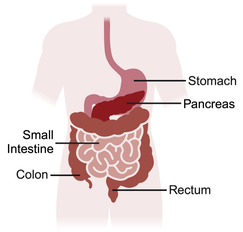
Use of patient-derived pre-clinical models to identify new and effective treatments for neuroendocrine tumors

Nilakshi Kulathunga[1], Zoey Wang[1,2], Justin Kale[1], Alberto Lens-Pardo[3], Rusha Chakraborty[1], Yan Li[1], Betty Li[1], Areeba Qureshi[1], Emmanuel Cherin[4], Christine E. Demore[2,4], Hubert Tsui[1,5], Rocio Garcia-Carbonero[3], Julie Hallet[6,7], Calvin Law[6,7], Simron Singh[6,8], David Andrews[1,9], Hon S. Leong[1,2], Iacovos P. Michael[1,2]

[1]Biological Sciences Platform, Sunnybrook Research Institute, Toronto, ON, Canada; [2]Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada; [3]Oncology Department, Centro de Oncología Experimental, Grupo de Investigación en Tumores Gastrointestinales y Neuroendocrinos, Instituto de Investigación Sanitaria Hospital 12 de Octubre (imas12), CNIO, UCM, Madrid, Spain; [4]Physical Sciences Platform, Sunnybrook Research Institute, Toronto, ON, Canada; [5]Laboratory Medicine and Molecular Diagnostics, Sunnybrook Health Sciences Centre, Toronto, ON, Canada; [6]Clinical Evaluative Sciences, Sunnybrook Research Institute, Toronto, ON, Canada; [7]Department of Surgery, University of Toronto, Toronto, ON, Canada; [8]Department of Medicine, University of Toronto, Toronto, ON, Canada; [9]Department of Biochemistry, University of Toronto, Toronto, ON, Canada

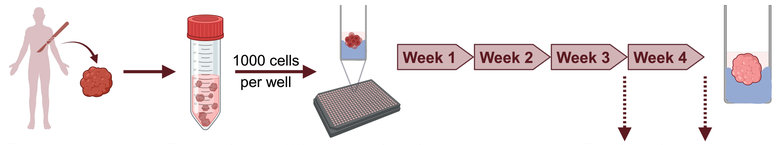
Background

- Neuroendocrine tumors (NETs) originate from hormone-producing cells located throughout the body, especially in the gastroenteropancreatic tract (i.e., **GEP-NETs**)
- GEP-NETs are highly heterogeneous and respond poorly to current therapies
- Here, we aim to establish **patient-derived organoids (PDOs) and xenografts (PDXs)** for GEP-NETs, and use these to identify novel treatment strategies

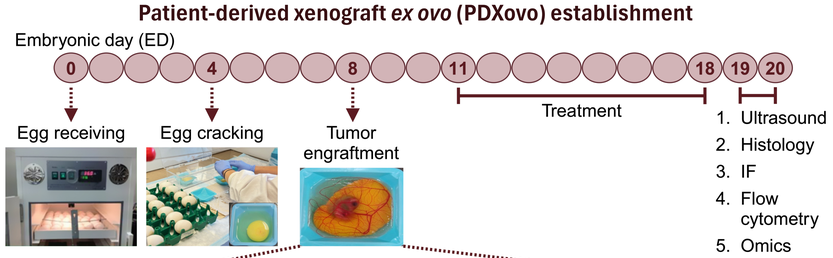


Methods

Patient-derived organoid (PDO) establishment



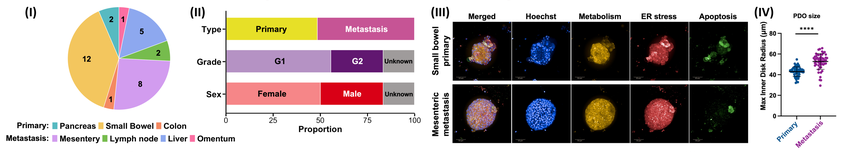
Patient-derived xenograft *ex ovo* (PDXovo) establishment



