classical and basal, which are associated with survival and response to palliative CTx. The molecular classification of PDAC patients could guide therapy selection and improve their prognoses. There are surrogate markers for this molecular classification, such as GATA6 (classical) and KRT5 (basal) expression. Therefore, molecular profiling may guide therapy.

OBJECTIVES: To classify pancreatic ductal adenocarcinoma (PDAC) molecularly using EUS-derived samples from INCA patients using GATA6 and KRT5 levels.

METHOD: This study included PDAC subjects from 2009-2024. Clinicopathological, social, and demographic data were obtained from medical records. Next, we will evaluate the GATA6 and KRT5 gene (digital PCR) and protein (immunohistochemistry) levels using EUS samples sent to the Division of Pathology (DIPAT) and with frozen material stored in the National Tumor and DNA Bank (BNT/INCA). Approved project CEP number: 47357415.3.0000.5274

RESULTS: A total of 86 PDAC patients were included, of whom 60.5% were women, with a median age of 60.5 years; 57.0% were white, 46.5% consumed alcohol, and 31.4% were smokers. At diagnosis, 37.2% were at stage IV. On endoscopic ultrasound evaluation, there was a predominance of tumors in the pancreatic head (72.1%), with vascular invasion (61.6%) and lymph node metastases (48.8%). Histopathological analysis revealed a median tumor cellularity of 12.5%, with a predominance of histological grade 2 (53.6%), and a significant association between cellularity and histological grade ($p < 0.05$). Regarding treatment, 61.7% received therapy; palliative CTx was the most common (71.7%), mainly focused on FFX (60.8%). The median overall survival was 5.5 months. Age (HR = 1.8; $p=0.03$), functional performance (HR=2.2; $p<0.0001$), and resectability status (HR=1.9; $p = 0.01$) were independent pre-treatment factors associated with worse overall survival.

CONCLUSION: PDAC patients treated at the INCA have a critical overall survival rates, which was associated with age, functional performance, and the possibility of surgical resection. It is an ongoing study, and the next steps include GATA6 and KRT5 expression analyses by digital PCR and immunohistochemistry to assess the feasibility of PDAC molecular classification using EUS and its clinical impact on prognosis and treatment response.

Keywords: pancreatic ductal adenocarcinoma; endoscopic ultrasound-guided biopsy; molecular biomarkers.

PAPER CODE: 106**Analysis of Oropharyngeal Squamous Cell Carcinoma by Exfoliative Cytology and HPV Genotyping in Patients from INCA**

OLIVEIRA, A.R.D.S.1; DA SILVA, B.N.M.2; CURVELO, J.A.D.R.2; DE MELO, A.C.2; SOARES, M.A.2; GOLDEMBERG, D.C.2. 1. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL; 2. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Oropharyngeal squamous cell carcinoma (OSCC) shows a rising incidence, with an estimated 106,400 new cases and 52,305 deaths in 2022. Molecular evidence consolidates human papillomavirus (HPV) as an independent etiologic agent, whose genotypic heterogeneity influences tumor biology and clinical outcomes. In this context, the adoption of an expanded genotyping approach capable of detecting 35 HPV genotypes represents a major advance over conventional programs limited to types 16 and 18, reducing false negatives and enhancing diagnostic accuracy. Exfoliative cytology, a minimally invasive and cost-effective method, when combined with HPV molecular genotyping, strengthens early detection of HPV-induced oropharyngeal alterations.

OBJECTIVES: The aim of this study is to correlate HPV genotypes with cytological findings in OSCC, identifying cytopathic effects by tumor stage and linking HPV subtypes to surveillance and screening parameters.

METHOD: The present study prospectively investigates HPV-positive and HPV-negative oropharyngeal squamous cell carcinoma patients at Cancer Hospital I/INCA. Oropharyngeal samples are collected using sterile brushes, fixed in 96% ethanol, and Papanicolaou-stained. HPV genotyping is performed by PCR and reverse hybridization (Multi HPV Flow Chip, XGEN®), detecting 35 HPV genotypes. Sociodemographic and clinical data are analyzed in SPSS v.30 ($p < 0.05$).

RESULTS: Morphological alterations typical of HPV infection, such as koilocytosis, mild nuclear hyperchromasia, multinucleation, and dyskeratosis, are expected to be identified in HPV-positive samples.

Genotyping is anticipated to reveal a predominance of high-risk genotypes, notably HPV-16 and HPV-18, as well as other oncogenic genotypes potentially underdetected by conventional assays restricted to types 16/18, thereby reducing false negatives. The possibility of coinfection by multiple subtypes, which may modulate tumor biological behavior, is also addressed. To date, five samples have been included in the study. Genotyping is currently being performed at the Tumor Genetics and Virology Program (INCA), while cytological analysis is underway at the Division of Pathology (DIPAT/INCA), with preliminary observation of morphological changes consistent with viral cytopathic effect.

CONCLUSION: The validation of cytopathic alterations through specific genotyping highlights the potential of exfoliative cytology as a precise tool for early OSCC detection. This study enhances the understanding of the role of HPV subtypes on oncogenesis and provides crucial insights for the development of targeted surveillance strategies. The findings have significant clinical implications, supporting early diagnosis, personalized therapeutic management, and improving patient prognosis and quality of life.

Keywords: Oropharyngeal squamous cell carcinoma; Human papillomavirus (HPV); Genotyping.

PAPER CODE: 107**Generation of Anti-GD2 CAR-T Cells and Characterization of Antitumor Activity**

VEIRA, L.M.1; ARAGÃO, E.1; DE LIMA, L.N.1; MACHADO, S.A.2; CORREIA, E.M.1; BONAMINO, M.H.3. 1. INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE DE BRASÍLIA, BRASÍLIA - DF - BRASIL; 3. INSTITUTO NACIONAL DE CÂNCER/FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Immunotherapy with T cells modified with Chimeric Antigen Receptors (CARs) is one of the most promising antitumor strategies. The CAR gene can be integrated into the genome using the Sleeping Beauty system, which employs

two vectors: one carrying the CAR gene within the transposon, and another encoding the transposase that mediates genomic integration. Some solid tumors, such as retinoblastoma, neuroblastoma, osteosarcoma, melanoma and glioblastoma, are known to commonly express the ganglioside GD2, making it good target for CAR-T cell immunotherapy.

Objectives: The aim of this work was to synthesize and validate an anti-GD2 CAR plasmid (14G2A clone) and to generate CAR-T cells from peripheral blood mononuclear cells (PBMCs).

METHOD: A total of 20 µg of the anti-GD2 CAR plasmid and 1 µg of the SB transposase plasmid were electroporated into 3×10^7 PBMCs pre-activated. The cells were then analyzed by flow cytometry for up to 8 days post-electroporation. Additionally, CAR-T cells were co-cultured with 3×10^4 T-98 glioblastoma cells at effector-to-target (E:T) ratios of 5:1 and 1:1. After 1, 3, and 5 days of co-culture, the remaining adherent tumor cells were stained with crystal violet, and absorbance was measured at 592 nm.

RESULTS: To evaluate the efficiency of CAR expression and the antitumor activity of the generated anti-GD2 CAR-T cells, PBMCs were electroporated with the 14G2A CAR plasmid and expanded in vitro. The average frequency (n = 7) of anti-GD2 CAR-T-positive cells was 7.63%, 17.9%, and 16.1% on days 1, 6, and 8 of expansion, respectively. After 72 hours of co-culture, the anti-GD2 CAR-T (14G2A) group reduced absorbance by 76.3% and 74.5% at the 5:1 ratio, and by 59.1% and 74.3% at the 1:1 ratio, compared to the T-98 alone and MOCK groups (PBMCs electroporated without the CAR plasmid), respectively.

CONCLUSION: These results demonstrate stable expression of the anti-GD2 CAR plasmid (14G2A) in PBMCs, following electroporation using the Sleeping Beauty system, and in vitro antitumor activity. Future steps in this project will include additional functional assays, such as more cytotoxicity assays using GD2⁺ cell lines and in vivo validations.

Keywords: CAR-T Cell; GD2; Sleeping Beauty.

