Title Of Talk

DPU
Dr. D. Y. PATIL BIOTECHNOLOGY & BIOINFORMATICS INSTITUTE

ACSTM
Asian Conference on Science, Technology & Medicine

(Metabopsy and Mimetics: Unlocking Cancer and metabolic diseases perspectives)

Prof. N.K. Sharma,
(Ph.D., FRSB., FMASc.,)

Metabopsy Health Services Pune, India

ORCID SCOPUS LinkedIn TWITTER

#CTRL #DYPBBI, #DPU #METABOPSY

Metabopsy Health Services Pune, India

Outline Of Talk

1) About our Department/Institute/University
2) ABOUT OUR CANCER AND TRANSLAIONAL RESEARCH LAB
3) Introduction/Problems slides
4) Novel tools and technologies VTGE and methodologies
5) Results
6) Conclusion
7) Acknowledgements

ABOUT THE UNIVERSITY

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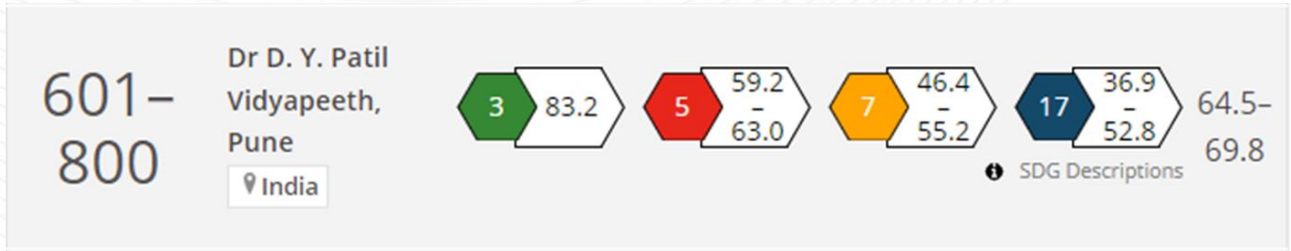


Times Higher Education
Impact Rankings 2024

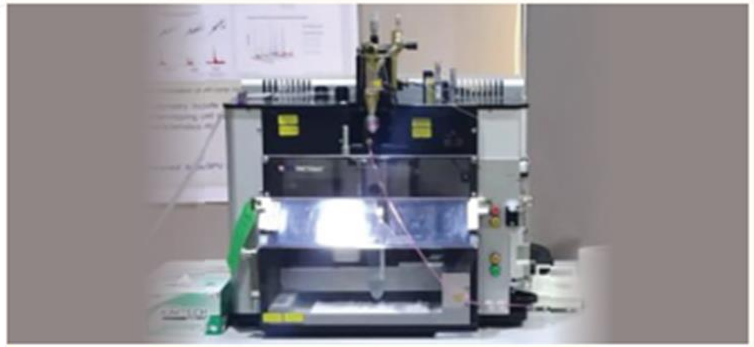
Metabopsy
Health Services
Pune, India

DST-FIST SUPPORTED LAB

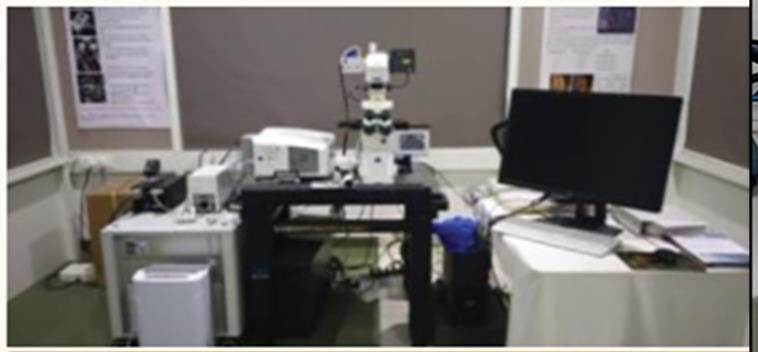
DST, DBT, ICMR, ICAR FUNDED RESEARCH PROJECTS

ELISA Reader & Washer

Quant Studio 12K Flex PCR



FACS JAZZ Flow Cytometer



Zeiss LSM710 Confocal Micrscope

Existing Students

- Ph.D. STUDENTS: 04
- UG/PG Research project students : 8, UG/PG SUMMER INTERNS : 11

Number of students trained in the last 10 years (Ph.D.-03, UG-30, PG-20, Summer interns-50)

CTRL ALLUMNI:

- 02-POST DOC FELLOW, USA, 01-INDUSTRY
- 20-MS/Ph.D. in IIT, IISER, USA, Canada etc.
- 10-Industry (BIOCON, RELIANCE LIFE SCIENCE, SPRINGER etc.)
- 10-NET/GATE qualified

RESEARCH GRANTS

- EXTRAMURAL DST/ICMR: 1.05 CRORE
- INTRAMURAL GRANTS-DPU PUNE: 40 LAKHS

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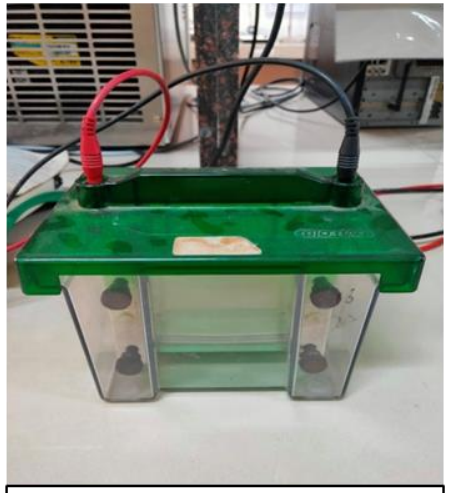
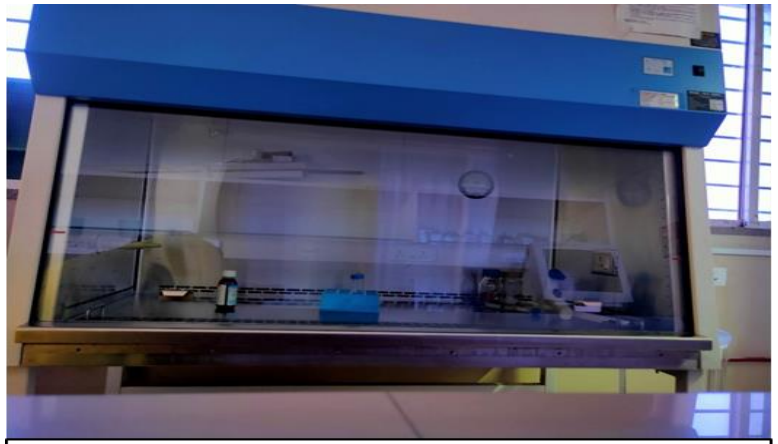
CTRL LAB FACILITIES