Results

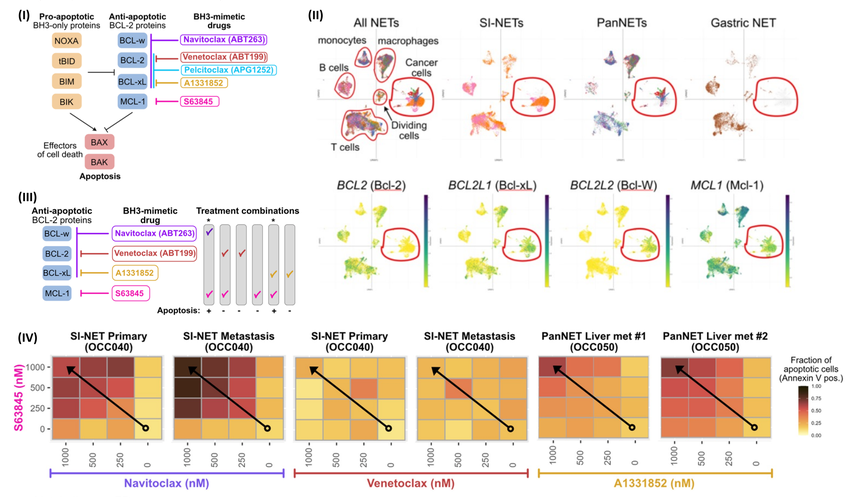
Patient-derived organoids (PDO)

1. PDOs can be successfully established from primary and metastatic GEP-NETs



(I) Number of PDOs established from different tissue sites between March and November 2024. (II) Proportion of PDOs established from different tumor types, grades, and patient sex between March and November 2024. (III) Representative images of PDOs stained stained for metabolic activity (ChromaLive yellow), ER stress (ChromaLive red), and apoptosis (Annexin V). (IV) Size of PDOs established from primary and metastatic tumors. Metastatic tumors form bigger organoids than primary tumors on average.

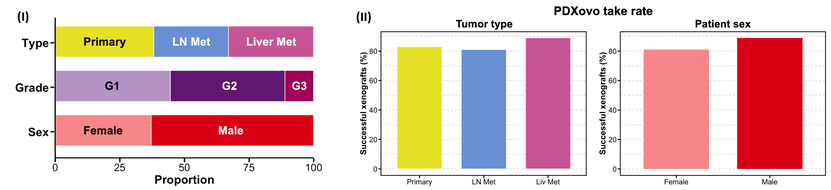
2. GEP-NET PDOs respond to anti-apoptotic drugs targeting the BCL-2 protein family (i.e., **BH3 mimetics**)



(I) Summary of apoptotic proteins and BH3-mimetic drugs. (II) Top: cell type composition in published GEP-NET single-cell RNA-sequencing data (PMID: 37756410). Cells are colored by patient origin. Bottom: Expression of BCL-2 proteins in GEP-NETs (yellow: low expression; purple: high expression). The cancer cell fraction is circled. (III) Summary of PDO drug treatment results. (IV) Representative data showing the fraction of apoptotic cells after treatment with BH3 mimetics as a single agent or in various combinations. (V) Representative images of PDOs treated with two different BH3-mimetics alone or in combination and stained for metabolic activity (ChromaLive yellow), ER stress (ChromaLive red), and apoptosis (Annexin V).