PAPER CODE: 112

EPIGENETIC AGING IS ACCELERATED IN TRIPLE-NEGATIVE BREAST CANCER AND DEFINES POOR PROGNOSIS

DE ARRUDA, L.P.; DE PAULA, D.J.G.; CHRISTIANES, A.L.D.S.; LIMA, S.C.S.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Breast cancer is the most incident malignancy and the leading cause of cancer-related death among women worldwide. Prognosis varies across subtypes, defined by hormone receptors, HER2 expression, or absence of all markers, which characterizes triple-negative breast cancer (TNBC). TNBC is linked to the poorest prognosis, occurs more often in younger women, and shows incidence and mortality differences across ethnic groups. Beyond these markers, other biological processes may shape tumor evolution. Age-related variation in subtype incidence suggests a role for cellular aging. DNA methylation, an epigenetic mechanism often linked to gene silencing, is increasingly being used to measure biological aging through “epigenetic clocks,” such as GrimAge, developed to estimate healthspan.

OBJECTIVES: This study aimed to investigate whether the acceleration of biological aging differs among BC subtypes and is associated with patient survival.

METHOD: Using TCGA data, we analyzed the epigenetic age of tumor samples (N=741) and non-tumor adjacent tissues (NTAT, N=89), including aging acceleration rates. Analyses were stratified by molecular subtype, clinical stage, and race. Kaplan–Meier survival curves were used to compare groups with accelerated versus deaccelerated aging. Next, we will extend the study to Brazilian patients, including tumors, adjacent tissues, and peripheral blood, to evaluate biological aging patterns.

RESULTS: Biological age was found to be higher than chronological age in both tumor ($p<0.01$) and NTAT ($p<0.01$). When stratified by subtype, TNBC showed the highest acceleration compared to Luminal A, Luminal B, and HER2 tumors ($p<0.01$). Moreover, patients with accelerated aging exhibited poorer overall survival compared to those with deaccelerated aging, even

after adjusting for molecular subtype and clinical stage (HR=2.01, 95% CI: 1.2-3.4, p=0.008).

CONCLUSION: These findings suggest that epigenetic ageing rates could provide an additional prognostic biomarker, complementing traditional subtype and clinical risk factors, and highlight the potential relevance of cellular aging processes in shaping tumor aggressiveness.

Keywords: Breast Cancer; DNA Methylation; Epigenetic Clock.

PAPER CODE: 113

Standardization of a Luminex assay for simultaneous detection of herpesviruses and polyomaviruses

RAULINO, R.C.S. 1; CABRAL-CASTRO, M.J.2; SANTOS, N.S.O.3; SOARES, M.A.4. 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. DEPARTMENT OF PATHOLOGY, SCHOOL OF MEDICINE, FLUMINENSE FEDERAL UNIVERSITY, NITERÓI, NITERÓI - RJ - BRASIL; 3. ³DEPARTMENT OF VIROLOGY – PAULO DE GÓES INSTITUTE OF MICROBIOLOGY – FEDERAL UNIVERSITY OF RIO DE JANEIRO,, RIO DE JANEIRO - RJ - BRASIL; 4. GENETICS AND TUMOR VIROLOGY PROGRAM, NATIONAL CANCER INSTITUTE (INCA), DEPARTMENT OF GENETICS, FEDERAL UNIVERSITY OF RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Immunosuppressed and transplanted patients are at high risk for viral reactivation, particularly by herpesviruses and polyomaviruses, which are associated with neurological disorders, nephropathies, and oncological complications such as post-transplant lymphoproliferative diseases. Early detection of viral replication is essential for clinical and therapeutic management; however, conventional methods, although highly sensitive, require multiple independent reactions for different viruses, increasing both cost and turnaround time. The xMAP® (Luminex) technology enables the simultaneous detection of multiple genetic targets in a single reaction, representing a promising approach for viral surveillance in immunocompromised patients.

OBJECTIVES: To standardize a multiplex assay based on Luminex technology for the simultaneous detection of herpesviruses and polyomaviruses in samples from transplanted and immunosuppressed patients.

METHOD: Specific probes and primers were designed for EBV, CMV, BKV, and JCV, targeting conserved genomic regions. Probes were amine-modified and coupled to carboxylated magnetic microspheres. Clinical samples were subjected to a pre-PCR using biotinylated primers, and the resulting amplicons were hybridized to the microspheres and analyzed using the MAGPIX® system. Clinical samples previously characterized as positive or negative by qPCR were included as controls.

RESULTS: The coupled microspheres exhibited high binding efficiency and a fluorescent response proportional to the concentration of the complementary oligonucleotide. The assay showed good sensitivity, with MFI values increasing up to the saturation point. In the analysis of 134 clinical samples, all JCV-positive (n=7) and EBV-positive (n=5) samples were amplified, while discrepancies were observed in EBV-negative samples (n=5) that tested positive, and are currently under investigation. CMV-positive (n=10) and CMV-negative (n=11) samples did not amplify, whereas assays for BKV (n=79) and BKV/JCV coinfections (n=14) remain under evaluation. The B95-8 cell line, known for producing EBV particles, confirmed the presence of the EBNA-1 gene by qPCR (Ct=22 for 50 ng DNA) and was validated as the positive control for EBV in the Luminex assay. Preliminary results demonstrate robust coupling efficiency and high diagnostic potential of the platform.

CONCLUSION: The multiplex Luminex assay represents a promising tool for the simultaneous detection of multiple opportunistic viruses in immunosuppressed patients. The methodological standardization demonstrated high hybridization efficiency and reproducibility. Ongoing validation with an expanded sample set will further assess its performance and support its use in viral monitoring within oncological and transplantation contexts.

Keywords: opportunistic viruses; multiplex; luminex.

PAPER CODE: 117

MOLECULAR PROFILE OF LOW-GRADE DIFFUSE GLIOMA IN PATIENTS TREATED AT THE BRAZILIAN NATIONAL CANCER INSTITUTE (INCA)

PESSÔA, C.F.; SANTOS, L.; GUIMARÃES, I.D.S.; TESSAROLLO, N.; RODRIGUES, E.M.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: In Brazil, it is estimated that approximately 11,490 new cases of malignant central nervous system (CNS) tumors will occur between 2023 and 2025. Among these, diffuse gliomas are the most common primary malignant brain tumors in adults, accounting for 25–30% of cases, with a predominance in the supratentorial region. With advances in molecular techniques, the World Health Organization (WHO) revised the classification of CNS tumors by incorporating genomic markers such as IDH mutations, 1p/19q codeletion, TP53, ATRX, among others, to refine the definition of diffuse glioma subtypes.

OBJECTIVES: This study aimed to evaluate the genetic profile and tumor immune microenvironment of WHO grade 2 diffuse gliomas in Brazilian patients.

METHOD: This retrospective, descriptive, single-center study included 20 adult patients treated at INCA (2009–2015) with WHO 2007 grade 2 diffuse gliomas. IHC evaluated IDH1(R132H), ATRX, p53, Ki-67, GFAP, and immune markers (CD4, CD8, PD-1, PD-L1, TREM2). Variant analysis used a custom 39-gene panel (Agilent) sequenced on NovaSeq6000 (Illumina). Quality control, trimming, and variant calling followed GATK best practices via the Sarek pipeline.

RESULTS: After record review, 66 patients were identified; 40 met eligibility criteria, and 29 had tumor samples, yielding 20 analyzable cases (12 men, 8 women). Most (17) were under 40 at diagnosis. In total, 25 samples were analyzed, including two from one surgery and four paired samples from initial and recurrent tumors. Missense mutations predominated (~80), followed by frameshift deletions (~30) and insertions (~18). Single-nucleotide polymorphisms (SNPs) were most frequent (~85), then deletions (~38) and insertions (~30). The main base substitution was

C>T (n = 61). Recurrent mutations involved IDH1 (67%), MTOR (52%), NF1 (48%), FAT1 (48%), and FAT2 (48%); one case had IDH2 mutation (LGG_G27). According to WHO CNS 2021, eight tumors were IDH-mutant astrocytoma grade 2, four IDH-mutant oligodendroglioma 1p/19q-codeleted grade 2, one reclassified as astrocytoma, two as glioblastoma grade 4, and three remained IDH-wildtype.

CONCLUSION: Both IHC and sequencing analyses met the study's objectives. Applying the WHO 2021 CNS classification led to a 30% (6/20) diagnostic reclassification, underscoring the impact of integrated molecular profiling on treatment decisions. These results highlight the importance of molecular classification for accurate diagnosis of diffuse gliomas. Future perspectives include deeper analysis of the tumor immune microenvironment.

Keywords: glioma; IDH; low grade glioma.

PAPER CODE: 125

Evaluation of Prognostic markers in Advanced Pancreatic Ductal Adenocarcinoma Patients Treated at the Brazilian National Cancer Institute

DE FARIAS, J.P.F.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies and the sixth leading cause of cancer death in Brazil. PDAC diagnosis commonly occurs at advanced stages, limiting treatment to palliative chemotherapy (CTx), usually with FOLFIRINOX (FFX) or gemcitabine (GEM). Recent studies have linked molecular profiles, such as the Moffitt classification (classical vs. basal), to therapeutic response and survival. Gene and protein expression of GATA6 and KRT5 have emerged as surrogate markers of this classification and as independent prognostic biomarkers in PDAC.