BIORAD Thermal Cycler

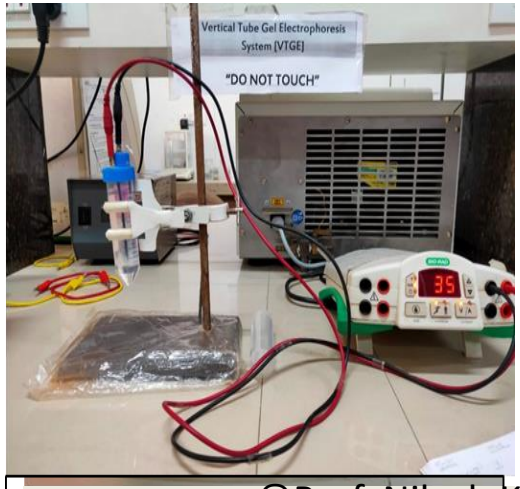


BIORAD SDS – PAGE system

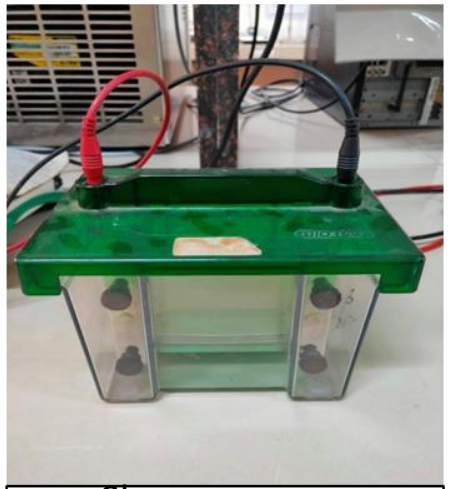
BIORAD Transblot Transfer Cell



MICROFILT INDIA Biological Safety Cabinet Class II



In-House developed VTGE (Vertical Tube Gel Electrophoresis) system

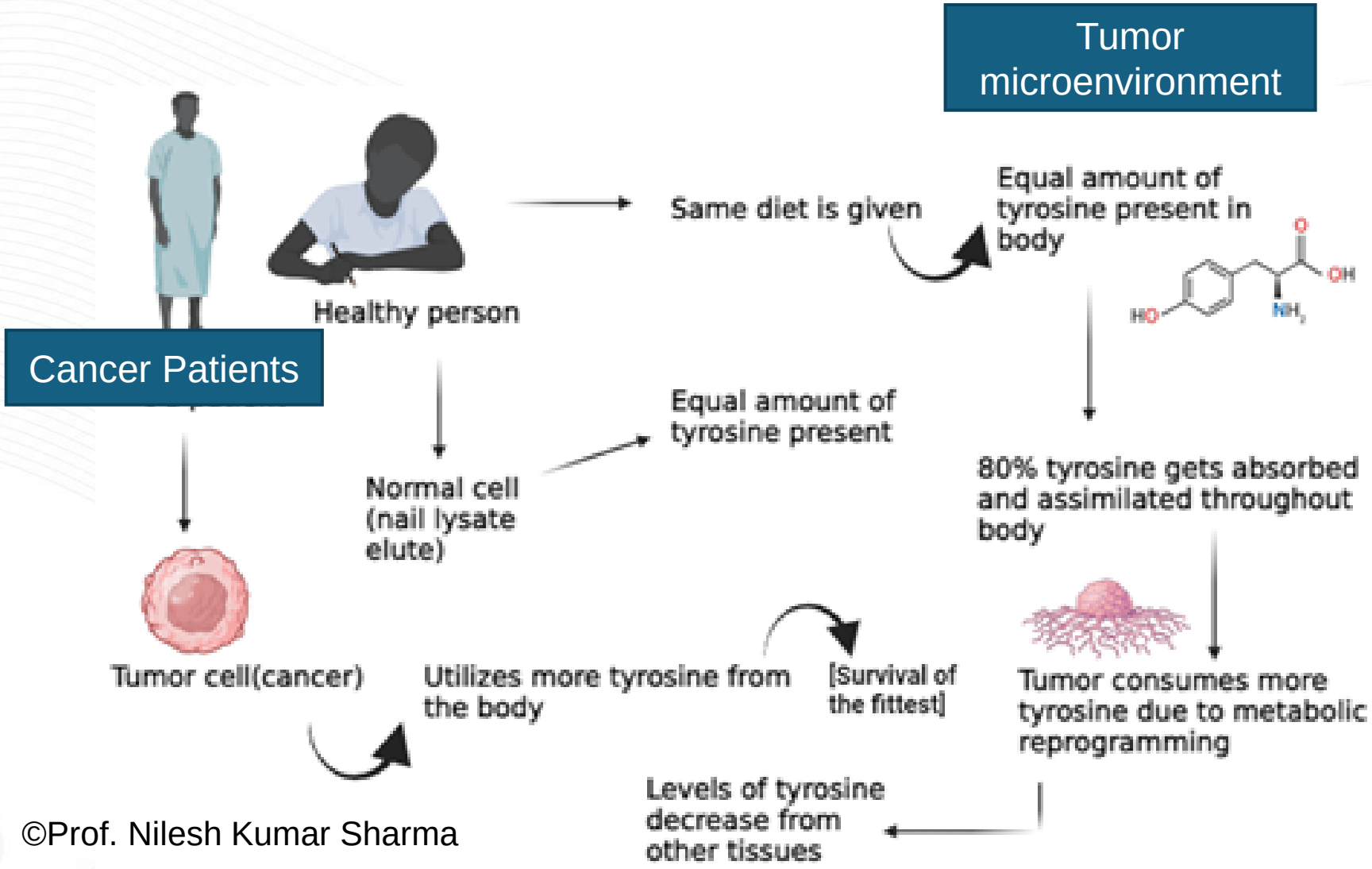


BIORAD SDS – PAGE system

BIORAD Transblot Transfer Cell

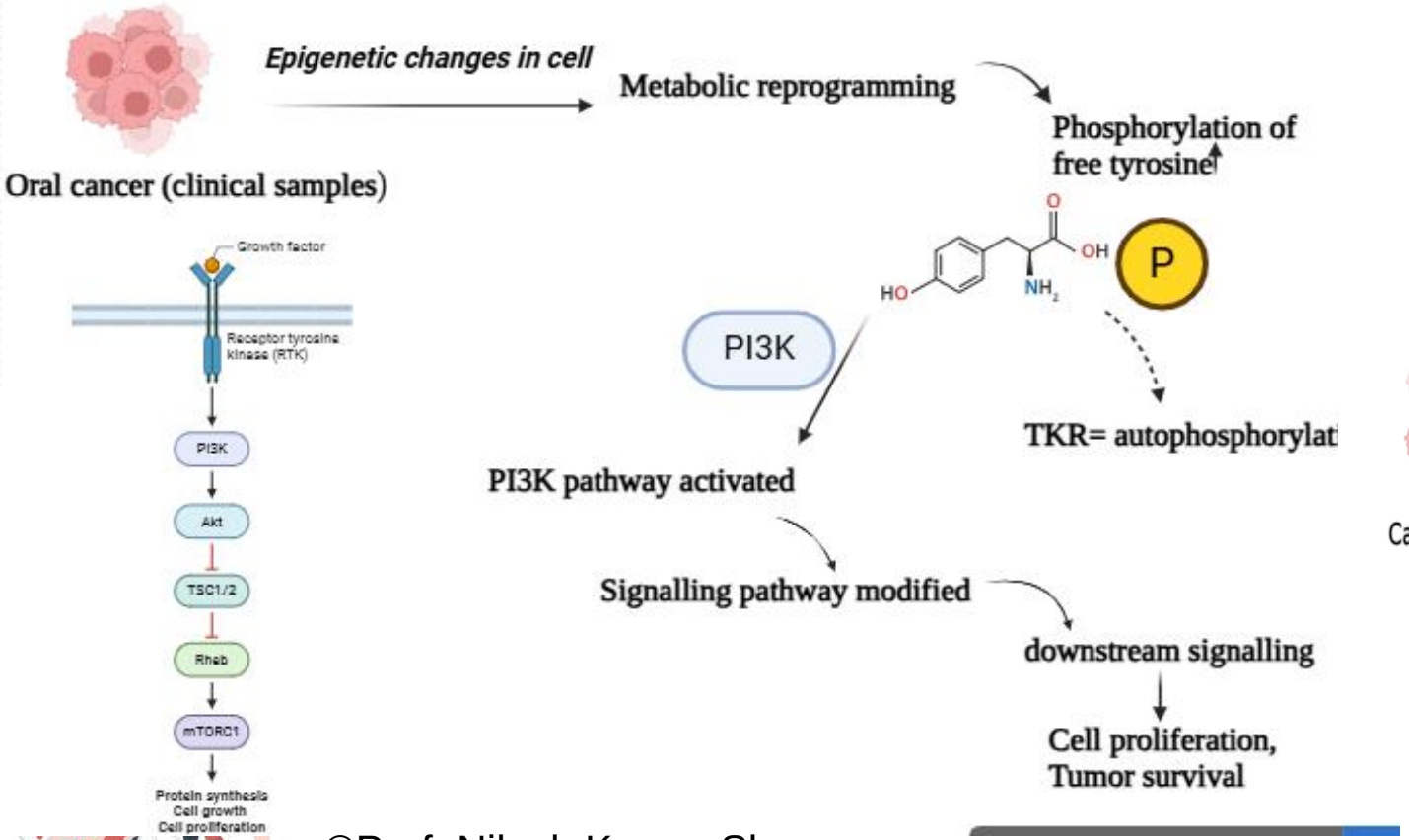
Eppendorf CO_2 incubator

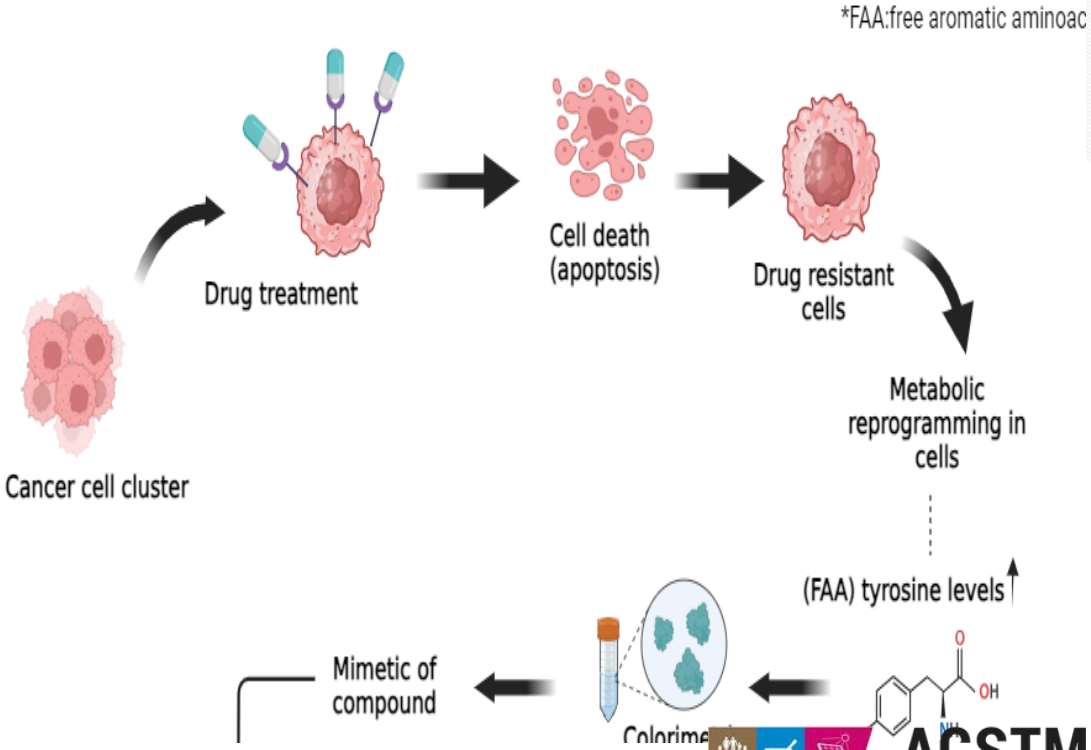
Rationale of Tyrosine mimetic (MITRO) in cancer



Rationale of Tyrosine mimetic (MITRO) in cancer

Tumor microenvironment





TO EXPLORE THE RELEVANCE TYROSINE AS METABOLITE BIOMARKERS AND DESIGN OF MIMETIC MITRO

Innovative Methodologies And Approaches Are Needed (Cellular Metabopsy Assisted By VTGE)

INTRODUCTION TO CELLULAR METABOPSY

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Cellular metabopsy define as a procedure that measures the metabolic intelligence of a tumor by analyzing metabolites in cells.

- **"METAB" is derived of "Metabolites" that is a well-known chemical compositions of Biological cell system.**
- **These Metabolites are used a Biomarkers for the detection of Cancer and other human diseases.**

"Opsy" is a Latin term that states medical examination or inspection

Rationale of "Metabopsy", since "METABOLOMICS" is already known.

©Prof. Nilesh Kumar Sharma

Metabopsy Journey

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Metabopsy is **a medical procedure that combines the terms "metabolic" and "biopsy"**. It's used to diagnose and study metabolic disorders and cancer.

