INSTITUTE OF MOLECULAR BIOLOGY

BIG BIOINFORMATICS GROUP

Human Genomics Laboratory

ИНСТИТУТ БИОМЕДИЦИНЫ И ФАРМАЦИИ

ABI

Molecular characterization of cancers with long-read RNA sequencing

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21AG-1F021

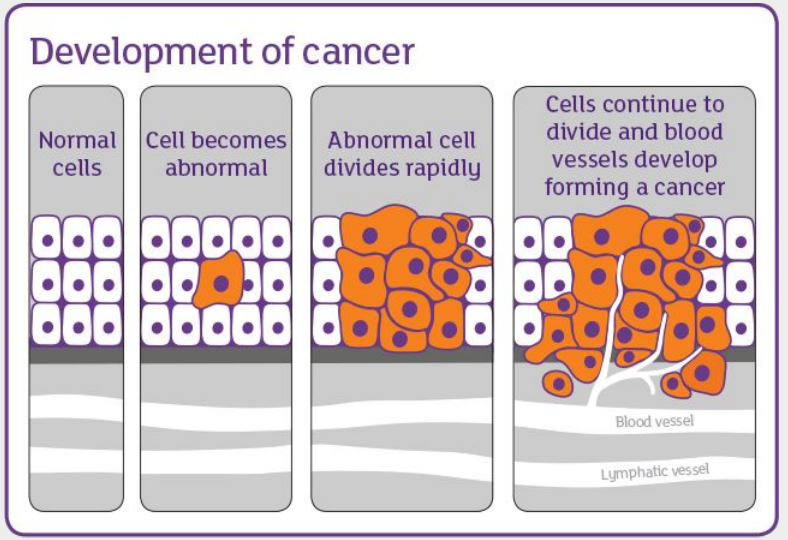
What is cancer?

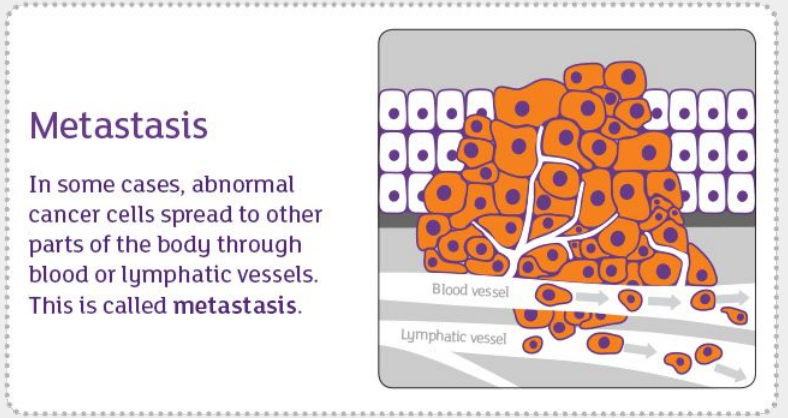
Uncontrolled Proliferation

The fundamental abnormality resulting in the development of cancer is the continual unregulated proliferation of cancer cells.

Regulatory Abnormalities

Cancer cells display mutations in mechanisms regulating cell proliferation, differentiation, and survival.

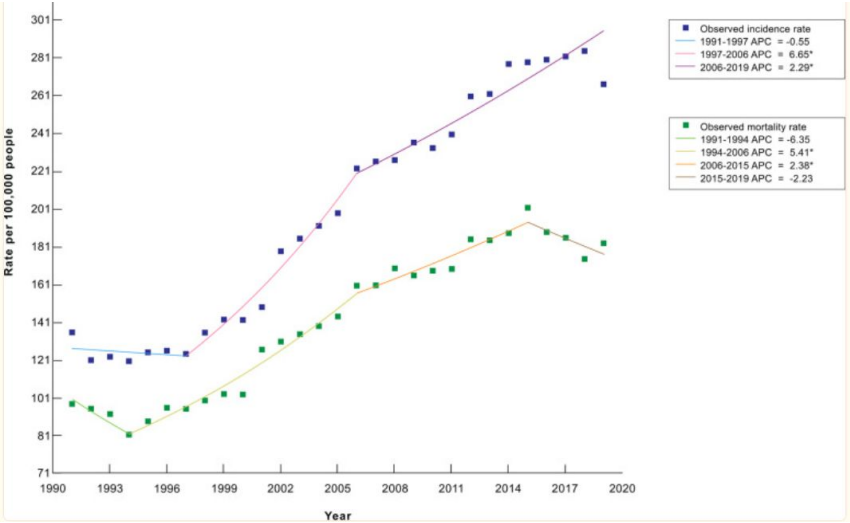




https://www.cancer.nsw.gov.au/about-cancer/cancer-basics/what-is-cancer

Cancer burden in Armenia

Trend of Incidence & Mortality (1990-2020)



Cancer Stage at Diagnosis (%)

Type of cancer	Stage I-II (%)	Stage III (%)	Stage IV (%)
Breast	76.8	6.9	16.3
Cervical	37.1	44.0	19.0
Lung	17.2	19.3	63.5
Colorectal	34.4	36.0	29.6
Stomach	30.6	26.9	42.5
Bladder	77.2	10.7	12.2
Prostate	35	28.8	36.1
All	50.4	19.5	30.1

Source: Bedirian K, et al. Front Oncol. 2022 Jan 11;11:782581.