OBJECTIVES: To evaluate clinicopathological and molecular features associated with therapeutic response and survival rates in patients with advanced PDAC treated at Instituto Nacional de Câncer (INCA).

METHOD: Observational, retrospective study including patients with advanced PDAC treated with palliative chemotherapy between 2000 and 2024 at INCA. Clinical and therapeutic data were reviewed. GATA6 and KRT5 expression will be analyzed by immunohistochemistry (IHC). Overall survival (OS) and progression-free survival (PFS) were estimated using the Kaplan–Meier method, and prognostic factors were assessed using Cox regression. Approved by the Institutional Review Board (IRB), number 47357415.3.0000.5274

RESULTS: Among the 223 eligible cases, 122 received GEM monotherapy, 95 FFX/FOLFOX6, and 6 with other protocols. Eighteen patients underwent curative-intent surgery followed by palliative CTx at relapse. Sixty patients received a second-line palliative CTx after progression. The median age was 61.1 years; 54% were female, 26% smokers, 30% diabetic, and 57% had a family history of cancer. PDAC were located in the head in 66%, and in the body/tail in 28%. At diagnosis, 10% were supposed resectable, 30% had locally advanced disease, and 60% had metastasis (44% liver, 20% non-regional lymph nodes, 13% peritoneum, and 3.6% lung). The performance status (PS) was 1 in 76.7%. The median OS was 7.63 months, and PFS was 7.5 months. The PS (HR=2.2), number of CTx cycles (HR=3.6), and type of CTx (HR=1.6) were independent predictors of OS. For PFS, the number of CTx cycles (HR=3.4), the type of CTx (HR=1.5), and PS (HR=2.2) remained significant independent predictors.

CONCLUSION: Our preliminary results with clinicopathological data highlight the poor prognosis of PDAC, with limited survival rates despite palliative CTx, which was associated with the PS, number of CTx cycles, and type of CTx. In the following steps, we will perform IHC analyses of GATA6 and CK5 and associate molecular findings with clinical data.

Keywords: Pancreatic ductal adenocarcinoma; palliative chemotherapy; palliative chemotherapy.

PAPER CODE: 126

Applying liquid biopsy to predict prognosis and treatment response of high-grade serous ovarian cancer patients in SUS

DE OLIVEIRA, K.P.V.; BORONI, M.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: High-grade serous ovarian cancer (HGSOC) has the worst prognosis among gynecological tumors, usually detected in late stages and presenting high genomic instability. The combination of nonspecific symptoms, the ovary's anatomical position, and lack of effective biomarkers results in a low 5-year overall survival rate, increasing the likelihood of metastasis. Although advances in omics research have had a profound impact on oncology, particularly in precision medicine, the invasiveness of surgery and biopsy remains an obstacle to treating these patients. In this context, liquid biopsy approaches may offer an alternative solution. Potential liquid biopsy biomarkers include circulating tumor DNA (ctDNA), whose genetic profile could unveil tumor evolution from the early stages to recurrence.

OBJECTIVES: We aim to develop and validate predictive models by applying machine learning (ML) to liquid biopsy data, improving risk stratification, and personalizing treatment of HGSOC patients within the SUS.

METHOD: A custom gene panel was developed by integrating somatic mutations and copy number alterations from multiple datasets and curated publications. DNA libraries will be prepared from matched-pairs samples (ctDNA, ovary tissue) of HGSOC naïve-treated patients, sequenced on the NovaSeq X Plus platform, and processed to identify the variants. These data will be combined with epidemiological information into ML algorithms to identify ctDNA variants that could help predicting patients' therapy response.

RESULTS: We integrated knowledge from 17 curated papers and 6 datasets generated either by the MSK or by the TCGA. We found 96 potential CNA genes and 35 frequently mutated ones. Although almost ubiquitously mutated in HGSOc biopsies, the TP53 gene had a lower frequency of mutation in the ctDNA MSK dataset (87%), which reinforces the importance of matched-pairs comparisons in liquid biopsy studies. Overall, from the 123 unique genes, 37 have already been target-druggable. Enrichment analysis revealed alterations in PI3K/AKT and in the JAK-STAT pathways, as well as in miRNAs, which are consistent with our previous findings. This panel will now be applied for sequencing DNA libraries from patients enrolled in this study, followed by variant comparison between ctDNA and solid tissue. Finally, the data will integrate ML models to identify therapeutic response and prognosis for HGSOc patients in the SUS cohort.

CONCLUSION: Liquid biopsy is a promising tool in clinics but still in its early stages. Genes consistently affected in HGSOc are targets of drugs for other cancers; however, it is also important to consider genes that are not yet druggable but have implications in the disease course. Selecting a sequencing panel is tricky, since there are differences between biomarkers of liquid biopsy and solid tumors. Our panel is being designed to help early detection of HGSOc, allowing a higher overall survival rate.

Keywords: liquid biopsy; ctDNA; ovarian cancer.

PAPER CODE: 127

Alternative Splicing Dynamics in Chronic Myeloid Leukemia

DE SOUSA, M.C.S.1; STELZER, G.T.2; FARIAS, L.1; SCHAFFEL, D.B.1; RENAULT, L.Z.2; PADILHA, T.F.1; ZALCBERG, I.1; SABIONI, B.3; GUTYAMA, L.M.1. 1. DIVISÃO DE LABORATÓRIOS ESPECIALIZADOS (DILABESP), INSTITUTO NACIONAL DE CÂNCER (INCA), RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO (UFRJ), RIO DE JANEIRO - RJ - BRASIL; 3. DEPARTAMENTO DE HEMATOLOGIA, HOSPITAL UNIVERSITÁRIO CLEMENTINO FRAGA FILHO, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Splicing factors are frequently altered in cancer, including myeloid malignancies. In Chronic Myeloid Leukemia (CML), canonical regulator genes such as SF3B1, HNRNPA1, CELF2, and others like EZH2 are often dysregulated. Although CML is driven by the BCR::ABL1 gene fusion, complex co-mutation patterns have been reported. Frequent alternative splicing variants include exon 7 skipping ($\Delta 7$) and a 35-bp insertion (INS35), detected in up to 70% of cases by NGS. Our previous data suggest these variants correlate with high BCR::ABL1 expression and may reflect intrinsic disease traits or secondary alterations. Additionally, on the occasion of the 25th anniversary of the TKI revolution, we revisited a machine learning study using mathematical models to predict Deep Molecular Response (DMR).

OBJECTIVES: To study longitudinal $\Delta 7$ and INS35 frequency in CML, and their correlation to splicing regulator genes. We also aim to explore mathematical and machine learning models to understand disease biology.

METHOD: Splicing variants were detected by Sanger sequencing (SS) or fragment analysis (FA), used for semi-quantification likewise. A total of 882 CML samples were analyzed: 263 at diagnosis, 352 at follow-up, and 267 longitudinally from 31 patients. EZH2 expression was quantified by SYBR-Green qPCR, normalized to HPRT and GAPDH (GeNorm). A mathematical model derived from the SIR equation was validated using machine learning, with slopes, halving time, and white blood cell counts as key predictors.

RESULTS: We demonstrated before that $\Delta 7$ was frequent in high %BCR::ABL1 samples. Herein, we further showed that $\Delta 7$ VAF increased with %BCR::ABL1 ($p=0.03$). The lowest $\Delta 7$ VAF statistically discriminating MR3 patients was 16.88% ($p=0.009$); INS35 does not correlate with %BCR::ABL1 - the lowest p-value was 0.16 at VAF 11.43%. From 533 CML samples analyzed by FA, 26.2% $\Delta 7$, 10.7% INS35, 4.8% had INS35+ $\Delta 7$, and 0.8% partial $\Delta 7$. $\Delta 7$ is frequent both at diagnosis and relapse, however, at different VAFs: 11% and 6%, respectively ($p=0.02$). Initial analysis of 18 INS35/ $\Delta 7$ -positive against 8 wildtype CML samples showed median EZH2 upregulation of 3.7 log2FC vs HPRT and 3.0 log2FC

vs GAPDH. Our machine learning predicted DMR at 36-months with 77% accuracy (XGBoost: 0.77 accuracy, 0.76 precision; Logistic Regression: 0.53, 0.39) on 40 patients. The model stratifies patients into susceptible, active disease, remission; transitions depend on WBC, BCR::ABL1, treatment, halving time, slope. Relapse risk under study.