How it works

- Metabopsy involves analyzing metabolites, which are a type of biomarker.
- It can be used to analyze discarded milk teeth and nail clippings.
- It can also be used to measure the metabolic intelligence of tumors.

History

- The term "metabopsy" was coined by Prof. Nilesh Kumar Sharma and his team.
- Sharma is a professor at the Dr. D. Y. Patil Biotechnology and Bioinformatics Institute in Pune.
- He has developed an in-house vertical tube gel electrophoresis (VTGE)-assisted metabopsy.

Translational Aspects of Biotechnology for Diagnosis and Prognosis
31 Mar 2023 — The sphere of biotechnology includes the following domains: * Medical...
Dr. D. Y. Patil Biotechnology and Bioinfor...

Prof. Nilesh Kumar Sharma (Ph.D., FRSB, FMASc.) - LinkedIn
2 Jan 2025 — It gives a positive sense to see the inclusion of a New Word "Metabopsy"...
LinkedIn · Prof. Nilesh Kumar Sharma (Ph.D.,...

Prof. Nilesh Kumar Sharma (Ph.D. FRSB., FMASc.) - X
10 Jan 2025 — Metabopsy to Measure #metabolic #intelligence of #tumor!...
X

Show all

DOMAIN OF METABOPSY

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"METAB"

- Cellular Metabopsy
- Urine Metabopsy
- Serum Metabopsy

"OPSY"

- Tooth Metabopsy
- Saliva Metabopsy
- Sweat Metabopsy
- Tear Metabopsy
- Nail Metabopsy

VTGE tool to assist Cellular Metabopsy

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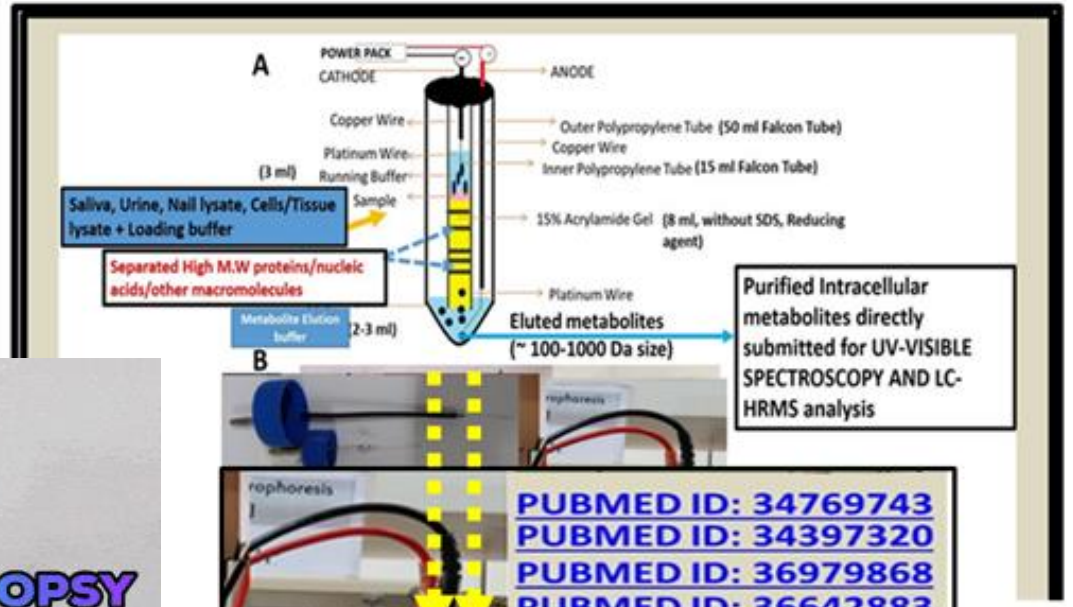

