International Workshop 'Ultrafast Beams and Applications'
2-5 July 2019, Yerevan, Armenia

Joint Laboratory of Experimental Biology
CANDLE SRI and IMB NAS RA

In vitro, in vivo, and in silico modelling for radiobiological research

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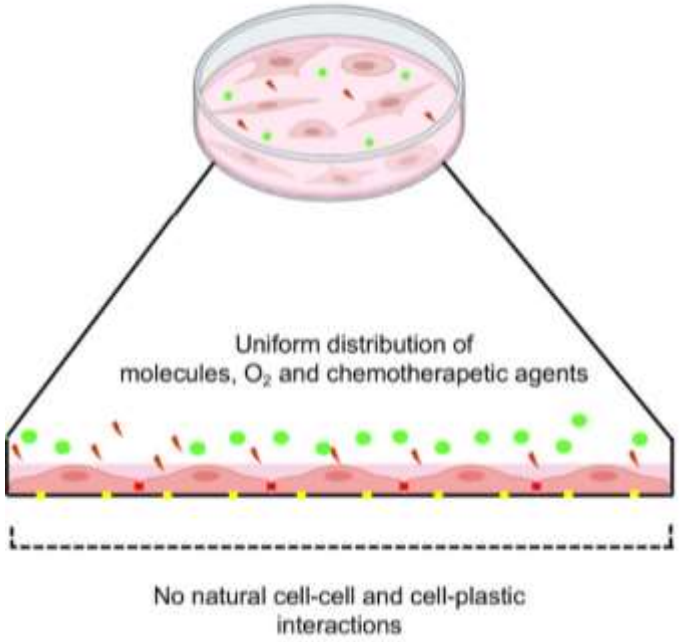
Yerevan, 2024

Models for Radiobiological Research

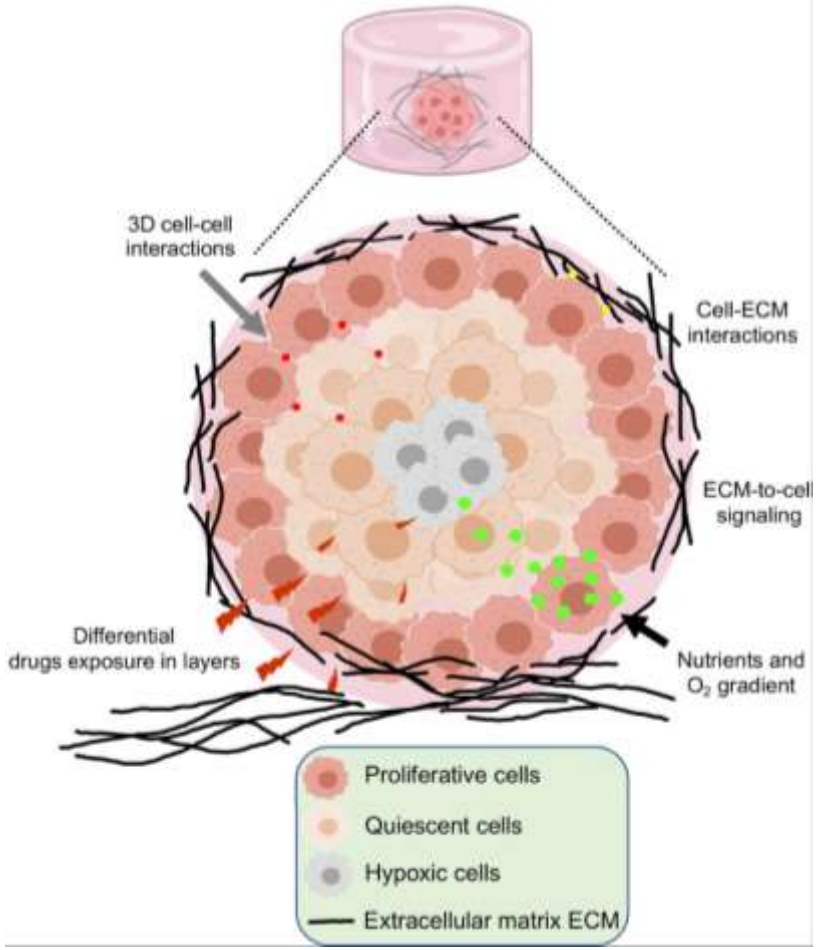
In vitro	In vivo	In silico
Isolated cells/tissues	Rats/mice	AI/ML
2D/3D cancer models	In vivo glioblastoma model	Drug-like properties prediction

In Vitro Studies

Conventional 2D cancer models



Advanced 3D cancer models



Advantages

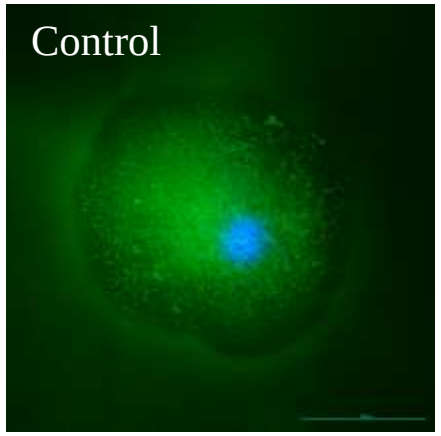
- Control and Precision
- Molecular mechanisms
- Extrapolation to Humans
- Ethical Considerations
- Cost and Time Efficiency