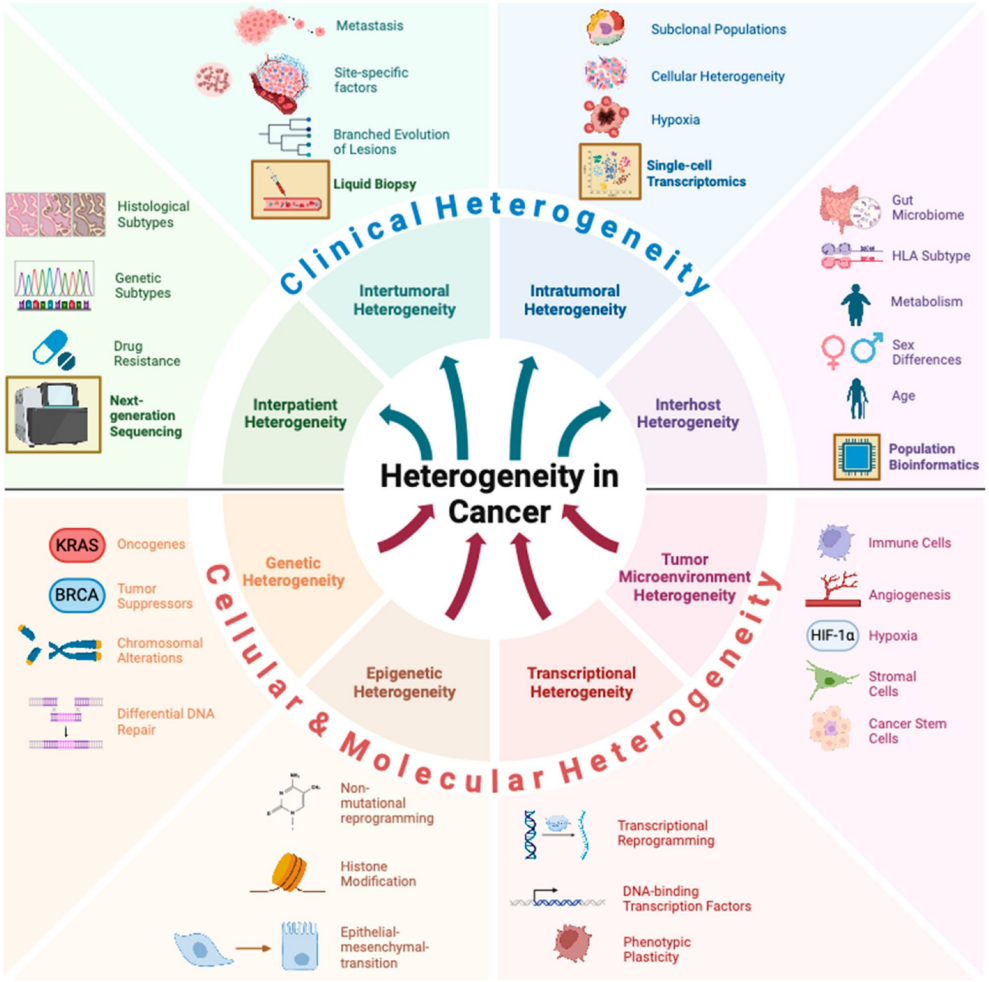
Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8787108/

Cancer heterogeneity

Intra-tumor: individual cells within a tumor exhibit genetic and non-genetic differences.

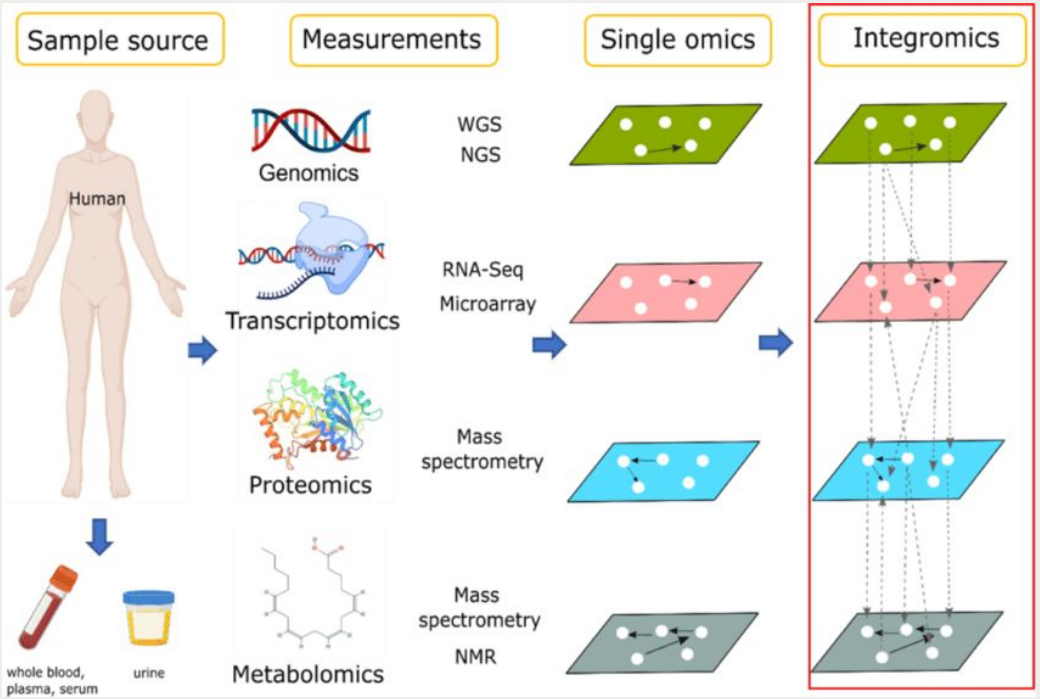
Inter-tumor: tumors within patients exhibit different characteristics.

Inter-patient: patients with same diagnosis differently respond to treatment.



https://doi.org/10.3390/cancers17030441

Cancer heterogeneity requires multimodal approach



Single omics approaches are not sufficient to uncover the cancer heterogeneity.

Pitaloka DAE, et al. Infect Drug Resist. 2022 May 28;15:2703-2711.

Cancer treatment

Precision oncology

Seeks to understand the unique genetic makeup of each patient's tumor to better tailor more effective treatments.

Precision oncology

Provides molecular profiling of tumors to identify best fit treatment.

https://www.lerner.ccf.org/immunotherapy/about/

https://nhcancerclinics.com/cancer-treatment-options/

Objectives

Establishment of the long-read RNA sequencing-based pan-cancer transcriptome catalog focusing on Armenian cancer cases to promote understanding the molecular mechanisms of selected cancers and extraction of novel “-omics” based markers for cancer molecular classification, diagnostics, and prognostics.

Tasks

Sequencing

Sequencing of the full-length transcriptome in selected solid and hematological cancers of patients in Armenia

Pan-Cancer Catalog

Creation of a pan-cancer catalog of RNA-sequencing-based genomic and transcriptomic features (Armenian Atlas of Cancer Genomes, AACG)