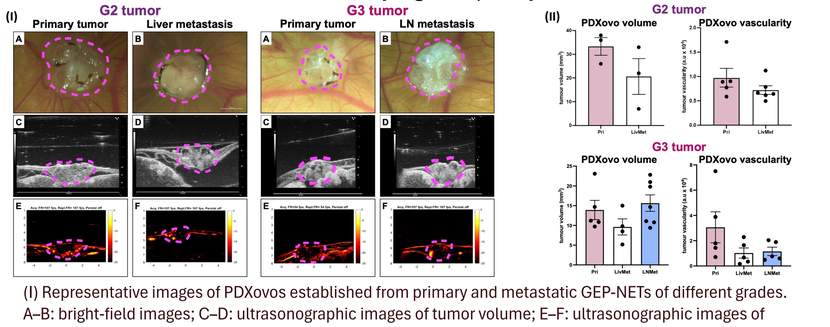
Patient-derived xenografts *ex ovo* (PDXovo)

1. PDXovos can be successfully established from primary and metastatic GEP-NETs



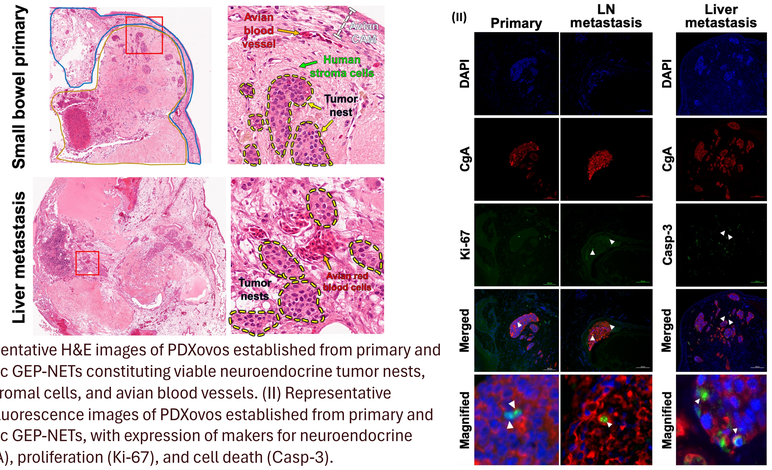
(I) Proportion of PDXovos established from different tumor types, grades, and patient sex between May 2023 and August 2024. (II) Take rate of PDXovos established from different tumor types and patient sex.

2. PDXovos are vascularised, as shown by high-frequency ultrasound



(I) Representative images of PDXovos established from primary and metastatic GEP-NETs of different grades. A–B: bright-field images; C–D: ultrasonographic images of tumor volume; E–F: ultrasonographic images of vascularity. (II) Quantification of PDXovo volume and vascularity.

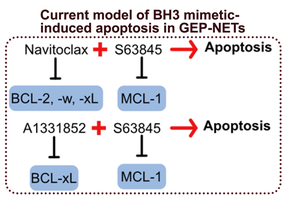
3. PDXovos maintain original tumor architecture and express neuroendocrine markers



(I) Representative H&E images of PDXovos established from primary and metastatic GEP-NETs constituting viable neuroendocrine tumor nests, human stromal cells, and avian blood vessels. (II) Representative immunofluorescence images of PDXovos established from primary and metastatic GEP-NETs, with expression of makers for neuroendocrine cells (CgA), proliferation (Ki-67), and cell death (Casp-3).

Conclusion

- We have established a large repository of two pre-clinical models for GEP-NETs, patient-derived organoids (PDO) and patient-derived xenografts *ex ovo* (PDXovo), with a respective success rate of over 90% and over 80%
- PDOs potentiate high-throughput drug screening
- Utilising the PDO model, we have found that BH3 mimetic drugs possess therapeutic potential against GEP-NETs
- PDXovos accurately recapitulate patient tumor architecture and expression of neuroendocrine cell markers, and represent an efficient, cost-effective method for further validation of candidate drugs



Future Directions

- Validate GEP-NET sensitivity to BH3 mimetics in the PDXovo model
- Identify currently approved GEP-NET therapies (e.g., Sunitinib, Cabozantinib, Everolimus, Peptide Receptor Radionuclide Therapy) that sensitise the tumor to BH3 mimetics
- Characterise the apoptotic mechanism in GEP-NETs and identify predictive biomarkers of response to BH3 mimetics
- Eventually, our results will translate to more effective treatment strategies for GEP-NETs and improved lives of GEP-NET patients

iacovos.michael@sri.utoronto.ca