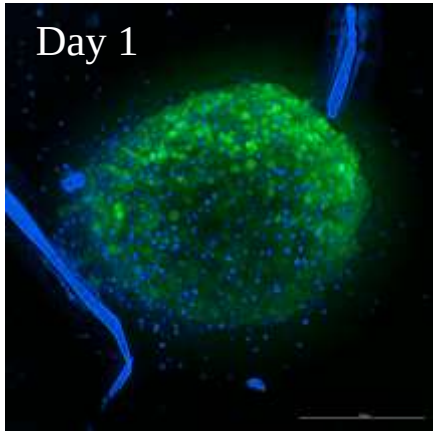
© https://doi.org/10.3389/fonc.2022.826113

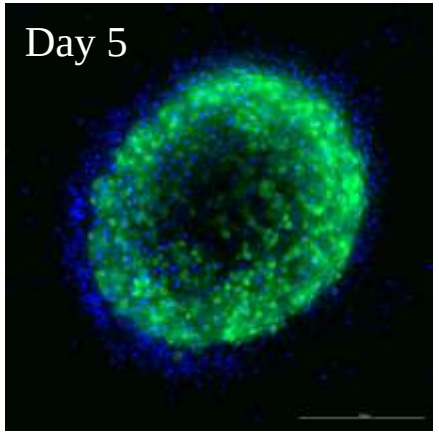
In Vitro Studies

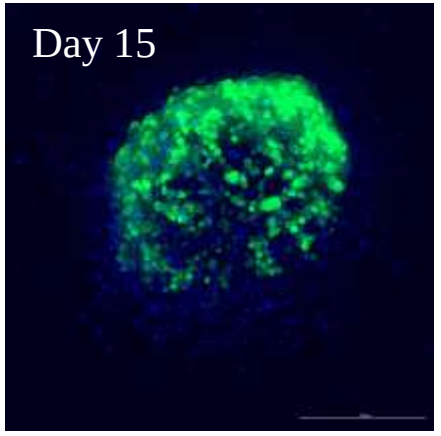
AREAL: UPEB irradiation
Irradiation dose: 3 Gy
Repetition rate: 20 Hz

Cell line: F98 (human glioblastoma)
Staining: Calcein AM (Live)/DAPI (Dead)
Imaging: Confocal (Cytation 10)