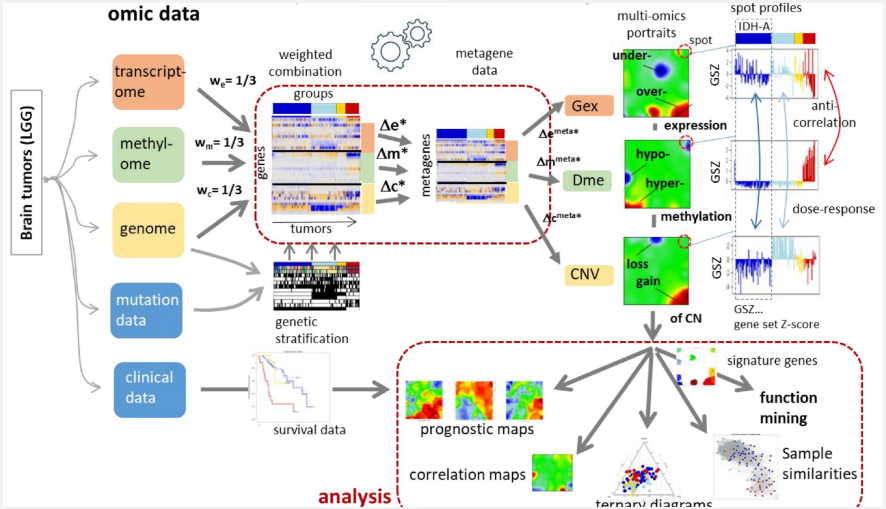
Bioinformatics Development

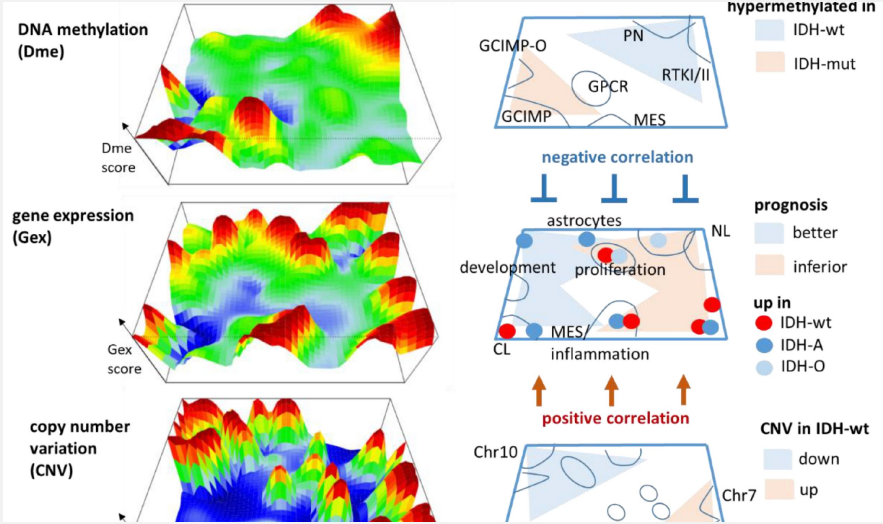
Development of bioinformatics methods for the analysis of genomic/transcriptomic data

Marker Extraction & Mechanisms

Understanding molecular mechanisms development, progression, and response to treatment of cancers, extract novel “-omics” based markers for cancer molecular classification, diagnostics, and prognostics

Integrated Multi-Omics Maps of Lower-Grade Gliomas

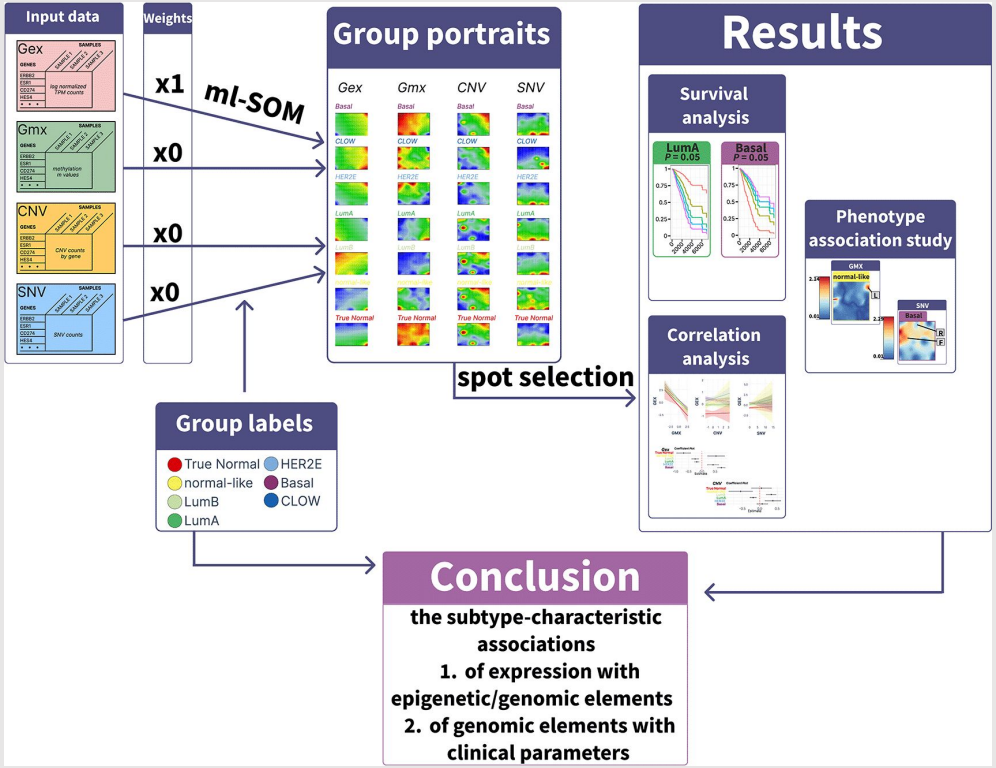




- **Lineage Differentiation:** Successfully distinguished astrocytoma- and oligodendroglioma-like lineages.
- **Cancer Hallmarks:** Genetic lesions and methylation independently or concertedly drive hallmarks like proliferation and blocked differentiation.
- **Clinical Utility:** Molecular landscape visualization is useful for subtyping, extracting prognostic markers, and understanding tumor heterogeneity.

Binder H, et al. Integrated Multi-Omics Maps of Lower-Grade Gliomas. Cancers (Basel). 2022;14(11):2797.

Integrated analysis of -omic landscapes in breast cancer subtypes



Molecular diversity: BC is characterized by diversity across expression, methylation, CNV, and SNV.

Perturbed modules: Identification of BC subtype specific gene modules and modalities that drive cancer progression.

Clinical implications: BC is a complex association between genomic factors that drive disease course.