CONCLUSION: $\Delta 7$ and INS35 are present in healthy donors and CML patients; are transient; independent of TKI response or disease phase, but enriched in BCR::ABL1+ samples. $\Delta 7$ VAF strongly correlates with %BCR::ABL1, while INS35 shows no correlation to CML burden. $\Delta 7$ VAFs are higher at diagnosis than relapse; INS35 VAFs show no significant change. Initial results show EZH2 upregulation in AS positive samples. Future work will expand samples, analyze others genes, improve ML predictions, and refine CML models.

Keywords: Alternative Splicing; Chronic Myeloid Leukemia ; Machine Learning models.

PAPER CODE: 137

Cytogenetic, Molecular and Cellular Alterations in Pediatric Patients with Ewing Sarcoma at a Reference Pediatric Cancer Centre in Rio de Janeiro

MOREIRA, V.G.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Ewing Sarcoma (ES) is a rare tumor, characterized by t(11;22)(q24;q12), resulting in EWSR1::FLI-1. About 30-40% of patients and non-metastatic disease develop local or metastatic recurrence within 2-10 years. Although there are few studies in metastatic disease, it has been showed that STAG2 is the most frequently mutated, and has been associated with metastasis, through the loss of its expression. Few studies to date have shown the expression pattern of STAG2 in pediatric patients with ES and its association with clinical progression. The majority of ES affects the bone, however 15% of tumors can arise in a variety of extraosseous locations. Other aspect that deserves investigation is ES cellular characterization of ES, suggesting a possible cellular origin for this aggressive tumor

OBJECTIVES: To analyze EWSR1 rearrangement in pediatric ES and STAG2 expression pattern; characterize the ES through cellular markers; associate cytogenetic, molecular and cellular changes with clinical features.

METHOD: A cohort of 90 patients aged 0-18 years diagnosed between 2010- 2020 was studied. Clinical and demographic characteristics were analysed from medical and electronic records. Histopathological and immunohistochemical (IHC) analysis were carried out for cellular characterization and evaluation of STAG2 expression pattern. The analysis of EWSR1 rearrangement was performed by fluorescence in situ hybridization (FISH) using EWSR1 break-apart probe on formalin-fixed paraffin embedded tissue sections.

RESULTS: The average age at diagnosis was 11 years, 47 (52, 2%) male and 43 (47.8%) female. Forty-six (51.1%) were white, 32 (35.5%) mixed race, 8 (3.4%) black, we do not have this information in 4 patients. The location of primary tumor was bone in 62 patients (70.5%) and soft tissue in 26 patients (29.5%). Of the 122 samples studied, 81 were biopsies of the primary tumor (66.4%), 33 excisions of the primary tumor (25.4%) and 8 biopsies of metastasis sites (8.2%). The presence of EWSR1 rearrangement was observed in 78.5% (33/42). STAG2 analysis by IHC showed high/moderate expression in 82.8% and low/absent expression in 17.2% of the 76 patients evaluated and associated with metastasis. Neuroectodermal markers were the most expressed (beta III tubulin n = 64/ 91.4% TRKA n = 32/ 64.6%).

CONCLUSION: FISH analysis using EWSR1 break-apart probe is an essential tool for ES diagnosis. Low/negative STAG2 expression was associated with metastasis. Our results suggest that neuroectodermal markers were the most expressed in pediatric ES. This study was the first in Brazil to address the cytogenetic, molecular and cellular alterations in pediatric ES. Our results have clinical implications and may contribute to a better understanding of the pathogenesis of pediatric ES

Keywords: Ewing sarcoma; STAG2; cellular origin.

PAPER CODE: 138

Genetic Profile of Patients with Complex Karyotypes in Acute Myeloid Leukemia

BLUNCK, C.B.; LOPES, B.D.A.; RODRIGUES, B.D.M.;
BARBOSA, T.D.C.; FAGUNDES, M.C.N.; DE SÁ, M.E.C..
INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO - RJ -
BRASIL.

INTRODUCTION: Acute myeloid leukemia (AML) is a hematopoietic disorder driven by genetic and epigenetic alterations. Prognostic classification relies on abnormalities detected by conventional cytogenetics and molecular genetics, dividing patients into favorable, intermediate, and adverse risk groups. AML with complex karyotype (CK-AML) represents 10–12% of cases and it is associated with high-risk. Typical CK involves losses in 5q, 7q, and/or 17p, while atypical CK lacks these changes. Key genes, such as TP53, are frequently altered, but not in all patients with CK-AML, suggesting distinct dysregulated pathways. Understanding these mechanisms is crucial to improve clinical management and develop targeted therapies.

OBJECTIVES: This study aims to characterize the genomic and transcriptomic landscape of complex karyotype AML to identify signaling pathways and molecular mechanisms linked to disease biology and prognosis.

METHOD: Two independent case series were evaluated: TARGET (discovery) and GenLAb (validation). Differential expression analyses in TARGET was used to identify differences between CK-AML and non-CK-AML. GenLAb samples are being characterized using RT-qPCR, FISH, cytogenetics, and MLPA. Chi-square and Fisher's exact tests assessed associations, with p-values <0.05 considered significant.

RESULTS: We analyzed 353 patients from the TARGET pediatric cohort, observing a higher frequency of patients older than 11 years (47% vs. 38.7% aged 0–10 years). A total of 85.5% were classified as low risk. Regarding the differential expression analysis, we identified 10 genes that showed significant expression differences in the CK-AML group. The analysis revealed that MSX1 ($p=0.0029$),

PAPA ($p=1e-05$), FRAS1 ($p=0.00082$), and C1CL3 ($p=0.00015$) exhibited increased expression, while TRH ($p=2.2e-05$), AIF1L ($p=1.9e-07$), AL590226.1 ($p=2.1e-06$), NPWI ($p=6.5e-09$), and AL592429.1 ($p=5.4e-09$) showed decreased expression. In addition, we included 111 from GenLAb so far, and the molecular characterization of this validation cohort is ongoing.

CONCLUSION: The identification of biomarkers in CK-AML contributes to a better understanding of disease progression. The integration of genomic analysis and experimental validation strengthens the findings and may support prognostic stratification. Further experiments are needed to confirm their clinical impact and therapeutic potential.

Keywords: Complex Karyotype AML; Genomic Profiling; Leukemia.

PAPER CODE: 144

Development of bispecific nanobody-derived CARs targeting B-cell leukemia markers: CD19, CD20, and CD22

MARQUES, J.D.D.P.C.1; BONAMINO, M.H.1; MARANHÃO, A.2. 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE DE BRASÍLIA, BRASÍLIA - DF - BRASIL.

INTRODUCTION: This project aims to contribute to the advancement of cell therapy for hematologic malignancies by using T cells expressing chimeric antigen receptors (CARs). CAR-T therapy is based on the genetic modification of T lymphocytes to express a transgenic receptor that enables them to recognize a specific antigen. Several CAR-T products have already been approved for clinical use in B-cell acute lymphoblastic leukemia (B-ALL) and multiple myeloma.

The CARs to be designed in this project will target simultaneously two out of three antigens expressed on malignant B cells: CD19, CD20, and CD22. This approach addresses the problem of leukemia relapse caused by antigen escape, a phenomenon in which tumor cells lose expression of the targeted antigen and evade the immune recognition.

OBJECTIVES: To develop and test bispecific nanobody-based CARs targeting CD19, CD20, and CD22 to reduce antigen escape rate and enhance CAR-T cell efficacy against B-cell malignancies.

METHOD: Bispecific CARs will be built using nanobody (VHH) domains selected by phage display from camelid libraries immunized with Raji cells. Selected VHHs will be humanized and cloned into the Sleeping Beauty vector pT4-19BBz. Modified T cells will be tested in vitro against Nalm-6 and Raji lines expressing different antigen combinations (GFP-labeled), and in vivo in NSG mice using luciferase-tagged tumor cells to assess cytotoxic activity and tumor control.

RESULTS: An immune response is expected in Lama glama against a wide range of antigens present on the Raji cell membrane, enabling the creation of a diverse VHH library. From this library, a few nanobody clones specific for CD19, CD20, and CD22 are expected to be selected. The designed CARs with the humanized versions of these VHHs are expected to be properly expressed in T cells. In vitro assays should demonstrate efficient cytotoxic activity of the CAR-T cells against tumor cells expressing the target antigen, while in vivo experiments in NSG mice are expected to show effective tumor control. It is expected to see a selection and a prevalence of cells that don't present the antigens targeted by the CARs used on each assay.