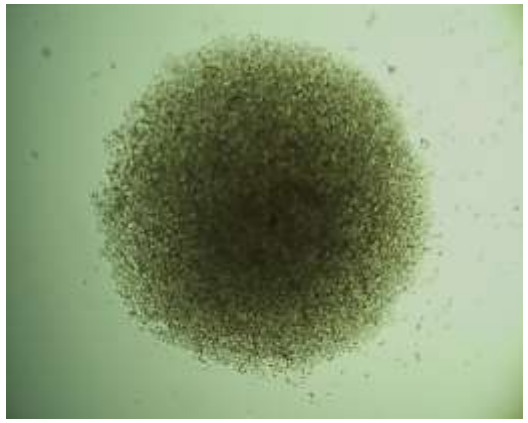


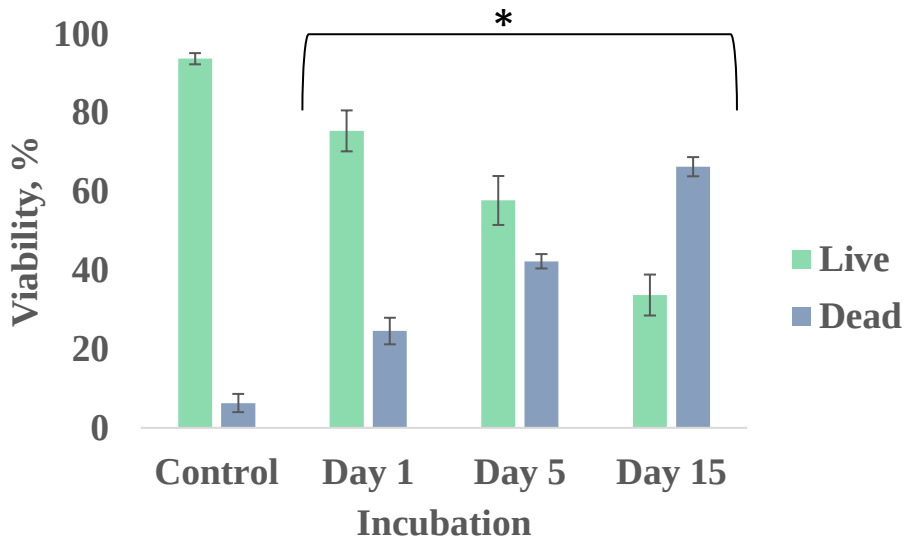


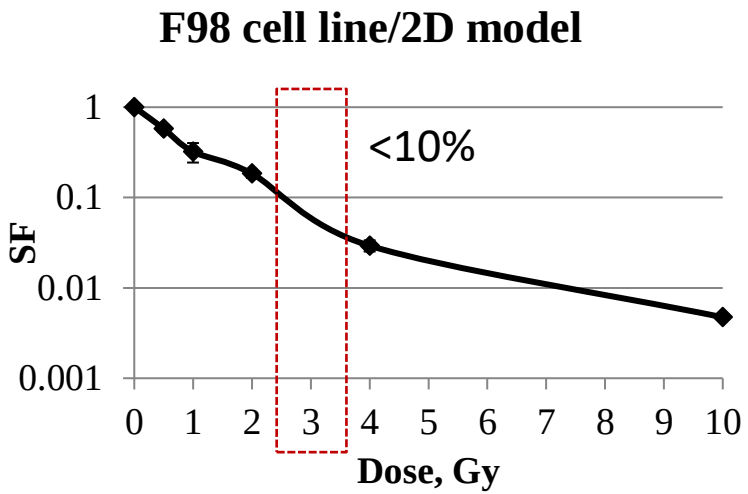




Unstained spheroid







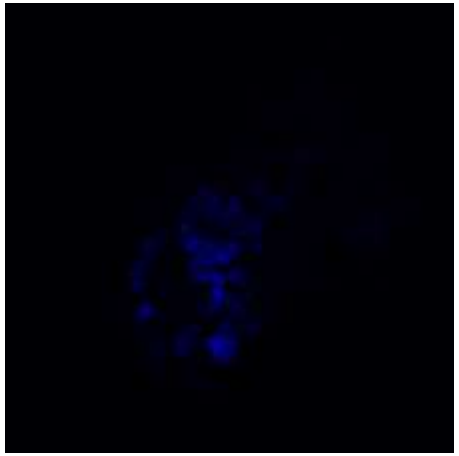
In Vitro Studies

AREAL UPEB irradiation
Irradiation dose: 3 Gy
Repetition rate: 20 Hz

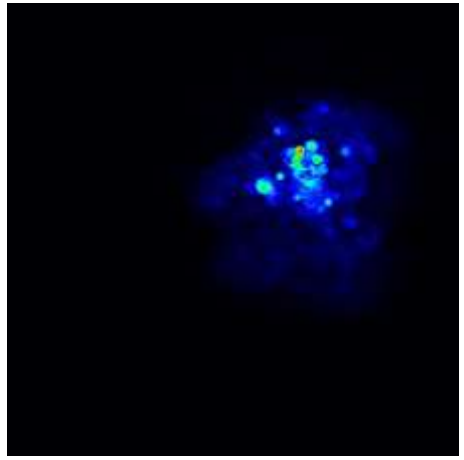
Cell line: F98 (human glioblastoma)
Staining: DC-FDA
Imaging: Two-photon microscopy

