Exploring Therapeutic Potential: Insights from Drug Penetration and Targeting using Nilogen's Ex-vivo 3D-EXpress Tumoroid Platform

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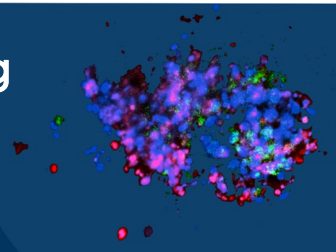

Background

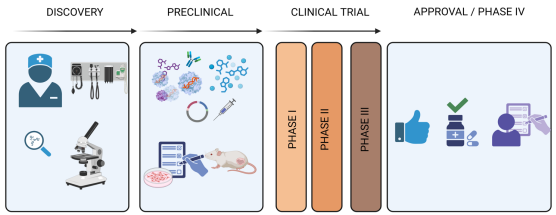

Figure 1: Drug Discovery Process. The schematic illustrates the sequential stages of the drug discovery process. It begins with the identification of a therapeutic target, followed by the screening of potential compounds and lead identification. Subsequent steps include lead optimization and preclinical testing to assess the efficacy and safety of candidate molecules. Promising candidates advance to clinical trials, which are conducted in phases (I, II, and III) to evaluate safety, dosage, and therapeutic efficacy in human subjects. The final stage involves regulatory review and approval before the drug is made available for clinical use.

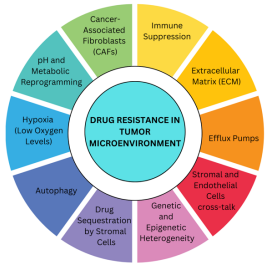

Fig 2: Labeling therapeutics with fluorochromes aids in understanding it's mechanisms. Nilogen's 3D-EX vivo models retain the tumor microenvironment found in a patient's tumor providing an optimal platform study therapeutic efficacy. Fluorescently labelled therapeutics can be used to study binding and internalization kinetics, in addition to tracking the penetration of the drugs within the tumor.

Type of Therapeutic	Preferred Labeling Technique	Description	Common Applications
Monoclonal Antibodies	Fluorescently Labeled Antibodies	Antibodies tagged with fluorophores for detection.	Flow cytometry, immune cell phenotyping, receptor binding studies
Small Molecule Drugs	Radioactive Labeling(e.g., ^{3}H, ^{14}C)	Incorporates isotopes into drugs for tracking uptake and metabolism.	Drug uptake studies, receptor binding assays, autoradiography
Cell Therapies	GFP or RFP (Fluorescent Proteins)	Genetically engineered fluorescent markers for tracking cell behavior.	Live-cell imaging, tracking cell distribution, studying cell proliferation
Peptides and Proteins	Biotin-Streptavidin Labeling	Biotin tags bind to streptavidin conjugates, amplifying signal detection.	ELISA, pull-down assays, protein interaction studies
Gene Therapy Vectors	Luciferase or GFP Labeling	Reporter genes inserted into vectors to monitor gene expression.	Gene expression analysis, transfection efficiency, promoter activity studies
Nanoparticles	Quantum Dot Labeling	Quantum dots provide stable and bright fluorescence for particle tracking.	High-resolution imaging, tracking nanoparticle uptake and localization
Immunotherapies	Mass Cytometry (CyTOF) with Metal-Labeled Antibodies	Uses metal-conjugated antibodies to analyze multiple markers without overlap.	Deep immune cell profiling, immunophenotyping, analyzing checkpoint markers
Cytokines and Growth Factors	Stable Isotope Labeling by Amino Acids (SILAC)	Uses stable isotopes to track protein synthesis and turnover in cells.	Quantitative proteomics, protein secretion studies, cytokine release studies
RNA-based Therapies	Click Chemistry Labeling	Uses bioorthogonal chemistry to label modified RNA molecules precisely.	RNA localization studies, interaction with proteins, stability analysis
Cell Surface Receptors	Magnetic Labeling (e.g., Magnetic Beads)	Conjugated beads isolate specific cell populations or proteins using magnets.	Cell sorting (e.g., MACS), receptor-ligand studies, enriching cell subpopulations

Table 1. Preferred Labeling Techniques for Various Therapeutics in *ex vivo* Experiments. The table summarizes the optimal labeling methods used for different types of therapeutics, including monoclonal antibodies, small molecule drugs, and cell therapies. Each therapeutic type is matched with its preferred labeling technique, a brief description of the method, and common applications in *in vitro* research. This comparison aids in selecting the appropriate labeling strategy to enhance detection, analysis, and understanding of therapeutic behavior and efficacy.