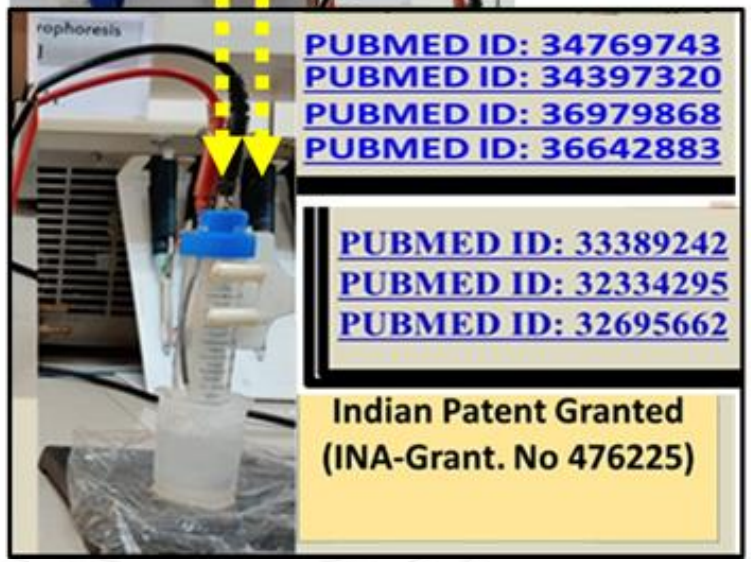

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A novel tool to assist Non-invasive, Discard to diagnosis metabopsy of diseases such as IMDs, Cancer, fibrosis) INA Patent Grant. No 476225.

Salient Features of VTGE system:

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- This system uses plastic ware (15 ml (Matrix) and 50 ml (Elution Buffer) Falcon Tube) to assemble VTGE and and Idea to use Beyond the Routine scope of Laemmli (1970) system.

- Important, use of 15% Acrylamide gel matrix (NO SDS, NO REDUCING AGENT) fractionate metabolites less than or equal to 1000 Da from various biological samples.

- The pH and salt compositions of Elution Buffer makes highly compatible and efficient for Ionization efficiency during LC-HRMS compared to existing Organic Solvents based extraction and analysis by LC-HRMS.

Salient Features of VTGE system:

- This VTGE system is validated by LC-HRMS that confirms the reliable and efficient ionization of known and some un-known metabolites including di- and tri-peptides from various biological samples (Cell lysate, urine, saliva, serum, nail clippings, culture media etc.).

- Easy to assemble, easy to run and NO TEAR AND WEAR TO THIS INSTRUMENT.

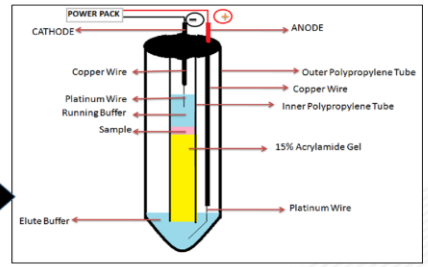
- The pH and salt compositions of Elution Buffer makes highly compatible and efficient for Ionization efficiency during LC-HRMS compared to existing Organic Solvents based extraction and analysis by LC-HRMS.

- Data collected from LC-HRMS suggest the accuracy of system by HIGHEST SIZE OF metabolite up to 973.116 Da is detected in the biological samples (Accuracy and precision are significant).

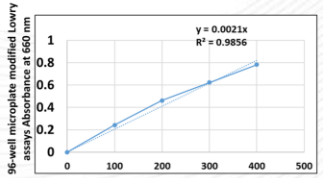
Rationale of Tyrosine mimetic (MITRO) in cancer

Step 1: Preparation of cell lysates, biological fluids and materials in modified Lowry buffer

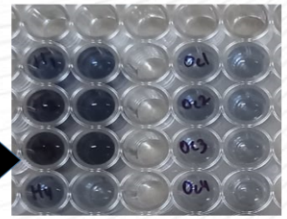
Step 2: Purification of cell lysates, biological fluids and materials with Vertical Tube Gel Electrophoresis (VTGE) in Lowry assay compatible elution buffer



Step 3: 96-well plate preparation of standard curve of tyrosine

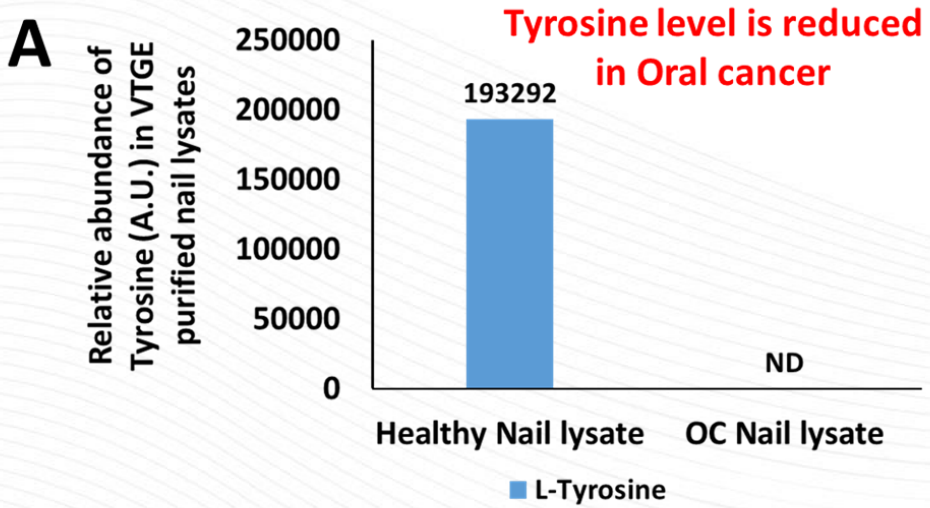


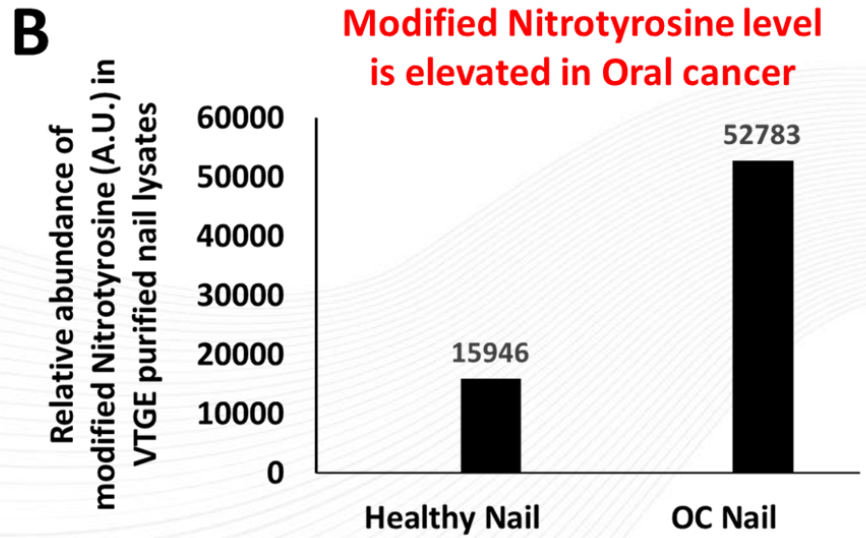
Step 4: 96-well plate free tyrosine estimation of VTGE purified elutes of cell lysates, biological fluids and materials using modified Lowry assay for various biological applications