Davitavyan S, et al. Integrated analysis of -omic landscapes in breast cancer subtypes [version 1; peer review: 2 approved with reservations]. F1000Research 2024, 13:564

Pan-cancer analysis of telomere maintenance mechanisms

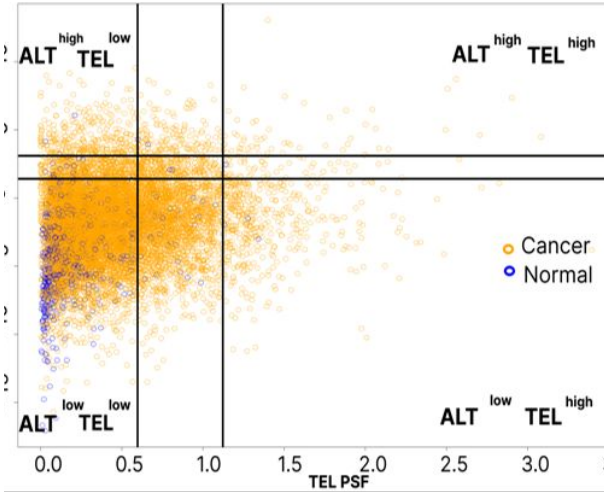
APPROACH

Quantified TEL and ALT pathway activity across 33 cancer types (TCGA), classified into 5 TMM phenotypes.

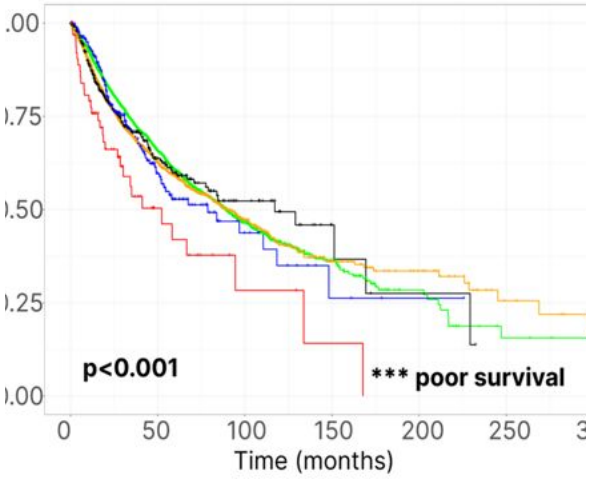
KEY FINDINGS

- Telomere maintenance in cancer is heterogeneous.
- TMM phenotype is a candidate prognostic biomarker.

TMM Phenotypes



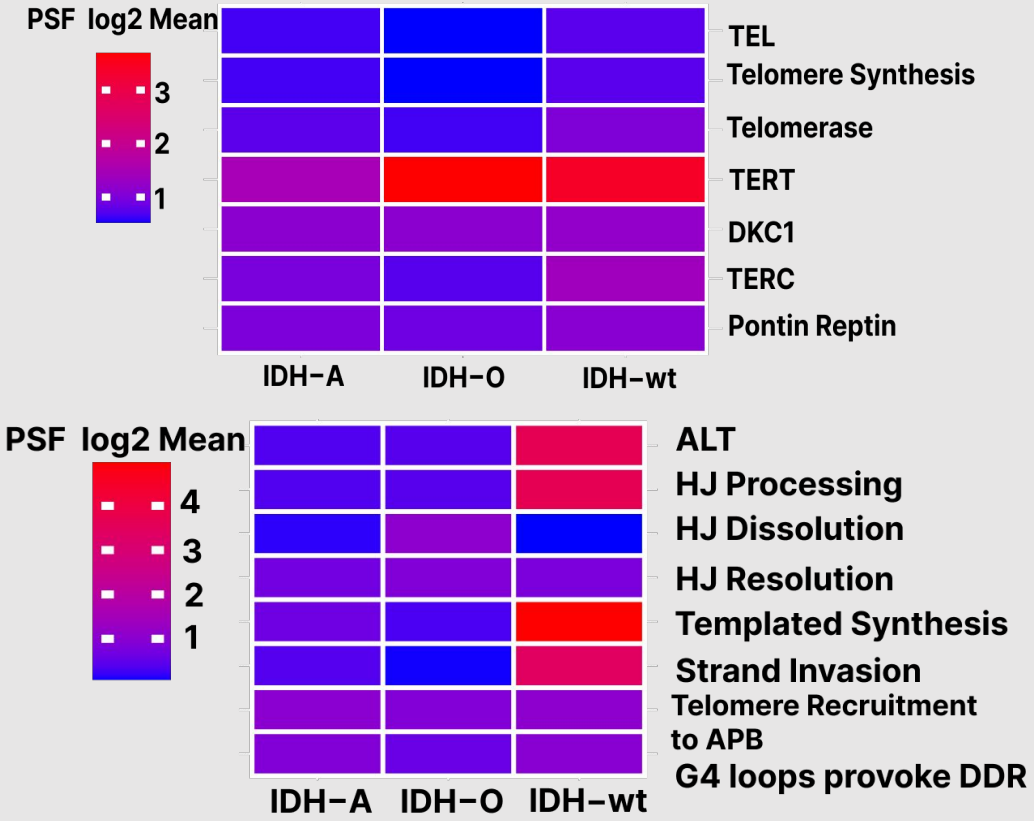
Overall Survival



Shortened survival for **ALT high TEL high** vs other phenotypes (p = 0.001)

Hakobyan, M.; Binder, H.; Arakelyan, A. Journal of Biological Chemistry, 2024; 300

Telomere Maintenance Pathways in Lower-Grade Gliomas



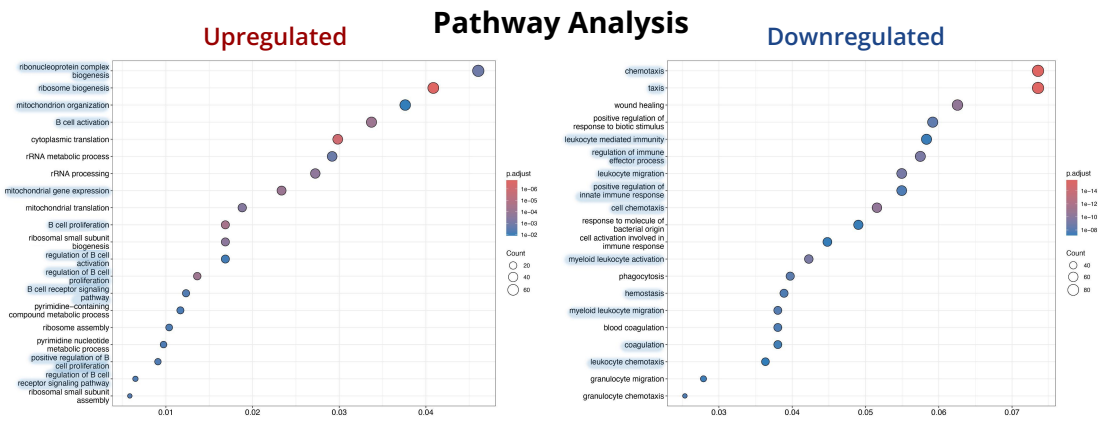
- Short telomeres correlate with increased **TEL** activity (mainly TERT).
- **ALT** activity depends on IDH/ATRX subtype, not just length.
- **IDH-wt** tumors exhibit peak ALT activation via RAD51.
- High **ALT + TEL** activity results in poorest prognosis.
- Moderate TMM activity yields best survival outcomes.