CONCLUSION: This work aims the development of brand new sequences of bispecific nanobody-based CARs targeting multiple B-cell leukemia antigens. The approach has the potential to improve the robustness and persistence of CAR-T cell therapy, offering a promising strategy to prevent tumor relapse due to antigen loss and to expand the therapeutic options for hematologic cancers.

Keywords: CAR-T; bispecific; ALL-B.

PAPER CODE: 149

Mapping Genetic and Environmental Risk Factors Related to Male Breast Cancer

TELES, K.D.A.1; FERNANDEZ, T.D.S.2; DOS SANTOS, A.C.E.1. 1. DEPARTMENT OF GENETICS AND TUMORAL VIROLOGY (CPQI-INCA), RIO DE JANEIRO - RJ - BRASIL; 2. CYTOGENETIC LABORATORY, CELL AND GENE THERAPY PROGRAM, (DILABESP-INCA), RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Male breast cancer (MBC) is a rare neoplasm, representing less than 1% of all breast cancer and male cancer cases. Although rare, cases have increased over the last 25 years (rate: 1% per year). The main risk factors include advanced age, ethnicity, radiation exposure, hyperestrogenism, family history (FH), and genetic syndromes. Mutations in the BRCA1/2 high penetrance genes to MBC are among the most established genetic risks for MBC. In addition, moderate-penetrance genes such as CHEK2, PALB2, and ATM are also implicated. Chromosomal abnormalities, such as the additional presence of an X chromosome in men (Klinefelter syndrome), increase the risk of MBC up to 50-fold. Identifying environmental and genetic factors for MBC can help from prevention to more personalized treatments.

OBJECTIVES: Describe clinical, sociodemographic, molecular and cytogenetic profile in patients assigned male at birth with BC at INCA.

METHOD: The current cohort includes 73 men with BC, diagnosed between 2002 and 2025 at genetic counseling appointments. Cytogenetic analysis is performed using blood samples and phytohemagglutinin. The karyotype pattern is identified using G-banding. DNA is extracted for NGS using a 24-gene hereditary cancer panel and MLPA for BRCA1/2 and CHEK2 in NGS-negative cases. Considering that certain variables are currently being collected, they will be expressed as a percentage according to the current number.

RESULTS: In a cohort of 73 patients, the mean age at diagnosis was 60 years. Approximately 55% of patients self-identified as black and 45% as white. Among patients with available data on HF, 81% (40/49) reported a positive history. BMI data were available for 59 individuals and 64% (38/59) had overweight or obesity. Additionally 57% (33/57) were smokers, and 50% (29/57) reported alcohol consumption. In the overall cohort, 72% had invasive ductal carcinoma, 97% had unilateral BC, and 70% had grade 2. Most tumors showed positive hormone receptor expression and negative HER2 status (91%). Of the 73 samples, 33 underwent genetic testing. Four patients had pathogenic variants (PVs) in a single gene: BRCA2, CHEK2, or POLE; two had PVs in two genes: one in BRCA1 and CHEK2, and another in ATM and a likely PV in CHEK2. Cytogenetic analysis was initiated and one patient showed a normal karyotype 46,XY[20].

CONCLUSION: These preliminary results reinforce the importance of molecular evaluation in men with breast cancer to improve biological understanding. Furthermore, the frequency of disease-related environmental factors found in this group appears to influence MBC.

Keywords: Male breast cancer; Genetic predisposition; Klinefelter syndrome.

PAPER CODE: 151

IDENTIFICATION AND CHARACTERIZATION OF PATIENTS WITH HEREDITARY BREAST CANCER IN BRAZIL

CABRAL, B.C.A.1; PEREIRA, A.S.1; TORRESAN, C.1; MARSILLAC, S.M.1; ZANELLA, K.M.1; GONÇALVES, C.N.1; DE PAULA, D.J.G.1; CONTEVILLE, L.C.1; DOS SANTOS, A.K.C.R.2; ANTONIAZZI, A.P.3; TORRES, L.C.4; DE ALBUQUERQUE, C.G.P.5; WEIS, L.N.6; DE OLIVEIRA, C.P.7; ADJUTO, G.M.A.F.7; FERRAGI, A.C.R.8; ASHTON-PROLLA, P.9; BORONI, M.1; DOS SANTOS, A.C.E.1; PINTO, L.F.R.1; MOREIRA, M.Â.M.1; PALMERO, E.I.1. 1. INSTITUTO NACIONAL DE CâNCER, RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO PARÁ, BELÉM - PA - BRASIL; 3. HOSPITAL DE AMOR DE BARRETOS, BARRETOS - SP - BRASIL; 4. INSTITUTO DE MEDICINA INTEGRAL PROFESSOR FERNANDO FIGUEIRA, RECIFE - PE - BRASIL; 5. INSTITUTO

DO CâNCER DO CEARÁ, FORTALEZA - CE - BRASIL; 6. HOSPITAL DE BASE DO DISTRITO FEDERAL, BRASÍLIA - DF - BRASIL; 7. HOSPITAL DE APOIO DE BRASÍLIA, BRASÍLIA - DF - BRASIL; 8. HOSPITAL MATERNO INFANTIL DE BRASÍLIA, BRASÍLIA - DF - BRASIL; 9. HOSPITAL DE CLÍNICAS DE PORTO ALEGRE, PORTO ALEGRE - RS - BRASIL.

INTRODUCTION: Cancer is a major public health problem and remains the leading cause of death worldwide. In Brazil, approximately 704,000 new cancer cases are expected to occur between 2023 and 2025, with breast cancer (BC) accounting for about 15% of these cases. Pathogenic (P) or likely pathogenic (LP) germline variants in high- and moderate-risk genes are among the most frequent causes of Hereditary Breast and Ovarian Cancer Predisposition Syndrome and explain roughly 25–50% of the familial risk of BC. Most studies characterizing the spectrum of germline variants in BC predisposition genes have focused on European and North American populations; however, few studies have investigated the highly admixed Brazilian population.

OBJECTIVES: Identify and characterize individuals at risk for hereditary breast cancer from the Brazilian Unified Health System (SUS) across the five Brazilian regions through whole exome sequencing.

METHOD: A total of 5,000 individuals at high risk for hereditary breast cancer in nine participating institutions are being included in the study. Sociodemographic, clinical-pathological, and cancer history data are stored on REDCap®. Blood samples are collected for DNA extraction. Exome sequencing is performed at the INCA using NovaSeq X Plus platform (Illumina) and processed with DRAGEN v4.3 software. Variant classification is carried out using Emedgene software, following the ACMG/CanVig guidelines.

RESULTS: A total of 2,447 individuals (2,396 women and 51 men) diagnosed with breast cancer were included in the study. Of these, 672 (27.5) were from the Northeast region, 136 (5.56%) from North, 19 (0.8%) from Central-West, 1,183 (48.3%) from Southeast, and 437 (17.8%) from South. Sociodemographic and clinical-pathological data from 2,211 participants were collected and stored in REDCap®. Study participants had

a mean age at diagnosis of 43 years (22–91; SD: 10.79). Regarding personal cancer history, 237 (14%) individuals had a second primary tumor. The most frequent tumor types were breast (188 cases, 70.7%) and ovarian (7 cases; 2.6%). 1,626 participants (86.6%) reported a family history of cancer, with 2,107 family members diagnosed with breast cancer. Until now 152 patients had exome sequenced and variants classified. Among them, 15.1% (n=23 individuals) have a pathogenic/likely pathogenic variant identified, mostly in BRCA2 (7 cases), BRCA1 (6 cases), PALB2 and CHEK2 (2 cases each).

CONCLUSION: This project, part of the Brazilian Genome Project, aims to characterize genomic diversity of families with hereditary breast cancer. Until now more than 2,000 samples from five Brazilian regions have been included. Then, 15% of participants carriers pathogenic germline variant in cancer predisposition gene. This project is enabling high-risk families and their relatives to monitoring and risk reduction according to genetic risk and understanding factors underlying hereditary cancer in Brazil.

Keywords: breast cancer; germline variants; oncogenetics.