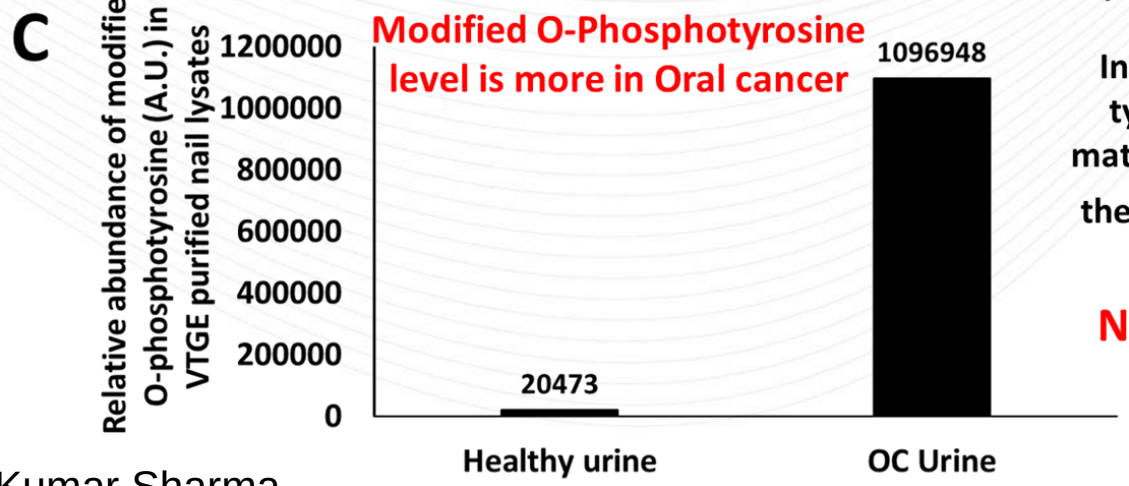


Design, of MITRO and evaluation as inhibitor of PI3K by Computational approaches

Reduced level of tyrosine, high modified Tyrosine in cancer







In summary, reduced level of tyrosine in the extracellular materials nail is compensated by the **cancer cell mediated modifications into Nitrotyropsine and O-phosphotyrosine**.

Reduced level of tyrosine, high modified Tyrosine in cancer

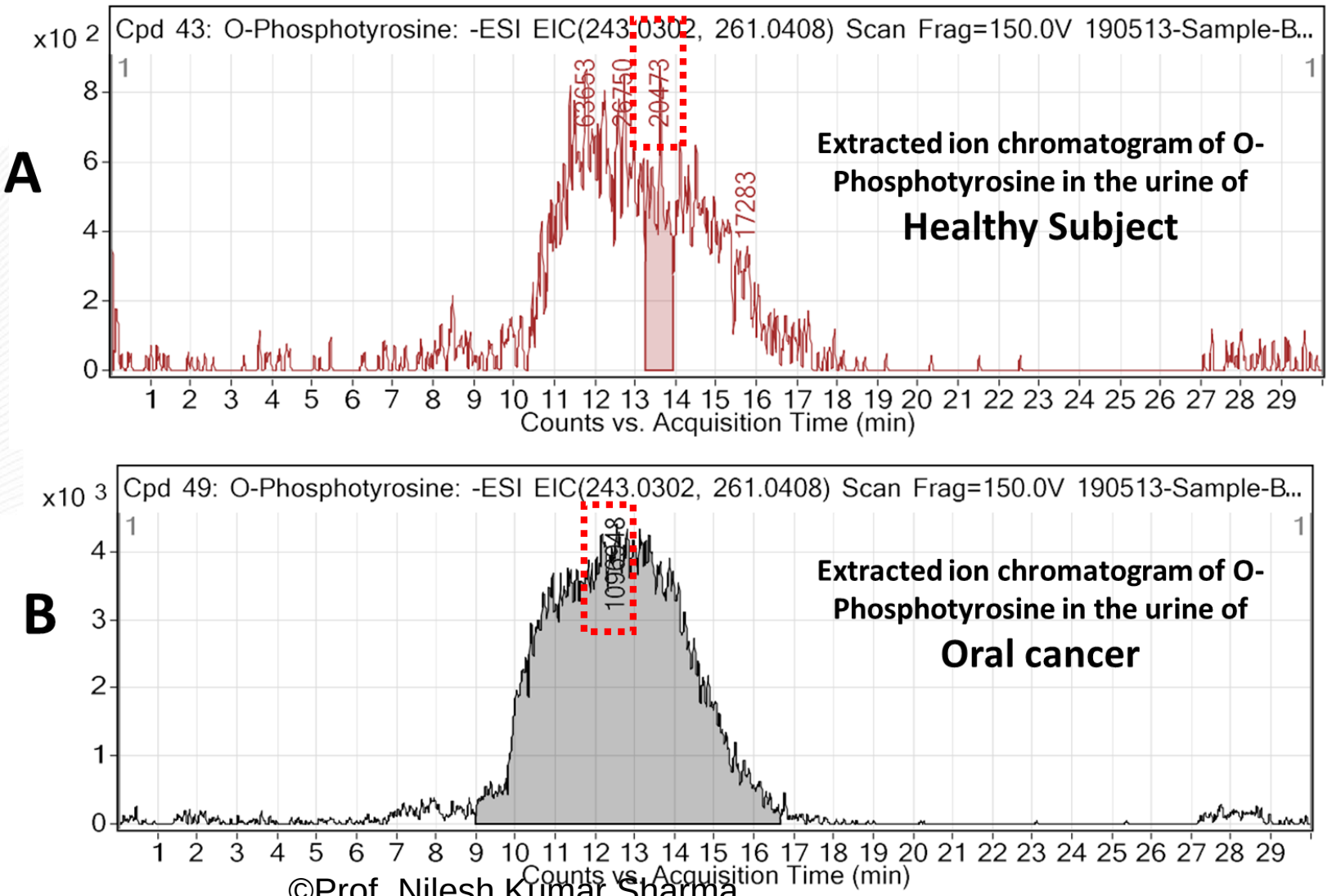


Figure: LC-HRMS profile of VTGE purified urine of oral cancer indicates elevated level of phosphotyrosine over healthy