Combined TEL/ALT activity, not length alone, identifies biologically distinct LGG groups.

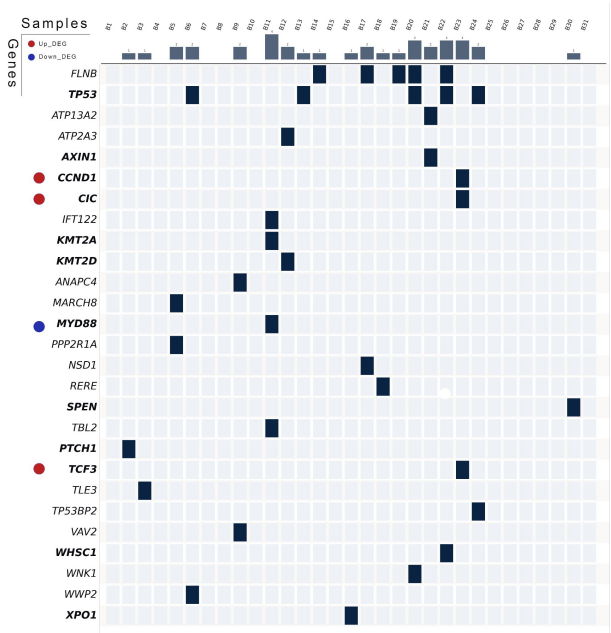
Hakobyan, M.; Binder, H.; Arakelyan, A. Int. J. Mol. Sci. 2025, 26, 4175.

Genomic landscape of Chronic lymphocytic leukemia

Pathway Analysis

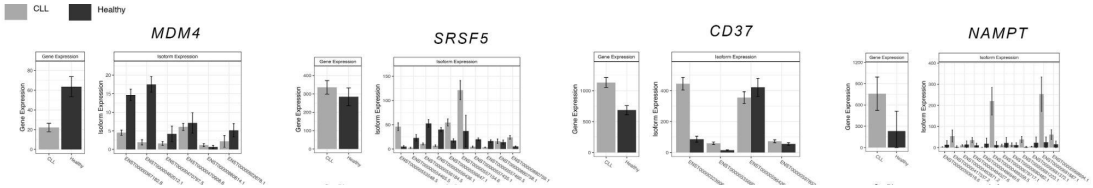


Somatic Driver Mutations



Key drivers: *TP53, XPO1, KMT2A/D, SPEN* mutated. *CCND1, TCF3, CIC* upregulated; *MYD88* downregulated.

Key Isoform Switches & Functional Impact

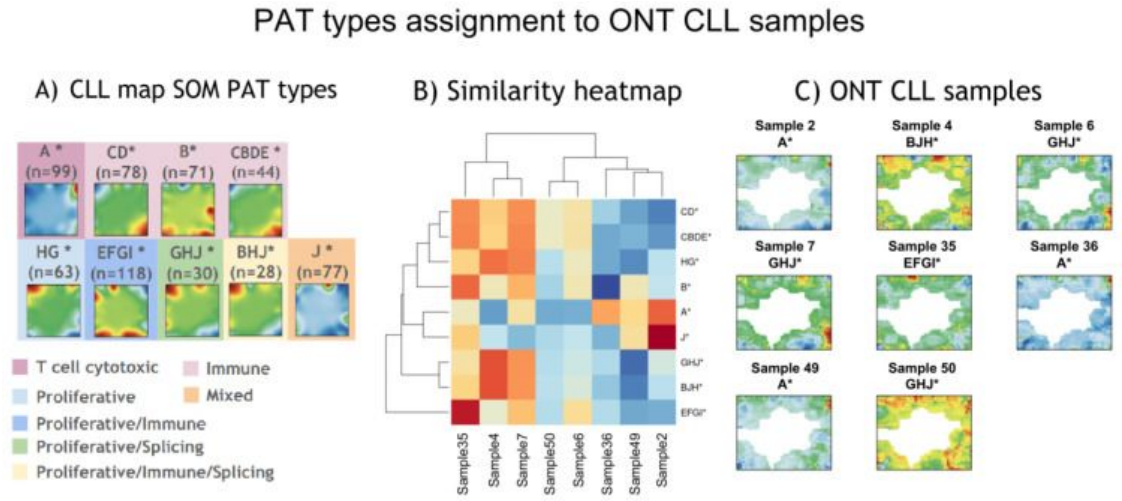


- Impairing DNA damage response and apoptotic pathways
- Altering B-cell receptor signaling & pre-mRNA splicing
- Disrupting NAD⁺ metabolism and mitochondrial homeostasis

in preparation

Assigning Transcriptomic Subtypes to CLL Using Nanopore RNA-Seq and SOM

Transcriptomic Landscape & Subtype Classification

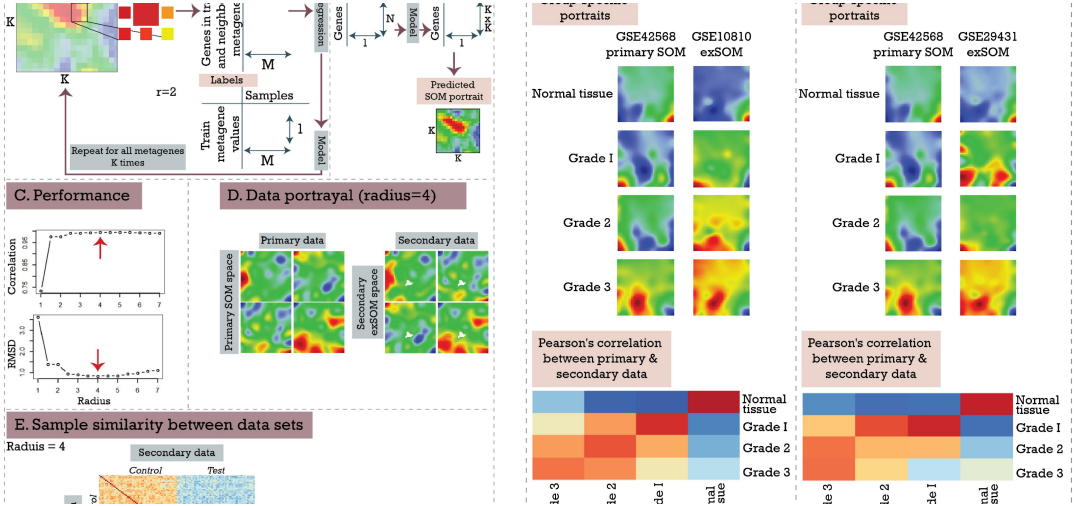


- Transcriptomic subtypes associate with survival, independent of gender and mutation status.
- Nanopore sequencing integrated with ML provides a cost-effective molecular subtyping approach.
- Facilitates more accessible and personalized CLL prognostic prediction and care.