PAPER CODE: 156

VALIDATION OF LAMP5 AND SKIDA1 BIOMARKERS FOR PREDICTING KMT2A-r IN ACUTE LYMPHOBLASTIC LEUKEMIA

SIMÕES, G.P.E.A.1; BLUNCK, C.B.1; BARBOSA, T.D.C.1; FAGUNDES, M.C.N.1; LINS, M.M.2; COSTA, E.S.3; FERRAZ, T.4; SCHRAMM, M.T.5; DE SÁ, M.E.C.1; LOPES, B.D.A.1. 1. INSTITUTO NACIONAL DE CâNCER, RIO DE JANEIRO - RJ - BRASIL; 2. IMIP, RECIFE - PE - BRASIL; 3. IPPMG-UFRJ, RIO DE JANEIRO - RJ - BRASIL; 4. HEMORIO, RIO DE JANEIRO - RJ - BRASIL; 5. PRONTOBABY HOSPITAL DA CRIANÇA LTDA; INSTITUTO NACIONAL DE CâNCER, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Acute leukemias (AL) are aggressive hematologic neoplasms driven by genetic alterations. Rearrangements in KMT2A (KMT2A-r) are common in patients under 2 years, occurring in ~80% of infants with

acute lymphoblastic leukemia, ~50% of infants with acute myeloid leukemia, and ~10% of patients with AL of ambiguous lineage. As KMT2A-r involves over 100 fusion partners, reliable biomarkers are crucial for identifying these alterations. Although CSPG4 expression detected by flow cytometry is widely used to predict KMT2A-r, previous studies have reported false-negative results associated with the use of this biomarker. Thus, a recent study conducted by our group used a machine learning approach and identified that LAMP5 and SKIDA1 genes have higher accuracy for predicting KMT2A-r.

OBJECTIVES: To validate LAMP5 and SKIDA1 as more efficient alternatives than CSPG4 for predicting KMT2A-r in AL.

METHOD: We used the TARGET-ALL-P2 dataset for validation, performing data analysis in R environment through differential expression analysis, ROC curve evaluation of the biomarkers with AUC calculation, and predictive values (PPV, NPV) estimation for each biomarker. A GenLab case series will further evaluate KMT2A status and LAMP5, SKIDA1, and CSPG4 expression by RNA-seq. The HTAN CHOP scRNA-seq dataset will also be analyzed to assess the biomarkers expression in leukemic blast and hematopoietic cells.

RESULTS: A total of 407 TARGET cases were included, 146 female and 261 male. Among them, the proportion of B-ALL patients was 44% and T-ALL 56%. Patients were divided into two groups: KMT2A-r (n=15) and KMT2A-WT (n=392). We identified 631 upregulated and 1318 downregulated genes, and determined that SKIDA1 ranked 20th among the most differentially expressed genes, LAMP5 ranked 2027th, and CSPG4 ranked 7939th. Next, we defined the expression differences between KMT2A-r groups: both SKIDA1 ($p=1.7e-9$) and LAMP5 ($p=0.0084$) were significantly overexpressed in the KMT2A-r group compared to KMT2A-WT, while no significant expression difference was observed for CSPG4 ($p=0.96$). Furthermore, biomarker performance analysis showed that SKIDA1 (AUC: 0.959) and LAMP5 (AUC: 0.719) had increased accuracy than CSPG4 (AUC: 0.480) to estimate KMT2A-r.

CONCLUSION: Preliminary results from the TARGET series suggest that SKIDA1 perform well in predicting KMT2A-r, potentially surpassing CSPG4. Differential expression analysis, combined with ROC curve and predictive metric evaluations, reinforces its potential as a diagnostic marker. However, definitive validation requires analysis of our laboratory case series, which is currently underway, to confirm the robustness and clinical applicability of these findings.

Keywords: Bioinformatics; Infant leukemia; KMT2A rearrangements.

PAPER CODE: 158

Integrating Gene Expression and Machine Learning for Lymphoma Diagnosis in Brazil

RANGEL, C.E.D.S. 1; ALBANO, J.M. 1; MILNOR, J.R. 1; FREIRE, E.C. 1; STUDART, M. 1; WERNERSBACH, L. 1; BRIERCHECK, E. 2; LEAL, F.E. 1. 1. INCA, RIO DE JANEIRO - RJ - BRASIL; 2. DANA-FARBER CANCER INSTITUTE, BOSTON - ESTADOS UNIDOS DA AMERICA.

INTRODUCTION: Lymphomas are heterogeneous neoplasms requiring complex diagnostic procedures often unavailable in low- and middle-income countries (LMICs). Inadequate access to immunohistochemistry, molecular panels, and expert pathology leads to misdiagnosis and treatment delays. Previous studies have demonstrated that low-cost gene expression assays can accurately classify lymphoma subtypes using FFPE tissue. This project expands upon that approach to include both common and rare lymphoma subtypes from Brazilian and international cohorts, aiming to improve diagnostic accuracy and equity in cancer care.

Objectives: To use a validation cohort to determine the performance of a low-cost molecular diagnostic assay capable of identifying lymphoma and accurately classifying its subtype using FFPE tissue samples.

METHOD: This retrospective analytical study uses FFPE samples stored at INCA. Eligible cases include 19 diagnostic categories representing common and rare lymphomas. Gene expression profiling will be performed using a Chemical Ligation Probe Assay (CLPA), and results analyzed via machine learning (super learner stacking model), supported by the Dana-Farber Cancer Institute. Clinical data such as age, sex, biopsy type/site, HIV status, CD4 count, viral load, and ART use will be collected.

RESULTS: A total of 2,758 cases were identified between 2010 and 2025 at INCA. The most frequent diagnoses included: Diffuse Large B-cell Lymphoma (1,420 cases), Reactive Lymph node (497), Burkitt Lymphoma (169), Kaposi's Sarcoma (129), Mycosis Fungoides (128), Plasmablastic Lymphoma (36), Angioimmunoblastic T-cell Lymphoma (20), Adult T-cell Leukemia/Lymphoma (73), B-cell Acute Lymphoblastic Leukemia (34), T-cell Lymphoblastic Lymphoma (82), Castleman's Disease (30), and Tuberculosis (139). Data on age, sex, sample type (incisional/excisional), site (nodal/extranodal), HIV status, CD4 count, viral load, and ART are under collection.

CONCLUSION: This project will generate a comprehensive national database of lymphoma and related conditions and establish the feasibility of a cost-effective, rapid molecular diagnostic tool. The results are expected to enhance early diagnosis and treatment decisions, particularly in settings with limited access to pathology infrastructure, supporting equitable global oncology care.

Keywords: Lymphoma; Molecular Diagnostics; Machine Learning.

PAPER CODE: 163**Detection of anti-Alternative Reading Frame protein (ARFp) antibodies as a biomarker of liver disease progression in patients with chronic HCV**

MIRANDA, T.D.S.1; DE SOUZA, S.L.2; VIDAL, L.L.3; MUNIZ, C.P.R.1; SCHIFFLER, F.B.2; PEREZ, R.D.M.2; NETO, J.M.D.A.2; CHINDAMO, M.C.2; F. JUNIOR, O.D.C.2; COELHO, H.S.M.2; MAVROMARA, P.4; DOS SANTOS, A.F.A.2; SOARES, M.A.1.
1. INSTITUTO NACIONAL DE CÂNCER (INCA), RIO DE JANEIRO - RJ - BRASIL; 2. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO (UFRJ), RIO DE JANEIRO - RJ - BRASIL; 3. FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRASIL; 4. HELLENIC PASTEUR INSTITUTE, ATHENS - GRECIA.

INTRODUCTION: Seventy-one million people are infected with the Hepatitis C virus (HCV), and 400,000 die from HCV-related diseases yearly. In chronic hepatitis C, liver fibrosis progressively develops, which can lead to cirrhosis and hepatocellular carcinoma (HCC). The Alternative Reading Frame protein (ARFp), an alternative reading frame -2/+1 in the region encoding the HCV core, is expressed during natural HCV infection and is recognized by serum antibodies. Recently, the role of ARFp in liver carcinogenesis has begun to be elucidated, including its involvement in cell cycle acceleration and increased formation of tumor foci. Previous studies have shown a higher prevalence of anti-ARFp antibodies in patients with HCC compared to patients without HCC.

OBJECTIVES: The present study aimed to characterize anti-ARFp antibodies in HCV-positive patients with different levels of fibrosis, assessing their potential as a biomarker of hepatic disease progression.

METHOD: Sera samples of 364 patients were collected from patients displaying different fibrosis stages (8 F0, 110 F1, 75 F2, 70 F3, 88 F4 without HCC, and 13 F4 with HCC). Plasmids containing the ARFp sequences of the HCV genotype 1a core were transformed into E. coli BL21 (DE3) to express ARFp and core proteins. Proteins were purified and used in ELISA assays to detect anti-ARFp and anti-core antibodies.