Reduced level of tyrosine, high modified Tyrosine in cancer

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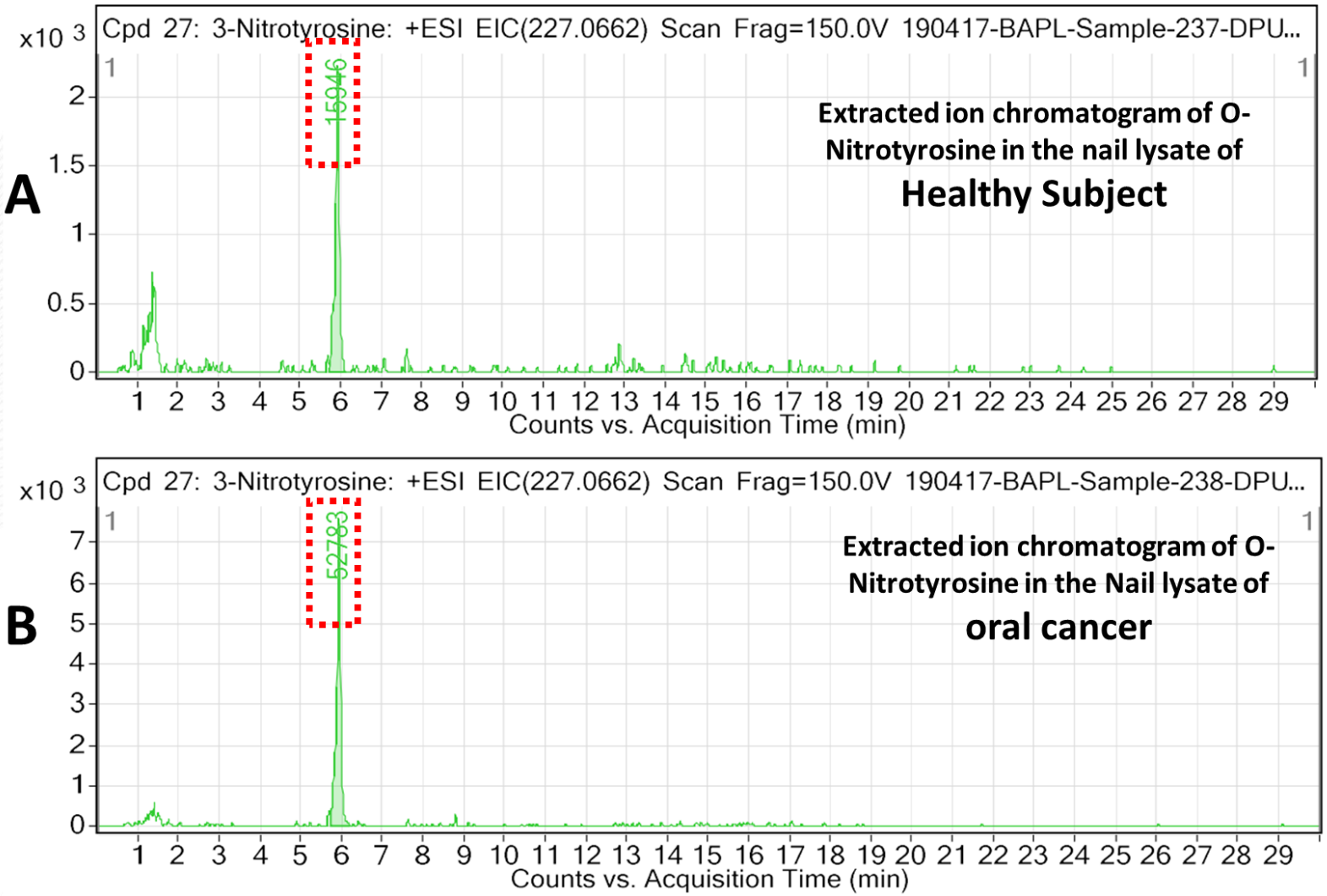

Figure: LC-HRMS profile of VTGE purified mail lysates of oral cancer indicates elevated level of Nitrotyrosine over healthy

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Reduced level of tyrosine, high modified Tyrosine in cancer

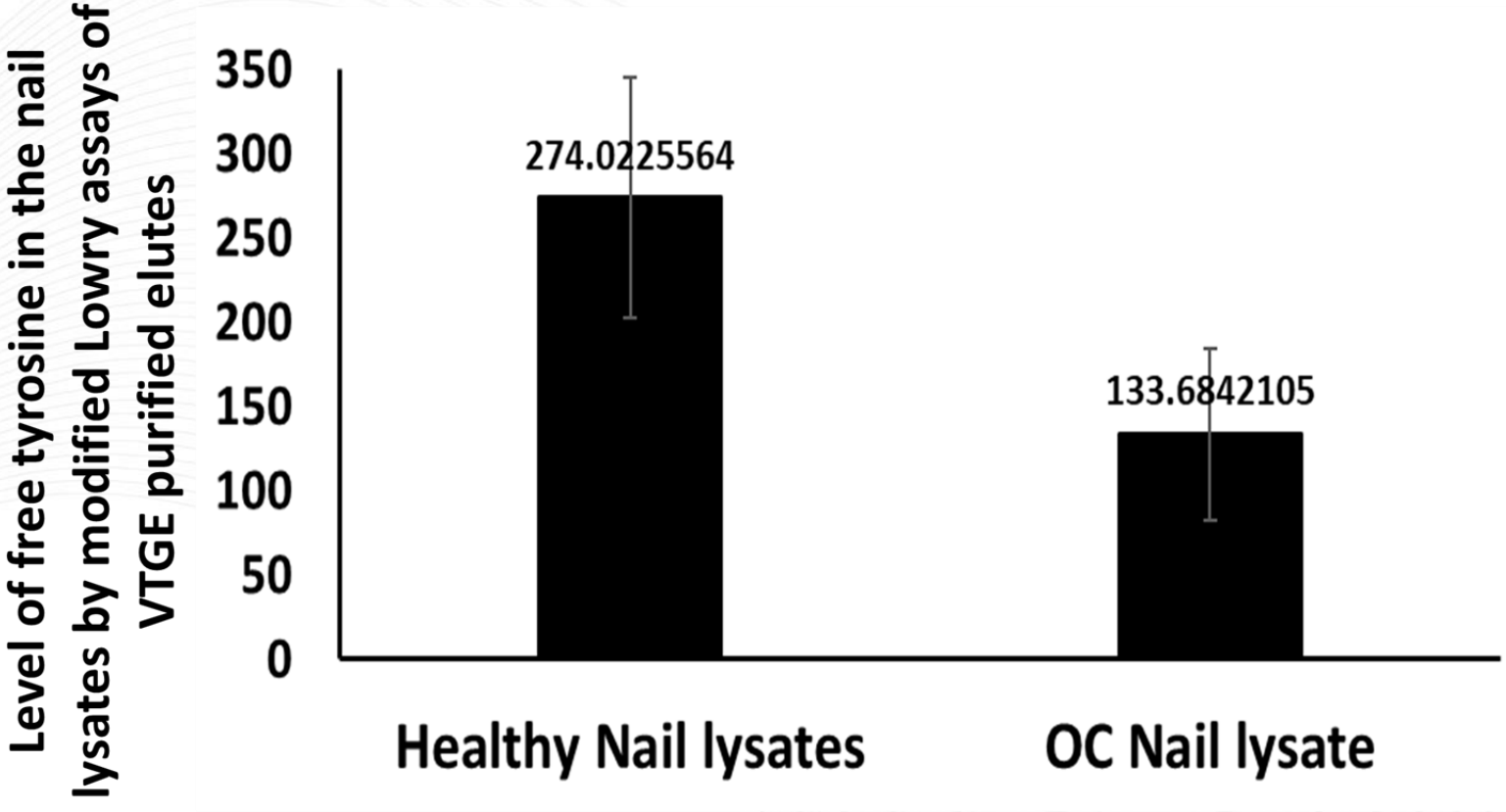

Figure: In-house developed novel Free tyrosine 96-well plate colorimetric assay suggest reduced amount of free tyrosine in the nail lysates of oral cancer patients.