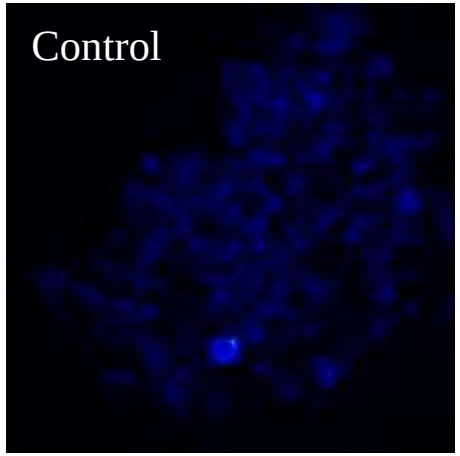
Real-time imaging

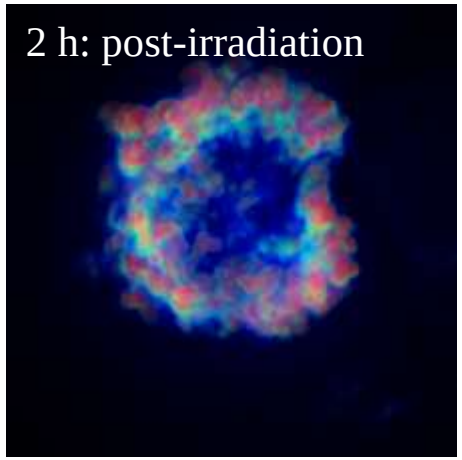
Control

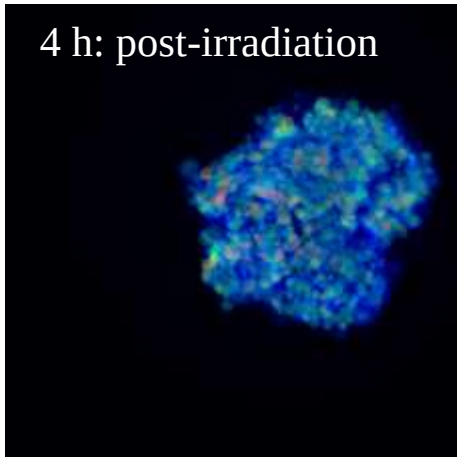


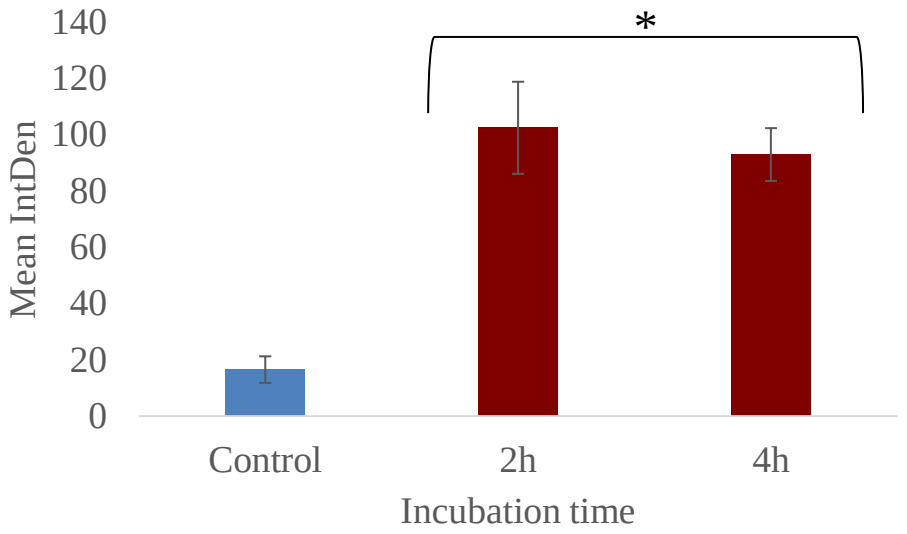
4 h post-irradiation









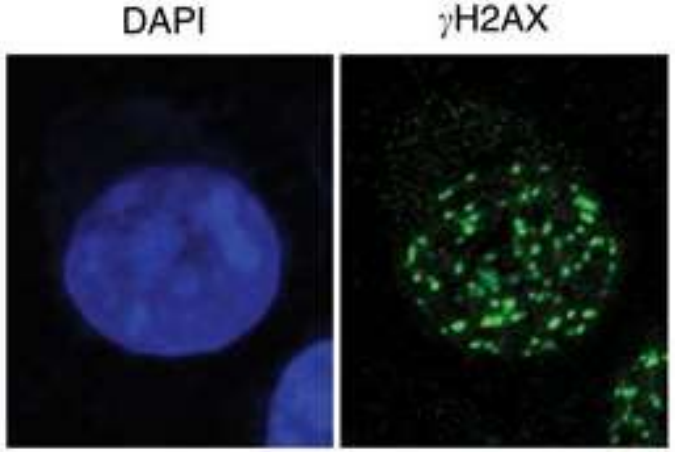


In Vitro Studies

AREAL UPEB irradiation
Irradiation dose: 3 Gy
Repetition rate: 20 Hz

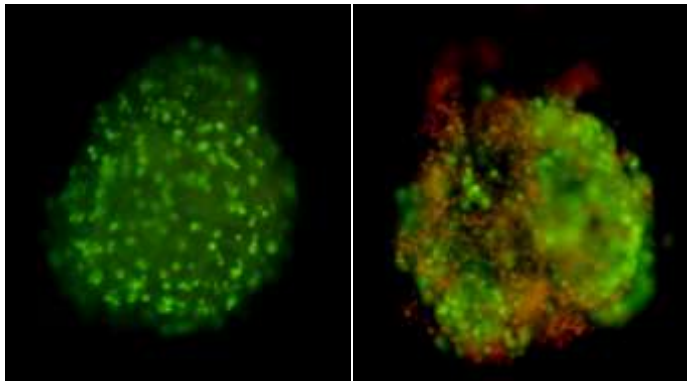
Ongoing/upcoming studies

DNA damage/repair analysis



Cell death/proliferation analysis

Live vs. Apoptotic cells



Co-culture studies

Co-culture with glial, endothelial cells, fibroblasts etc.

In vivo studies

Dr. Gohar Tsakanova
Former head of the lab of Experimental Biology, CANDLE SRI

The LD50 was estimated

- Dose: 2 Gy
- Pulse repetition rate: 2 Hz
- 50% of the animals survive 30 days post-irradiation