Arakelyan A, et al. Assigning Transcriptomic Subtypes to Chronic Lymphocytic Leukemia Samples Using Nanopore RNA-Sequencing and Self-Organizing Maps. Cancers. 2025; 17(6):964.

Projection of High-Dimensional Genome-Wide Expression on SOM Transcriptome Landscapes

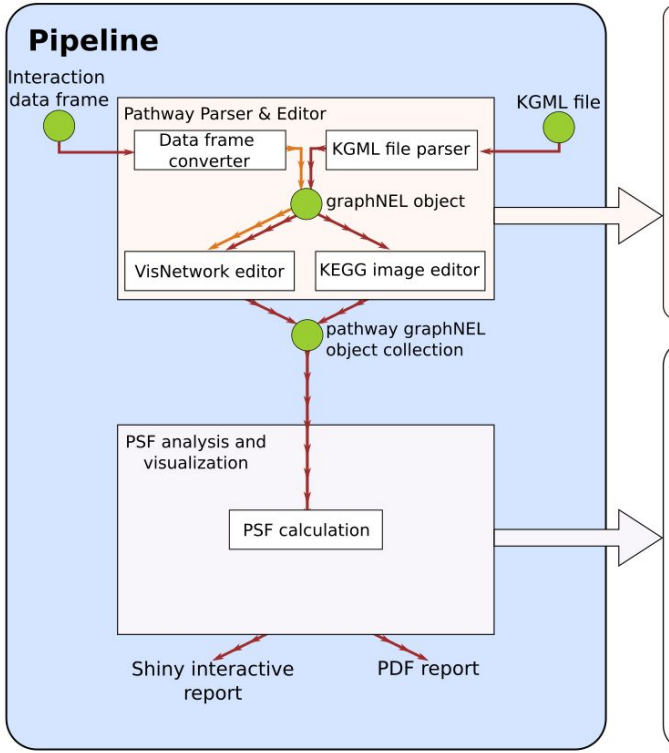
Methodology & Results: exSOM and supSOM Pipelines

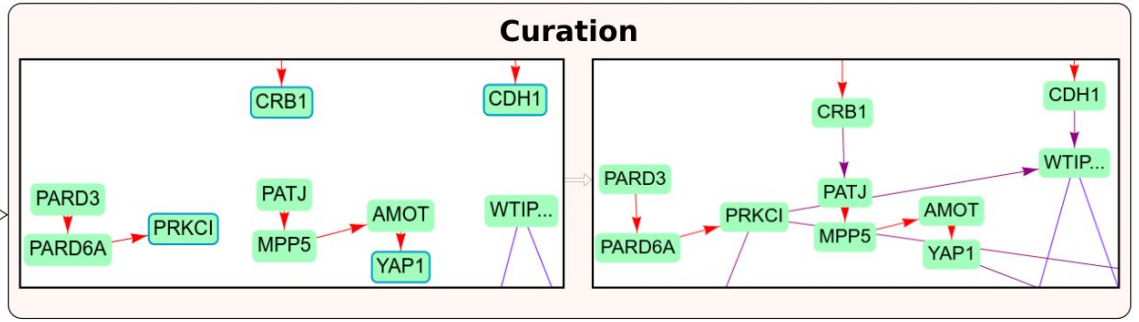


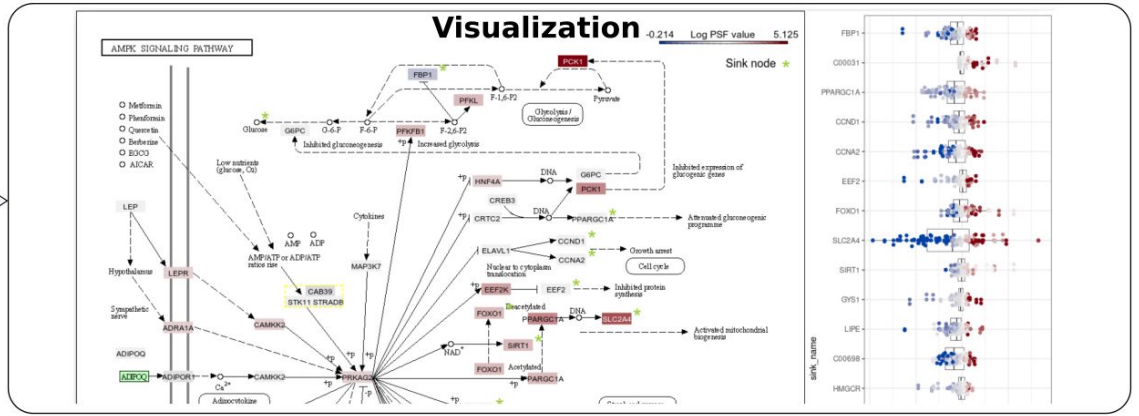
- exSOM and supSOM facilitate reusable SOM-based transcriptome maps.
- Ensures comparability across diverse datasets.
- Enables scalable genome-wide expression analysis for large-scale studies.

Nikoghosyan, M.; Loeffler-Wirth, H.; Davidavyan, S.; Binder, H.; Arakelyan, A. Projection of High-Dimensional Genome-Wide Expression on SOM Transcriptome Landscapes. BioMedInformatics 2022, 2, 62-76.