RESULTS: The overall seroprevalence for the HCV Core protein was 97.2% (354/364). In contrast, the seroprevalence for the ARFp varied across fibrosis stages. The prevalence of anti-ARFp antibodies was 40.0% (44/110) in F1, 41.4% (31/75) in F2, 47.1% (33/70) in F3, and 53.4% (47/88) in F4 patients without HCC. Patients with F4 fibrosis and HCC showed the highest prevalence (76.9%, 10/13), with significantly higher odds of ARFp positivity compared to those with F1–F3 fibrosis (OR = 4.69, 95% CI: 1.23–16.07; p = 0.004). A significant linear trend was observed with increasing fibrosis severity and HCC presence (p-trend = 0.002). The odds of ARFp positivity were approximately threefold higher in patients with HCC compared to those without (OR = 0.34, 95% CI: 0.08–1.38). However, ROC analysis showed that anti-ARFp levels did not discriminate F4 fibrosis from earlier stages (AUC = 0.568; cutoff ≥ 1.15 ; sensitivity 45.5%; specificity 70.2%).

CONCLUSION: These findings, together with the high prevalence and elevated levels of anti-ARFp antibodies in HCV patients with F4 fibrosis, with or without HCC, suggest that the ARFp protein may play a role in virus-associated pathogenesis. Although preliminary analyses indicate that ARFp alone may not serve as a reliable biomarker, studies involving larger cohorts and the integration of serum hepatic parameters could support its potential use as a diagnostic cofactor for liver disease progression.

Keywords: Hepatocellular carcinoma; Hepatitis C virus; ARFp antibodies.

PAPER CODE: 175

Identification of the BCR::ABL1-like subgroup in adolescent and young adult B-ALL patients within the Brazilian public healthcare system (SUS)

BARBOSA, T.D.C.; LOPES, B.D.A.; BLUNCK, C.B.; LUCAS, M.D.V.; DOS SANTOS, R.L.; AMARAL, T.F.; SIMÕES, G.P.E.A.; DE SÁ, M.E.C.. LABORATÓRIO DE GENÉTICA DAS LEUCEMIAS AGUDAS (GENLAB), COORDENAÇÃO DE PESQUISA E INOVAÇÃO, INSTITUTO NACIONAL DE CâNCER - INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: B-cell precursor acute lymphoblastic leukemia (B-ALL) in adolescents and young adults (AYA) presents a distinct epidemiological and molecular profile. In addition to a therapeutic response that is significantly inferior to pediatric B-ALL, there is a high frequency of molecular alterations associated with poor prognostic outcomes in AYA patients. In approximately 42% of AYA B-ALL cases, the BCR::ABL1-like subgroup is characterized by a gene expression profile and unfavorable prognostic impact similar to BCR::ABL1 B-ALL. However, identifying this subgroup remains a complex and high-cost task, requiring resources and methodological strategies that are unavailable in most treatment centers integrated into the Brazilian health system (SUS).

OBJECTIVES: To establish a prognostic genetic test, as it will identify the BCR::ABL1-like subgroup in AYA B-ALL patients, which is feasible to be used within the SUS using a simple DNA-based technology.

METHOD: We are conducting a discovery phase with RNA-seq, WGS, and SNP array data, available in the St. Jude Cloud database, to identify SNPs exclusively associated with BCR::ABL1-like in AYA patients, using pediatric patient data as a comparative group. Subsequently, a validation phase will be conducted, using Brazilian samples that will be simultaneously characterized through the gold standard panel and the SNPs based panel defined by the present study using TaqMan allelic discrimination assays.

RESULTS: A genome-wide association analysis (GWAS) was performed using PLINK v1.9, assuming an additive genetic model based on .vcf files from germline samples. A total of 551,592 autosomal SNPs were successfully imputed in 592 B-ALL individuals (53 cases and 538 controls). We identified the most significant SNPs and the alterations with a marked difference in the minor allele frequency (MAF) of the mutant allele. The six most significant SNPs were: rs10760512 (CYP27B1), rs200804895 (CBDW1), rs209433 (HS3ST2), rs2200843 (MFSD8), rs220860 (CADM1), and rs3818509 (ST6GALNAC4). Curiously, in the global analysis of all significant SNPs, we did not detect rs3824662 in the GATA3 gene, which is the only variant previously reported as strongly associated with the BCR::ABL1-like group. In parallel, 96 B-ALL samples were molecularly characterized for use in the validation phase, utilizing the gold-standard panel for identifying BCR::ABL1-like, genetic additional alterations and the GATA3 SNP.

CONCLUSION: We are expanding the GWAS, including .vcf data from somatic samples, to check the non-identification of this known associated variant in BCR::ABL1-like. We expect to identify genetic risk factors that predispose to BCR::ABL1-like B-ALL and to establish a genetic panel that may assist in the risk stratification of AYA patients. Importantly, our goal is to ensure that this panel is feasible for use in the public healthcare system (SUS) across different regions of the country.

KEYWORDS: B-ALL;BCR::ABL1-LIKE;GWAS.

PAPER CODE: 177

The Extent of Extranodal Extension as a Prognostic Indicator in Papillary Thyroid Cancer

GOMES, M.A.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Extranodal extension (ENE) is recognized as an important prognostic factor related to recurrence, distant metastasis, and decreased disease-

specific survival in patients with papillary thyroid carcinoma (PTC). However, the role of the extent of extranodal extension in the clinical outcome of these patients is still not well understood.

OBJECTIVES: To estimate the risk of developing distant metastasis (DM) in patients with different degrees of ENE according to a new stratification.

METHOD: This is a retrospective study based on medical records and slide reviews of papillary thyroid cancer patients who underwent therapeutic neck dissection. A new stratification based on the circumferential rupture of the lymph node capsule was developed, defined as Focal ENE when $< 1/3$ of the lymph node capsule was ruptured and Diffuse ENE when it happened with $\geq 1/3$ of the lymph node capsule.

RESULTS: 89 patients were included in the study, of whom 19% presented distant metastasis within 96 months of follow-up. The presence of diffuse extranodal extension represented a risk about six times higher than patients without ENE for the development of distant metastasis at 96 months, after adjusting for age group (HR=6.41; 95% CI: 1.7-23.8; p-value=0.006).

CONCLUSION: A higher extent of extranodal extension confers a higher risk of developing distant metastasis and should, therefore, be considered in the therapeutic decision-making process.

Keywords: thyroid cancer; extranodal extension; prognosis.

PAPER CODE: 179

Generation of CAR-NK92mi cells as an off-the-shelf approach using nonviral minimized backbone vector

Paula D. Alves, Emmanuel Aragão e Martín H. Bonamino

INTRODUCTION: Autologous CAR-T cell therapy is highly effective against certain hematologic tumors but

raises concerns regarding safety and cost. CAR-NK92 cells, which have been tested in clinical trials for CAR-based therapies, offer the advantage of allogeneic use and a lower risk of adverse effects. The NK-92mi clone is an IL-2independent NK cell line that reduces costs associated with expansion and genetic modification, making it a practical off-the-shelf CAR carrier.

OBJECTIVE: To reduce costs related to viral vector scalability, this study tested two transposon/transposase-based systems — Sleeping Beauty (SB) and piggyBac (PB) — to generate anti-CD19 CAR-NK92mi cells.

METHODS: In the SB system, we used PT3, PT4, and an innovative minimized version of the PT4 backbone vector called a minicircle (PT4-MC). The transposons were combined with the SB100x or PB transposases. To generate anti-CD19 CAR+ cells, 1×10^6 NK92mi cells were allocated to each condition (mock, PT3, PT4, PT4MC, and PB) and electroporated using the 2b Nucleofector (Lonza) with the NK92 program. Subsequently, cell viability and anti-CD19 CAR expression were assessed by flow cytometry.

RESULTS: As expected, NK92mi cells were highly sensitive to electroporation, and viability across all conditions was reduced to approximately 20% of the initial count. Moreover, while the other plasmids showed ineffective transfection, the PT4-MC condition achieved a stable 5% CAR+ NK92mi cell frequency. Thus, the use of PT4-MC for transfection shows improved efficiency and transgene stability.

CONCLUSION: NK cells possess innate immune mechanisms that hinder the transfection process; therefore, our results suggest that the reduced size of minicircle may offer an advantage in the genetic modification of NK cells. Enrichment assays for CAR-NK92mi cells are currently underway, along with in vitro and in vivo cytotoxicity tests against B-cell cancer lines and a xenograft mouse model, respectively.