Reduced level of tyrosine, high modified Tyrosine in cancer

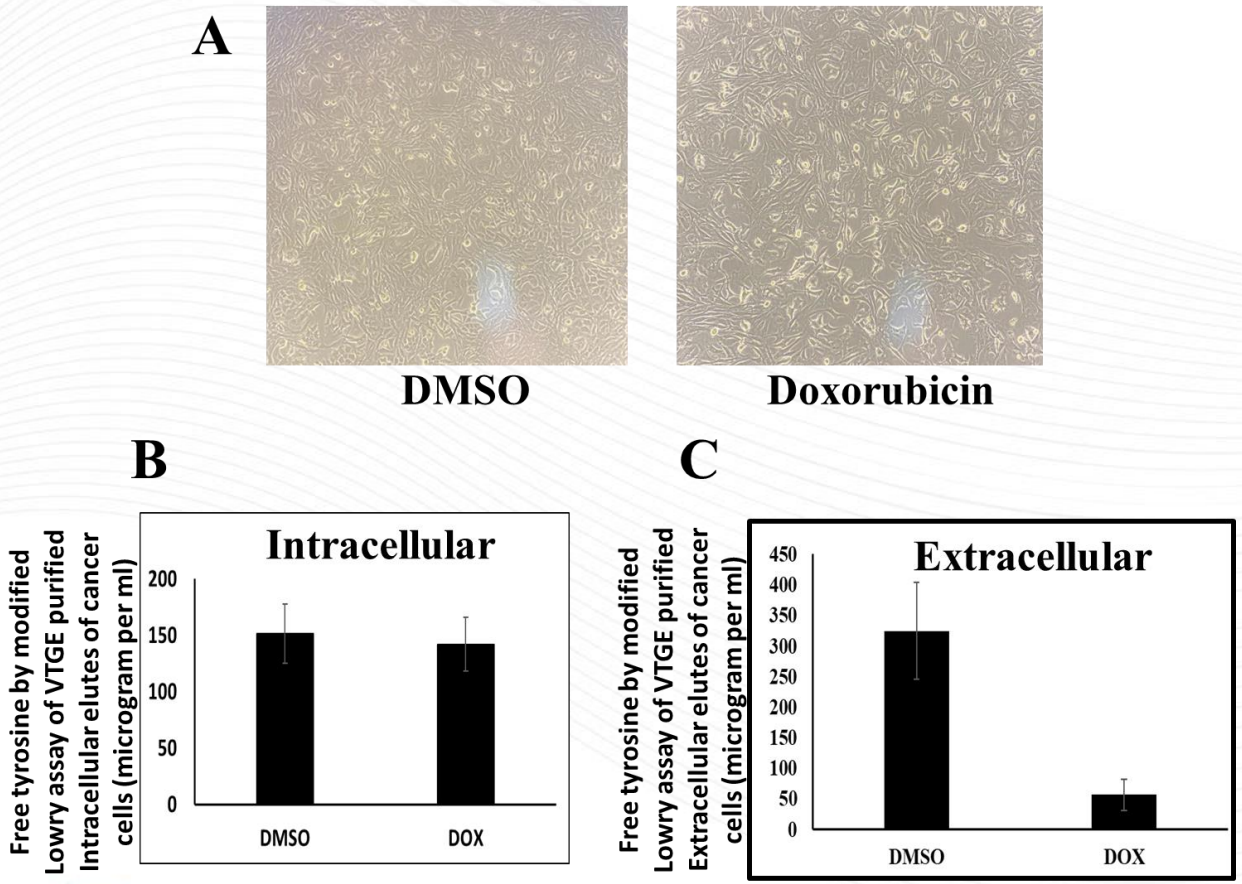

Figure: We extended the relevance of In-house developed novel Free tyrosine 96-well plate colorimetric assay to estimate the level of tyrosine in cell culture model and suggest that DOX induced cell death lead to high intake of tyrosine over control.

Reduced level of tyrosine, high modified Tyrosine in cancer

Mimetics of Tyrosine	Binding Affinity	Total no. of bonds	No. of H-bonds	Binding Amino acids(H-bonds)	Distance of H-bonds
Phospho Tyrosine (Known Ligand)	-6.1	7	4	GLU 849 (2 H-BONDS), VAL 851, SER 854, ILE 848, ILE 932, ASP 810, ASP 933	2.40, 2,28, 2.66, 2.23
MITRO1	-6.7	10	4	LYS 802, ASP 810 (2 H-bonds), CYS 838, ILE 848, ILE 932, ILE 800, PRO 778, MET 772	2.33,2.70, 3.02, 3.09
MITRO2	**-7.9**	**9**	**3**	**VAL 851, MET 922, ILE 932, TRP 780, ILE 848, GLU 849, TYR 836**	**2.16, 2.54, 2.21**
MITRO3	-6.3	7	3	ASP 915, ARG 916, PRO 953, VAL 955	2.32, 3.01, 1.97
MITRO4	-6.8	7	2	VAL 851, TYR 836, ILE 848, ILE 800, LYS 802, PHE 930, GLU 849	2.93, 2.25
MITRO5	-6.5	6	3	VAL 851, SER 854 (2 H-bonds),	1.84, 2.27, 2.39
Known Inhibitor of PI3K (buparlisib)	-9.3	9	3	GLN1014, ASP1018 LYS943	2.40, 2,28, 2.66, 2.23

Reduced level of tyrosine, high modified Tyrosine in cancer

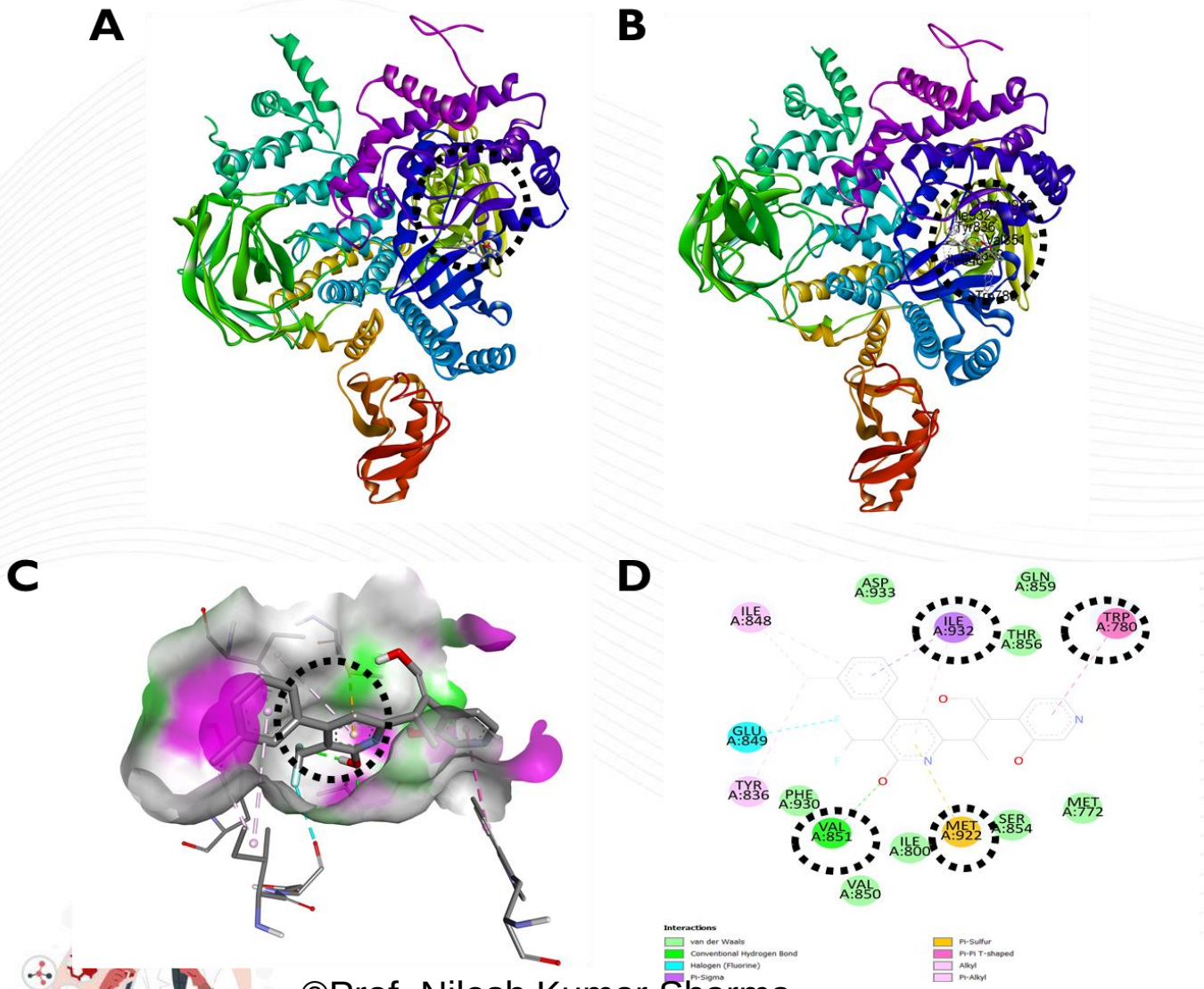

Figure: Designed novel MITRO2 displayed specific and strong binding as an inhibitor of PI3K enzyme. VAL851 and MET922 are known for their role in the inhibitory pocket.