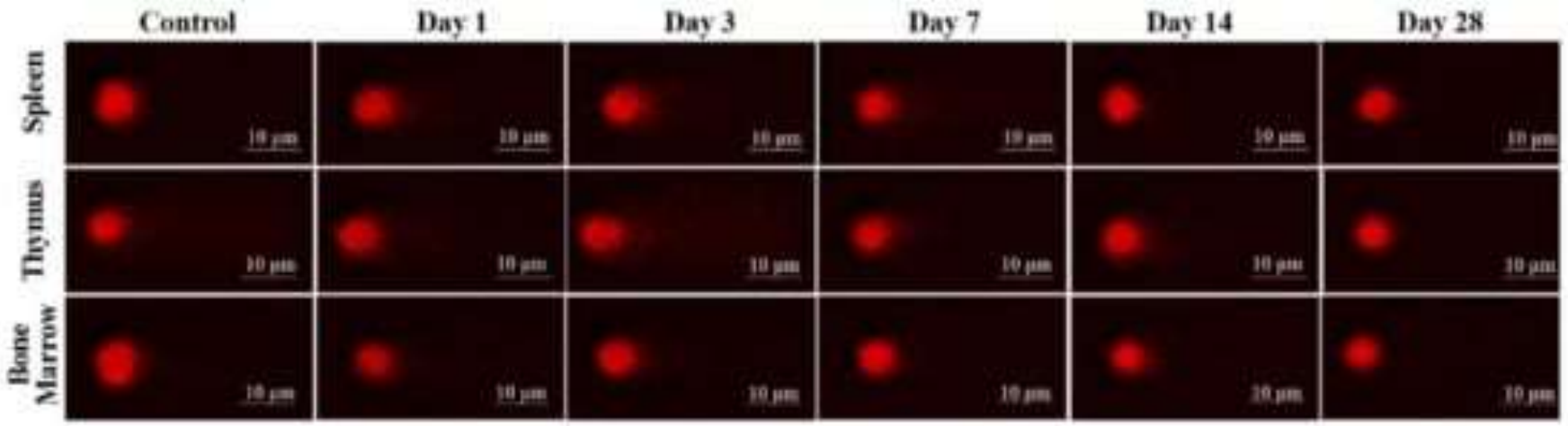
Immune system response

- Alterations in the rat immune system: 3 days post-irradiation
- Total recovery: 28 days post-irradiation