PAPER CODE: 180

OPTIMIZATION OF CAR-T CELL GENERATION EFFICIENCY FOLLOWING ULTRA-RAPID MANUFACTURING PROTOCOLS

ARAGÃO, E.; BONAMINO, M.H.. INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Since the 1980s, immunotherapy has emerged as a promising strategy for cancer treatment, culminating in the development of adoptive transfer of T lymphocytes engineered to express chimeric antigen receptors (CAR-T). This approach enables T cells to be redirected toward specific recognition and elimination of tumor cells. In this context, our research group recently demonstrated the feasibility of producing and applying CAR-T cells in a preclinical B-ALL model using a non-viral transfection method followed by only 24 hours of culture, thereby establishing an ultra-rapid CAR-T manufacturing protocol. Notably, the study revealed no significant differences in therapeutic efficacy compared to the conventional 14-day expansion process, underscoring the potential for substantial cost reduction.

OBJECTIVES: Scaling up this protocol is limited by low transfection efficiency of non-viral systems. This study aims to improve it through targeted optimizations in the CAR-T manufacturing process.

METHOD: The evaluated strategies included: (1) adjusting the T-cell activation period prior to electroporation, (2) comparing plasmid formats (full vs. minicircle) and electroporation platforms (Lonza Nucleofector 4D vs. MaxCyte), and (3) testing large-scale production parameters for clinical translation.

RESULTS: The results indicated that electroporation 48 hours after activation yielded the highest number of CAR⁺ T cells. Furthermore, the minicircle plasmid combined with the Nucleofector 4D system markedly improved CAR expression (up to 70%) and cell recovery while maintaining high viability.

CONCLUSION: Ongoing efforts will test IL-7/IL-15 supplementation, alternative promoters to enhance transgene expression, and improved large-scale

electroporation. In vitro and in vivo analyses will validate the optimized protocol, supporting a cost-effective, scalable, and clinically feasible ultra-rapid CAR-T platform to advance clinical translation and expand access to therapy in Brazil.

Keywords: CAR-T; ULTRA-RAPID MANUFACTURING; OPTIMIZATION.

PAPER CODE: 187

Identification of Patients with Hereditary Colorectal Cancer in a Public Oncology Referral Hospital in Brazil

GRASEL, R.S.. INSTITUTO NACIONAL DO CÂNCER, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Colorectal cancer (CRC) ranks as the second leading cause of cancer deaths worldwide. Around 20–30% of cases are linked to germline alterations. Recently, CRC in young individuals has drawn increasing attention due to its growing incidence and mortality, especially under 35. Hereditary CRC carries a higher lifetime risk, earlier onset, and multiple primaries. Identifying at-risk individuals enables early diagnosis and targeted prevention for patients and families.

OBJECTIVES: To identify patients with hereditary CRC and novel genes associated with high or moderate risk for CRC development.

METHOD: This was an ambispective and exploratory study. Patients with CRC were included, and their tumor samples were tested for the BRAF V600E mutation and for microsatellite instability (MSI). Patients with tumors lacking BRAF mutations and showing MSI, regardless of age at diagnosis, as well as patients with BRAF wild-type tumors diagnosed at ≤40 years of age, underwent hereditary cancer panel testing. All identified variants were classified according to the ACMG guidelines.

RESULTS: The mean age at diagnosis was 59 years; most were female (50.4%), of mixed race (53.0%), and overweight (53.8%). Colon cancer was more common (55.1%), mainly left-sided (65.9%), and diagnosed at stage III–IV (60.3%). Overall survival was higher for colon

vs. rectal cancer (64 vs. 43 months; $p=0.13$). The BRAF mutation was found in 8 samples (1.4%). Among 72 analyzed samples, 12 P/PP variants were detected in APC, MLH1, MSH6, MSH2, TP53, and MUTYH. Patients with P/PP had lower median age (43 vs. 55; $p=0.371$) and a significant association with mucinous histology ($p=0.011$). No statistical difference was found for MSI and age, but most were <40 years and MSI. Families showed 8 Lynch, 1 FAP, and 1 Li-Fraumeni case.

CONCLUSION: We identified high-risk hereditary cancer patients in an unselected cohort, regardless of family history. Molecular analysis found 12 P/PP variants (16.6%) in high/moderate-risk genes, six diagnosed before age 50 and three before 40. Universal somatic screening for colorectal tumors excluded sporadic cases. Adding age at diagnosis as an extra criterion may help improve hereditary syndrome detection.

Keywords: Colorectal cancer; hereditary cancer; Early onset cancer.

PAPER CODE: 188

VST.CD19 CAR-T Cells for Treatment of Viral Reactivation and Disease Relapse Post Allogeneic Stem Cell Transplantation

MAZZI, M.T., INCA, RIO DE JANEIRO - RJ - BRASIL.

INTRODUCTION: Stem Cell Transplant (SCT) remains the only curative option for relapsed and refractory hematological malignancies. However, it is a complex procedure with high rates of morbidity. Two of the main complications are profound immunosuppression, leading to viral infections and reactivations, and disease relapse. Adoptive cell therapy (ACT) for viral infections has been proposed for decades, and consists in isolating viral memory cells from a compatible healthy donor and expanding them in vitro before patient infusion. Recently, CD19 malignancies treatment paradigm has changed with the approval of anti-CD19 CAR-T cells. The combination of ACT and CAR-T cells could address two main complications of SCT with a single product, capable of recognizing viral infected cells and CD19+ malignant cells.

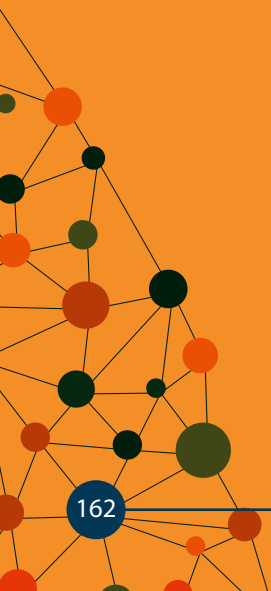
OBJECTIVES: Create a GMP-grade protocol for generation of virus-specific CD19 CAR-T cells (VST. C19 CAR-T), that maintains the functionality of both receptors, viral specific TCR and anti-CD19 CAR.

METHOD: Peripheral mononuclear cells (PBMCs) are isolated from whole blood by Ficol gradient. Memory cells are separated through magnetic columns with RA beads. The RA negative fraction is activated and electroporated with minicircle plasmid vectors containing the CD19.CAR and SB transposase. After 10-14 days in vitro expansion, the VST.CD19 CAR-T cells are analysed for CAR expression, immunophenotype, in vitro lysis, ELISpot, and IFN production after stimulation with viral pepmixes and CD19 lineages.

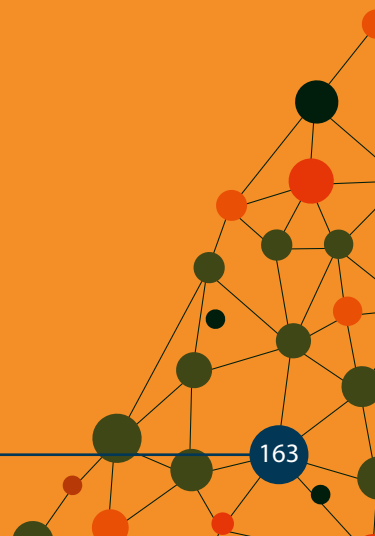
RESULTS: The initial protocol was developed at Baylor College, and adjustments were necessary. After 14 days of expansion in the adapted protocol, cell viability was above 90% and cell expansion varied from 0,5 to 1,5-fold. Second simulation of VST-C19 CAR-T cells with irradiated, peptide-pulsed activated T cells and a CD19 cell lineage (LCL) increased modestly the CAR expression from 12 to 17% on D11, but cell expansion increased 3-fold. ELISpot showed preserved viral specificity for CMV and EBV, which generally constitute a larger proportion of the T-cell memory compartment, but not for adenovirus and BK virus. Immunophenotype shows predominance of terminally differentiated CD8+ T cells (Tem and Temra) with low population of exhausted T cells (less than 1%). In vitro lysis of Nalm6 cells at four time points (24h, 48h, 72h and 96h), shows effective cytotoxicity at E:T ratios of 1:1 to 1:4.

CONCLUSION: The proposed protocol is GMP-grade compatible and generated VST CD19.CAR-T cells that seem to preserve the functionality of both the TCR and CAR. Cell expansion is not ideal, but scalable for clinical use. The VST.CD19 CAR-T cells show preserved cytotoxicity capacity and low exhaustion markers, which was a great concern since the CAR-T is generated from memory populations. Next steps are ongoing to analyse IFN production after double receptor challenge and in vivo lysis essays.

Keywords: CD19 CAR-T; Virus Specific T Cell; Stem Cell Transplant.



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