Reduced level of tyrosine, high modified Tyrosine in cancer

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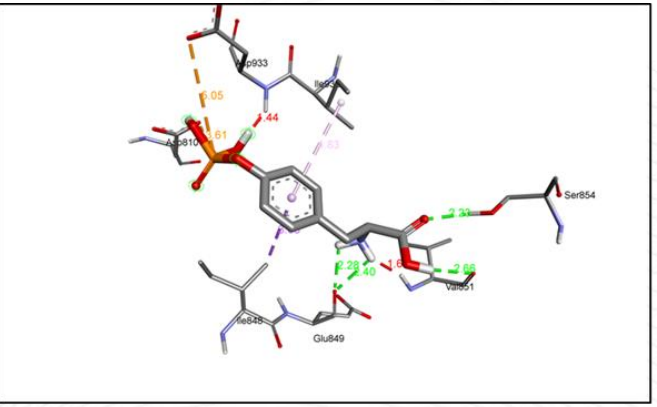
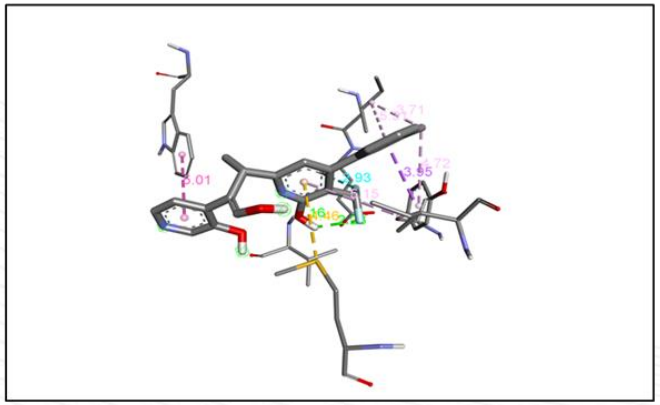
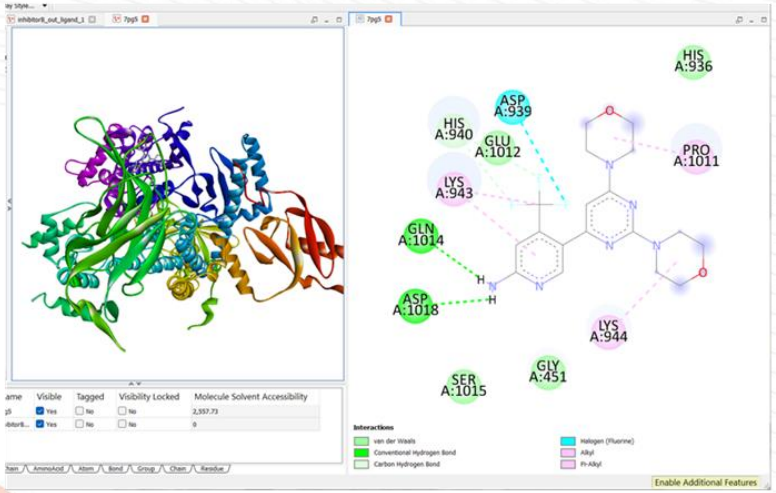


A known ligand Phospho-tyrosine (Binding affinity)= -6.1 kcal/mol



Designed Tyrosine mimetic (MITRO2) (Binding affinity)= -7.9 kcal/mol



A known inhibitor (Binding affinity)= -9.3 kcal/mol

Figure: Computation approaches suggest the Designed novel MITRO2 is comparable with known ligand and inhibitors in terms of binding affinity and specific binding pocket.

Reduced level of tyrosine, high modified Tyrosine in cancer

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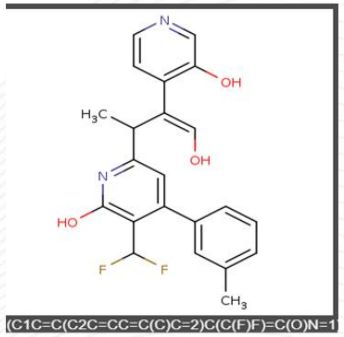
Designed Tyrosine mimetic (MITRO2

CC(\C(=C/O)C1=C(O)C=NC=C1)C1=NC(O)=C(C(F)F)C(=C1)C1=CC(C)=CC=C1



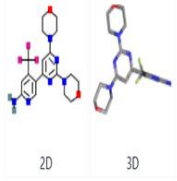
Query	Liver Toxicity		Metabolism						Membrane Transporters			Others			
			Cyp Inhibitors for												
	DILI	Cyto-toxicity	HLM	1A2	3A4	2D6	2C9	2C19	BBB	P-gp Inhibitor	P-gp Substrate	hERG Blocker	MMP	AMES	MRTD (mg/day)
	No	No	Yes	No	No	No	No	No	Yes	No	Yes	No	No	No	205

A known inhibitor Bupaslisib

Buparlisib

PubChem CID 16654980

Structure



ADMET Predictions

Job Name: -

Include model results based on a restricted applicability domain only

Include model results based on an unrestricted applicability domain

Query	Liver Toxicity		Metabolism						Membrane Transporters			Others			
			Cyp Inhibitors for												
	DILI	Cyto-toxicity	HLM	1A2	3A4	2D6	2C9	2C19	BBB	P-gp Inhibitor	P-gp Substrate	hERG Blocker	MMP	AMES	MRTD (mg/day)
	No	No	Yes	No	No	No	No	No	Yes	No	Yes	Yes	No	Yes	174

Figure: ADMET profile predicts better properties in terms of toxicity and carcinogenicity over a known inhibitor of PI3K.

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Conclusion/Future prospects

- Cellular metabopsy to profile intracellular and extracellular metabolites from fluids, cells and tissues lysates assisted VTGE and in-house 96-well plate colorimetric assays may be useful for diagnostic biomarkers and better understanding of metabolic changes in cancer cells.

- MITRO as a mimetic to modulate tyrosine metabolism as an inhibitor of PI3K enzyme may be explored as a component of combinatorial anticancer therapies along with PTX.

- The proposed in-house in-house 96-well plate colorimetric assays for the estimation of free form of Tyrosine with modification of conventional Lowry Assay is a novel and first of kind to measure the amount of tyrosine in cells, tissues and biological fluids.

- Proposed approaches and methodologies can be extended to other cancer types and anticancer drugs.

Acknowledgements

- We acknowledge State of the Art facilities and funding from DPU, Pune
- Extramural Grant from DST-SERB, ICMR-DHR, DST-FIST
- Importantly, more than 100 Summer interns/UG/PG/PhD students

Research Collaborators

1. Prof. J. K. Pal
2. Dr. Ramesh Bhonde, Regenerative Medicine Laboratory
3. Prof. Sachin C Sarode
4. Prof. C. R. Gore
5. Dr. Yogesh B Shinde
6. Dr. Birandra K Sinha (NIH, USA)
7. Dr. Pankaj Poddar (Senior Principle Scientist, NCL, Pune)

Research Institutions

We Welcome Potential Collaborators from DPU

i) Medical College,
ii) Dental College,
iii) Ayurvedic and Pharmacy College,
ix) ABMH, Pune
x) JCDC, Pune
x) NIEHS, NIH, USA
xi) NCL, Pune

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Thank you