Advantages

- Complexity
- PK/PD
- Long-term Studies

DNA damage rate

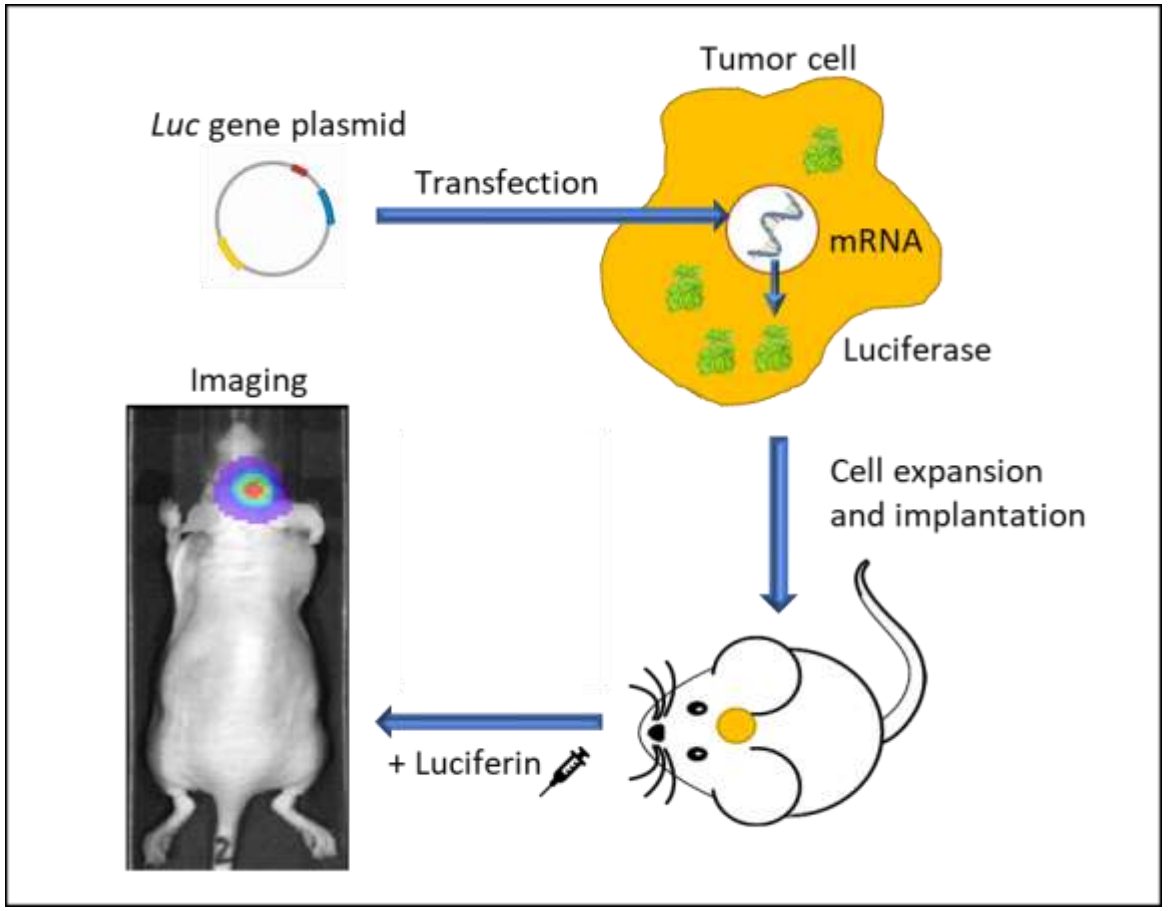


In vivo studies

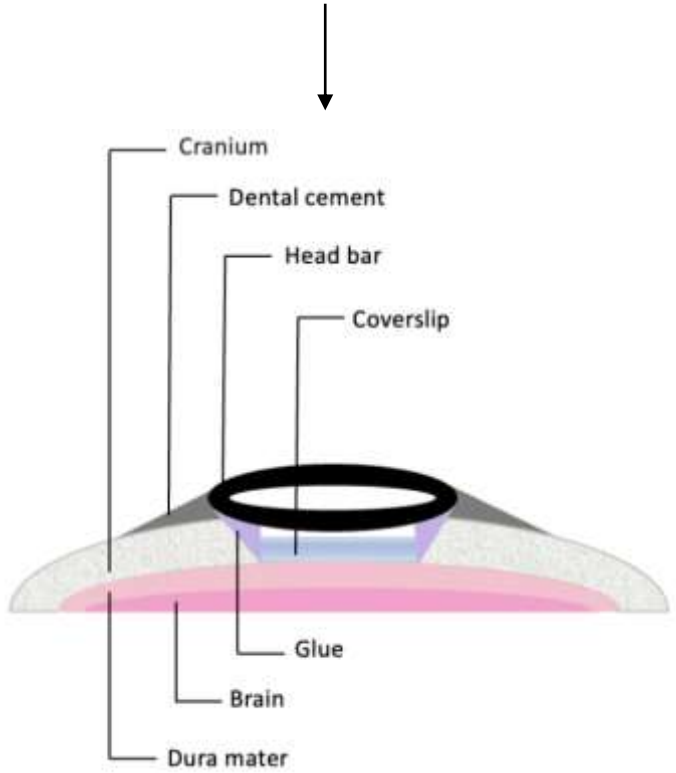
In collaboration with Dr. Aurelie Tchoghandjian
Head of GlioME project at the Institute of Neurophysiology, Aix-Marseille Université, France

Upcoming studies

Glioblastoma in vivo model



Two-Photon Intravital Microscopy of Glioblastoma



In silico studies

In Silico Pipeline for Predicting ADMET Properties

Absorption

- Aqueous solubility (logS)
- Lipophilicity (logP)
- Human intestinal permeability (CACO-2) permeability
- Human Intestinal Absorption (HIA)
- MDCK cell line permeability
- Human Oral Bioavailability
- P-glycoprotein inhibitor

Distribution

- Plasma protein binding
- Blood Brain Barrier permeability
- Pharmacokinetic volumes of distribution (Vd)
- Steady state volume distribution (VDss)

Metabolism

- CYP Substrates (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4)
- CYP Inhibitors (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4)
- Drug-drug interactions
- OATP1B1/OATP2B1/OATP1B3 inhibition
- Metabolism by human UGT enzymes (Phase II)

Excretion

- Human microsomal stability (CLint)
- Elimination half-life Human
- Elimination half-life Rat
- Human renal clearance (CLr)
- P-glycoprotein substrates and inhibitors

Toxicity

- Mutagenicity (Bacterial Reverse Mutation (AMES) test)
- Genotoxicity (in vitro micronucleus assay)
- Genotoxicity (in vivo micronucleus assay)
- Carcinogenicity (rodent)
- Cardiotoxicity (hERG inhibition)
- Hepatotoxicity (DILI)
- Neurotoxicity (AChE inhibition)
- Nephrotoxicity
- Acute oral toxicity (LD50, GHS)
- Developmental toxicity potential
- Endocrine toxicity (ER, TPO)

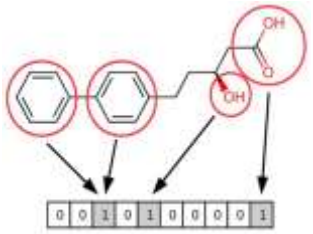
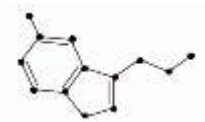
RISK SCORE: estimated based on ADMET properties along with physicochemical and medicinal chemistry properties

Advantages

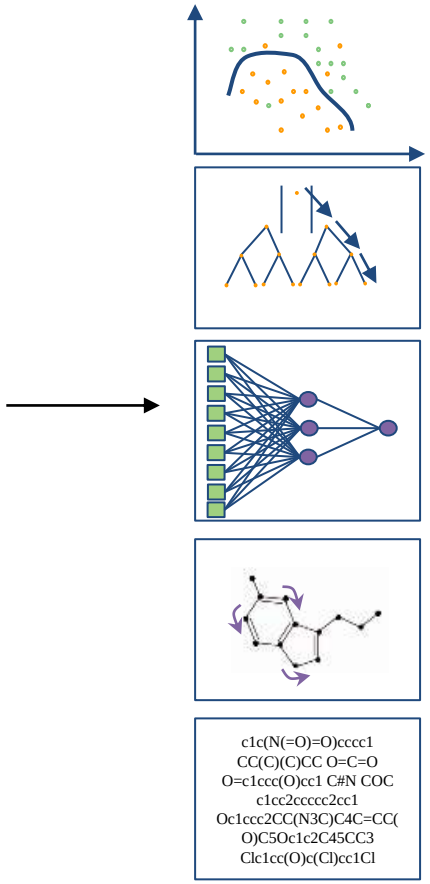
- Speed and Efficiency
- Cost-Effectiveness
- Ethical Considerations
- Risk Reduction
- Integration of Diverse Data

In silico studies: Models / Predictions

AI/ML models

Name	Description	Example
Descriptors	Physicochemical properties	Molecular weight Topological polar surface area Number of hydrogen bond acceptors Number of hydrogen bond donors Number of sp hybridized carbon atoms Number of rotatable bonds etc.
Fingerprints	Encoded molecular fragments	
Graphs	Nodes as atoms and edges as bonds	
Large Language Models	Simplified molecular-input line-entry system	CC(=O)Oc1ccccc1C(O)=O