Topology Based Pathway Analysis (PSF toolkit)



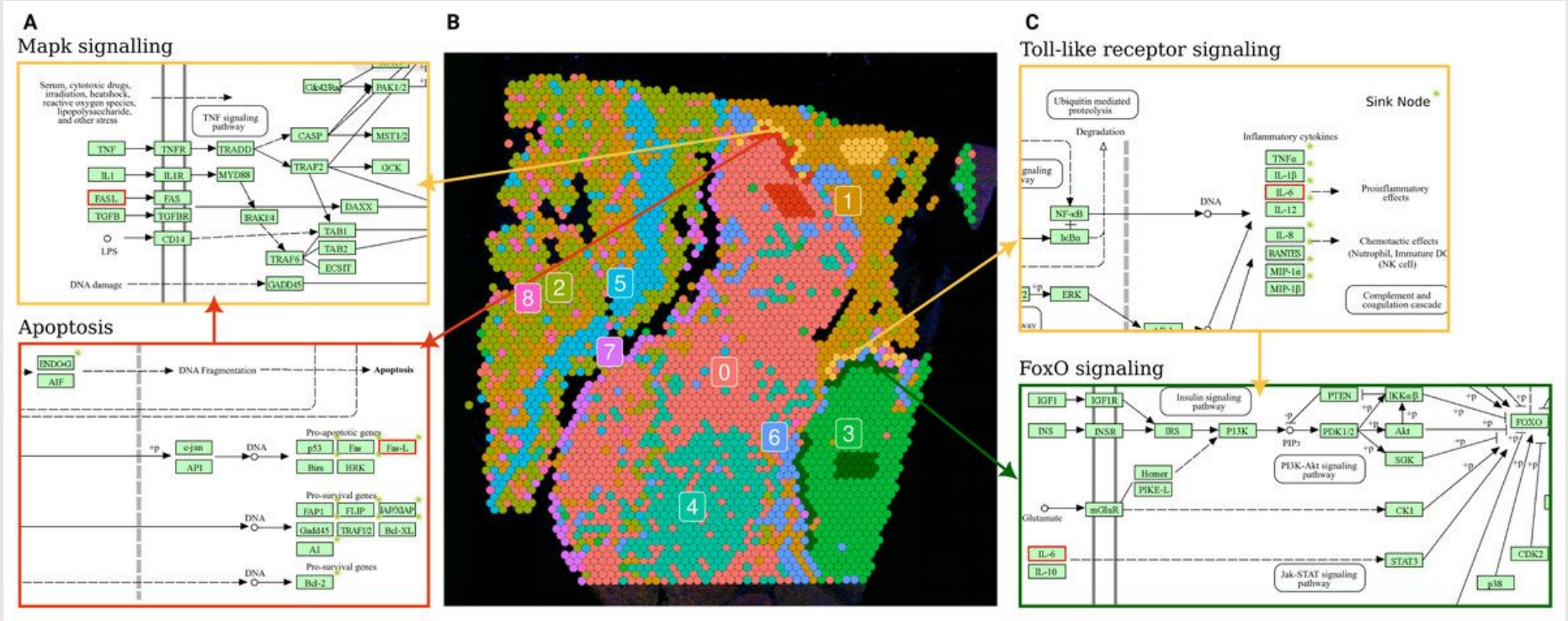




https://github.com/hakobyansiras/psf

Hakobyan S, et al. (2023) Front. Genet. 14:1264656.

Pathway Activity Analysis of Spatial Transcriptomics

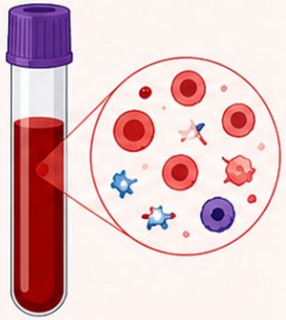


Hakobyan S, et al. Topology-aware pathway analysis of spatial transcriptomics. PeerJ. 2025 Aug 14;13:e19729.

Relevance of Pre-analytical Factors in Multiomics: Toward a Standardized Blood Processing Protocol

WHY PRE-ANALYTICAL FACTORS MATTER

Pre-analytical variations can introduce bias and compromise data quality across all omics layers.



Key sources of variability

- Tube type and additives
- Clotting and platelet activation
- Processing delay and temperature
- Centrifugation conditions
- Storage and freeze–thaw
- Hemolysis, icterus, lipemia

IMPACT ON MULTIOMICS

GENOMICS

Contamination with leukocyte DNA can bias variant calling and methylation profiles.

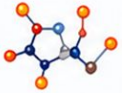
TRANSCRIPTOMICS

Highly sensitive to platelet and leukocyte RNA contamination and RNA degradation.

PROTEOMICS

Affected by protein release from blood cells (especially platelets) and proteolysis.

METABOLOMICS

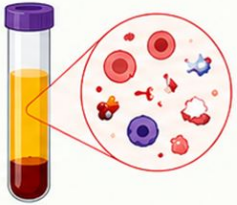


Metabolite levels change rapidly due to cellular metabolism and enzymatic activity.

Delays, inappropriate handling and storage can alter molecular profiles and reduce reproducibility.

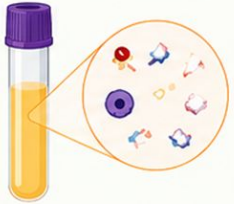
WHY PLASMA IS PREFERRED

SERUM (after clotting)



Risk of contamination from lysed cells during clot formation.

PLASMA (anticoagulated)



Cleaner composition, better for all omics layers.

Platelet-poor plasma is ideal, especially for transcriptomics.

HARMONIZED BLOOD PROCESSING PROTOCOL (RECOMMENDED)

1 COLLECTION

Collect blood in K_2EDTA tubes.

2 FIRST CENTRIFUGATION

1600 × g
10 min
4 °C

3 SECOND CENTRIFUGATION

16,000 × g
10 min
4 °C

4 PLASMA ALIQUOTING

Aliquot supernatant (plasma) into clean polypropylene tubes.

5 QUALITY CHECK

Visually inspect and record for:

- Hemolysis
- Icterus
- Lipemia

6 STORAGE

Store at −80 °C until analysis.

DOCUMENT EVERYTHING: SPREC

Use the Standard PREanalytical Code (SPREC) to ensure transparency and reproducibility.

P · L2 · P · D · A1 · C · J · A · D

- P — Specimen: Peripheral blood
- L2 — Specimen component: Plasma
- PED — Collection tube: K_2EDTA
- A1 — Processing: 2-step centrifugation
- C — Centrifugation conditions
- J — Storage: −80 °C
- A — Atmosphere: Ambient (processing)
- D — Quality control: Visual check

KEY TAKE-HOME MESSAGE

Following a standardized blood processing protocol—or carefully documenting any deviations—is essential to minimize pre-analytical variability and improve comparability and reproducibility in multiomic studies.

Developing Biobanking Infrastructure in Armenia

Opportunities and Challenges

1,600+

Samples Collected

Network Integration

The Biobank integrates with national and international clinical registries and research projects, supporting ongoing biospecimen collection efforts.

Clinical Registries

NAREG • ARMI • EUSTAR

GFM • JIR & more

Research Projects

AGP • AACG • AWGP

Driving multi-omics innovation

Mkrtchyan G, et al. *Front. Public Health*, 2026; 13:1749588.