Ensemble of AI/ML models (combination of all models)



Final Predictions

Classification task
Mutagenicity: positive/negative
hERG inhibition: active/inactive
Nephrotoxicity: toxic/non-toxic

Regression task
Solubility: logS values
Renal clearance: mL/min/mg values
Plasma protein binding: PPB % values

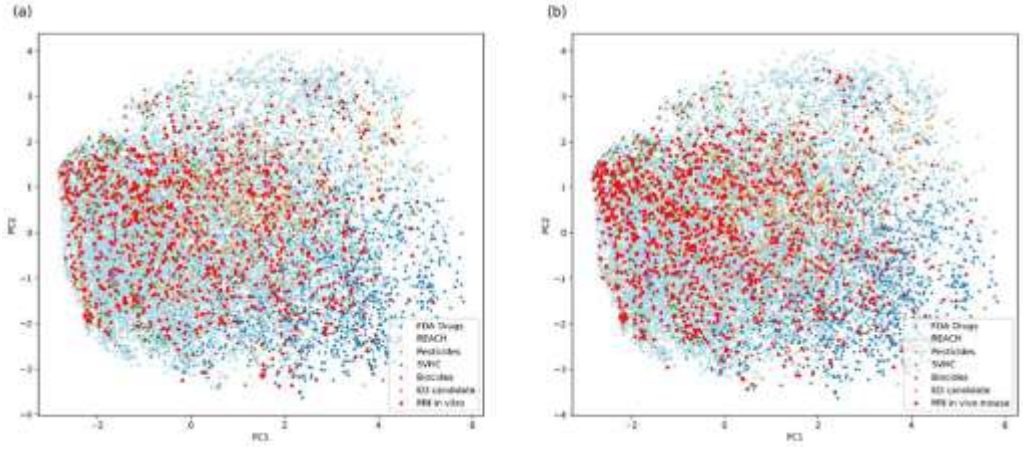
Our Paper

Datasets Construction and Development of QSAR Models for Predicting Micronucleus In Vitro and In Vivo Assay Outcomes

Lusine Khondkaryan, Ani Tevosyan, Hayk Navasardyan, Hrant Khachatrian, Gayane Chilingaryan, Gohar Tadevosyan, Lilit Apresyan, Nelly Babayan, Zaven Navoyan, Helga Stopper

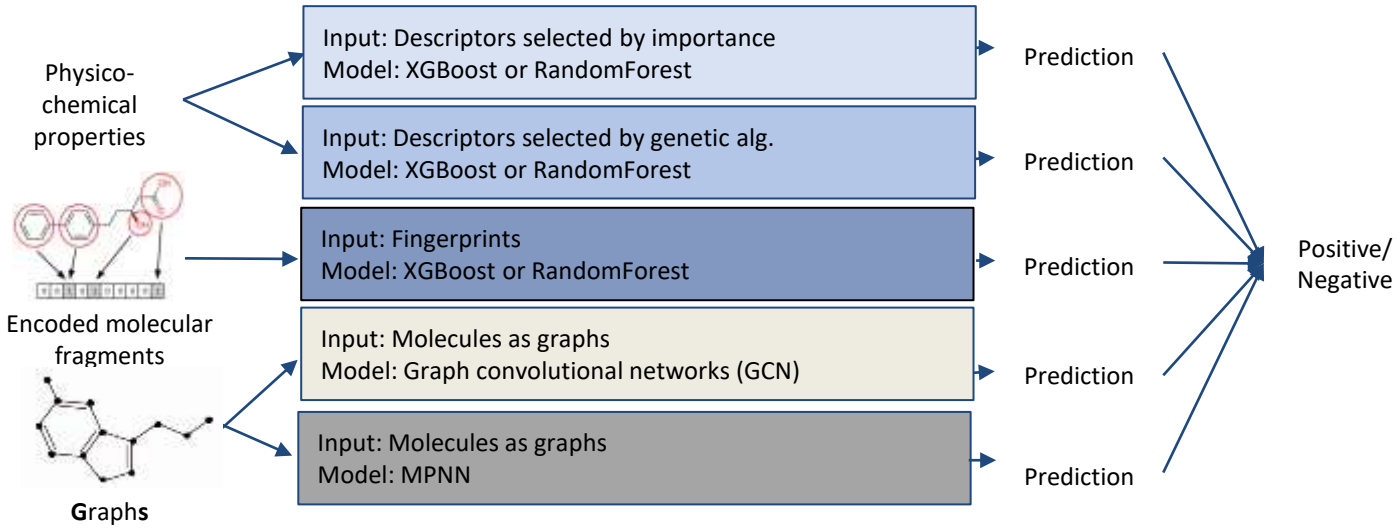
Toxics 2023, 11(9), 785; https://doi.org/10.3390/toxics11090785

2D PCA visualization of chemical space of compounds found in MN in vitro (**a**) and in vivo (**b**) datasets



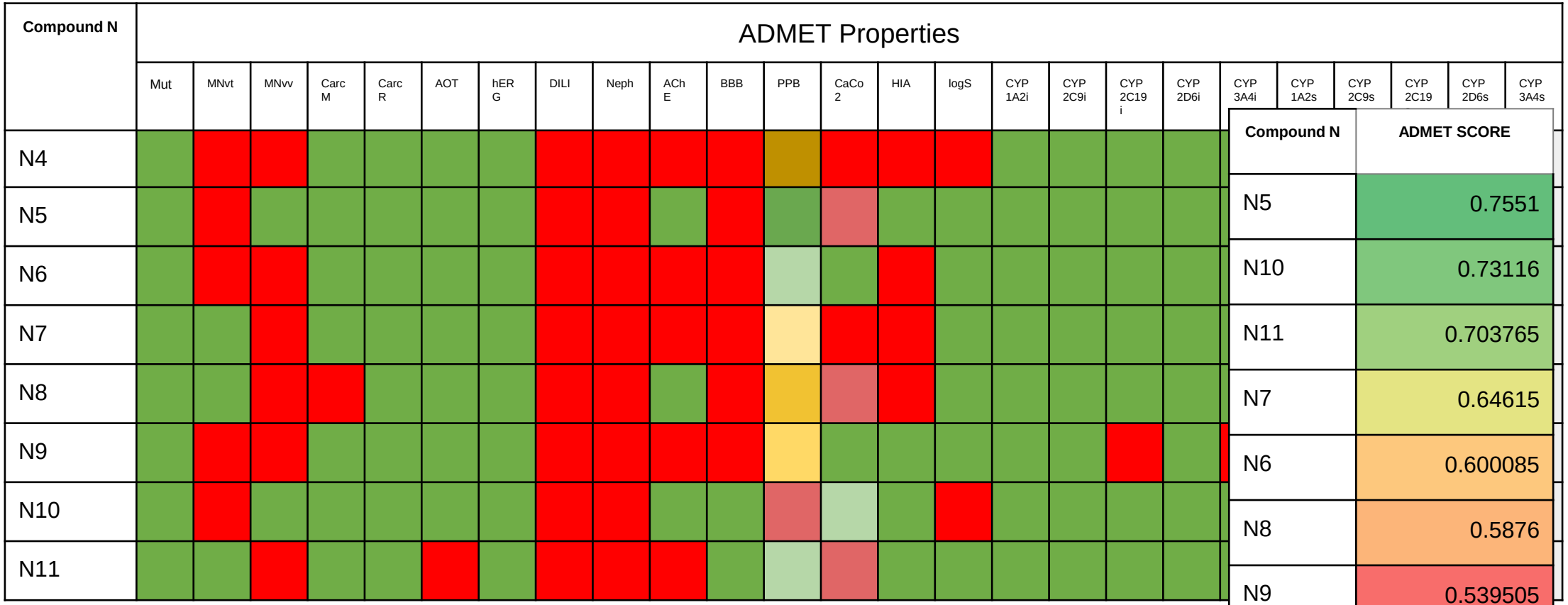
Extracted from 35 million PubMed papers using BioBERT pretrained large language model

Developed ensemble model for toxicity prediction



In silico studies: Risk Score

In collaboration with
INSTITUTE OF FINE ORGANIC CHEMISTRY NAMED AFTER A.L. MNJOYAN OF SCIENTIFIC TECHNOLOGICAL CENTER OF ORGANIC AND PHARMACEUTICAL CHEMISTRY, RA



Compound N	ADMET SCORE
N5	0.7551
N10	0.73116
N11	0.703765
N7	0.64615
N6	0.600085
N8	0.5876
N9	0.539505
N4	0.536185

In silico studies: Additional Scoring Functions

In collaboration with
INSTITUTE OF FINE ORGANIC CHEMISTRY NAMED AFTER A.L. MNJOYAN OF SCIENTIFIC TECHNOLOGICAL CENTER OF ORGANIC AND PHARMACEUTICAL CHEMISTRY, RA

Physicochemical Properties

- MW
- Number of hydrogen bond acceptors
- Number of hydrogen bond donors
- Number of rotatable bonds
- Number of rings
- Number of atoms in the biggest ring
- Number of heteroatoms
- Formal charge
- Number of rigid bonds
- Number of stereocenters
- Topological polar surface area
- logP

Compound N	PhCh SCORE
N11	0.75
N6	0.75
N7	0.75
N5	0.67
N8	0.67
N9	0.67
N4	0.67
N10	0.58

Medicinal Chemistry Properties

- QED
- SAscore
- Fsp3
- Lipinski Rule
- GSK Rule
- Pfizer Rule

Compound N	MedCh SCORE
N6	0.70
N5	0.69
N9	0.69
N11	0.69
N7	0.68
N8	0.68
N4	0.50
N10	0.50

ADEMT properties

Compound N	ADMET SCORE
N5	0.76
N10	0.73
N11	0.70
N7	0.65
N6	0.60
N8	0.59
N9	0.54
N4	0.54

Final Score

Compound N	Final Score
N11	0.713266
N5	0.704444
N7	0.693989
N6	0.681815
N8	0.645637
N9	0.631633
N10	0.601178
N4	0.567102

Lab members:

Biologists
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E. Avagyan, PhD student
A. Hovakimyan, PhD student
M. Melkumyan, MSc student
T. Nazaryan, BSc student
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AI/ML
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V. Atoyan, MSc student
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Thank you!

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