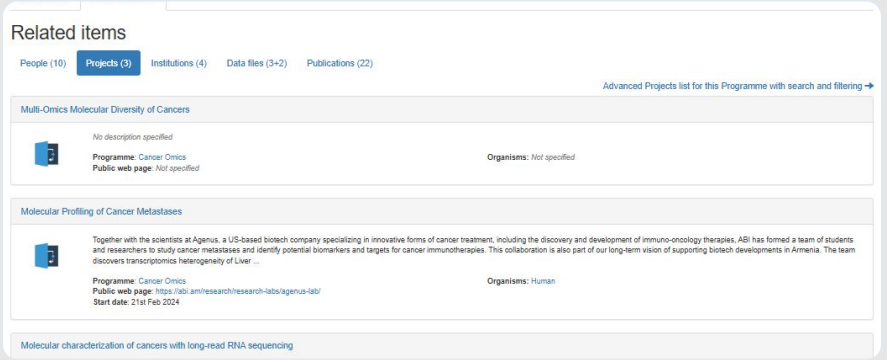
ArmLifeBank: A FAIR-Compliant Data Reuse Platform

For Biomedical and Multi-Omics Research in Armenia

ArmLifeBank integrates genomic, transcriptomic, and bioinformatics research outputs from Armenian laboratories.

FAIR Principles Implementation

- **Findable** through global indexing
- **Accessible** for secure collaboration
- **Interoperable** across platforms
- **Reusable** for continued analysis

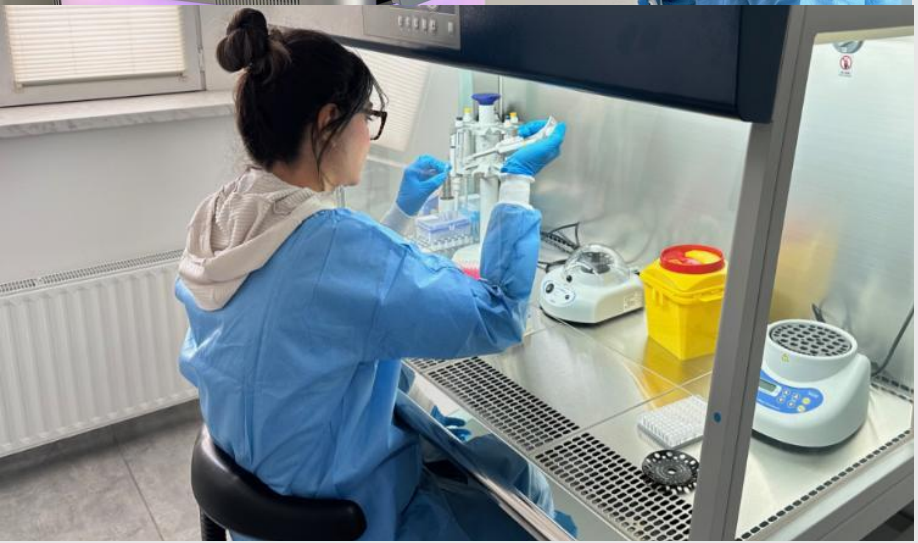


Platform Interface: Multi-Omics Dataset Browser

Data Distribution: Submission Analytics & Tracking

in preparation

Laboratory of molecular genomics (Lic. N Կ-ՖՕ-000135)



- Whole exome sequencing - rare diseases (~20k genes)
- Inherited cancer syndromes - cancer predisposition (~20k genes)
- Tumor comprehensive genomic profiling (627 genes)
- Targeted oncogene panels
- Fusion transcripts (RNA)

https://imb.am/mgc/

Team

Siras Hakobyan

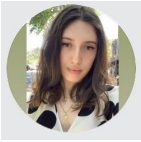
Yeva Bareghamyan

Yervand Hakobyan

Meline Hakobyan

Gevorg Martirosyan

Andranik Chavushyan



Tamara Sirunyan

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Collaborator

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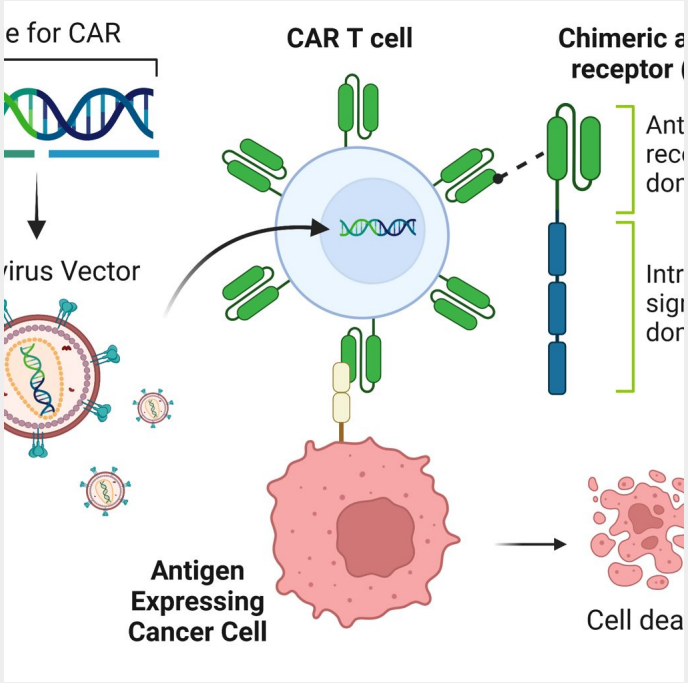
PD Dr. rer. nat. habil.
Interdisciplinary center for bioinformatics, Leipzig University

Senior scientist & managing director of IZBI

Chairman of Armenian Bioinformatics Institute Board

Quo vadis? Յո՞ երթաս:

Towards establishing capacity and infrastructure for adoption of advanced cancer cell therapies in Armenia



Institutionalizing CAR-Based Cancer Cell Therapies in Armenia (27TARGET-1F090)

Affordable point-of-care CAR-T treatment delivery

Macrophage-based cell therapy research for solid tumors (26RL-1F039)

Target prioritization and engineering: New promising research direction for solid tumors

Acknowledgements

INSTITUTE OF MOLECULAR BIOLOGY

Karine Mayilyan
Maria Nikoghosyan
Diana Avetyan

Meline Melkumyan

Santé Arménie

Arsen Mekinian
Anna Martirosyan

IZBI

Henry Löffler-Wirth
Maria Schmidt

ABI

Arpine Grigoryan
Gayane Ghazaryan

Ashot Davidyants

HistGen pathology center

Armen Mkhitaryan

UNIVERSITÄT LEIPZIG

Toralf Kirsten

Շնորհակալություն