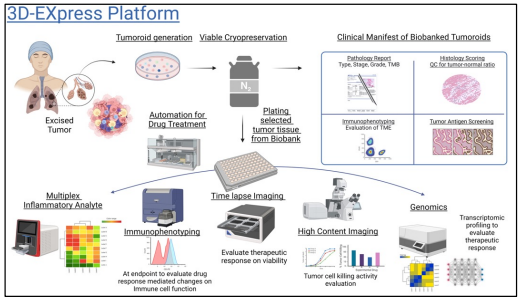

Fig 3: Nilogen's 3D-EXpress tumoroid platform capabilities. The diagram illustrates the capabilities of the Nilogen 3D Express platform, which enables comprehensive analysis of drug responses using 3D tumor models. Key features include high-throughput screening, immune cell infiltration assays, cytokine profiling, and assessment of immune checkpoint activity. The platform facilitates the evaluation of therapeutic efficacy, immune modulation, and biomarker identification in a physiologically relevant 3D microenvironment, providing valuable insights for oncology research and drug development.

Results

Assay Optimization

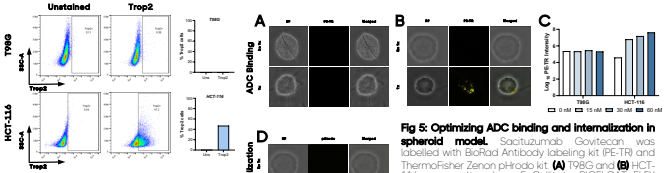

Fig 4: Evaluating expression of Trop2 on T98G and HCT-116 cell lines. HCT-116 and T98G cell lines were stained with fluorescently labelled Trop-2 antibody and evaluated for expression of Trop2 by flow cytometry. Flow plots showing the expression of Trop2, which is enumerated in adjacent bar graph.

Fig 5: Optimizing ADC binding and internalization in spheroid model. Sacituzumab Govitecan was labelled with BioRad Antibody labeling kit (PE-TR) and ThermoFisher Zenon pHrodo kit **(A)** T98G and **(B)** HCT-116 were cultured on FaCellitate BIOFLOAT FLEX coated wells to facilitate spheroid formation and treated with labelled Govitecan for 48hrs. Representative images of two spheroids with Govitecan binding using BioRad labelling kit (PE-TR) **(C)** MFI of labelled Govitecan in both the spheroids. **(D)** Evaluating internalization of Govitecan using Thermofisher Zenon pHrodo kit in HCT-116 spheroid.

Assay Validation

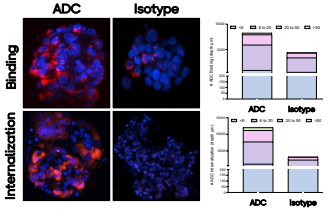

Fig 6: Optimizing ADC binding and internalization in tumoroids. Endometrial tumoroid tissue were treated with therapeutics labelled to study binding *(top)* and internalization *(bottom)*. Representative images showing the bound and internalized therapeutic. Graphical representation showing the cell count in each zone of penetration (µm) within the tumoroids.

How labelled therapeutics enhance research

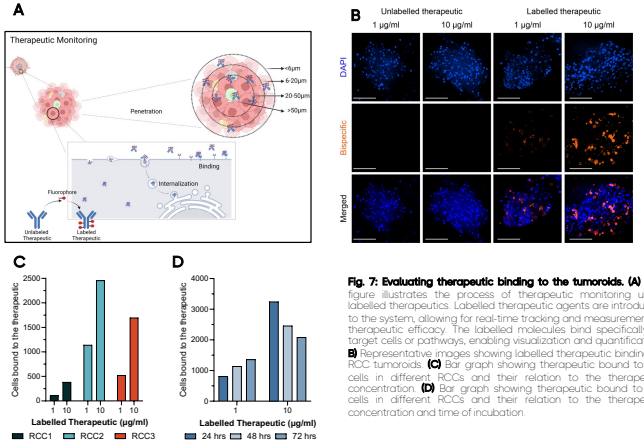

Fig. 7: Evaluating therapeutic binding to the tumoroids. (A) This figure illustrates the process of therapeutic monitoring using labelled therapeutics. Labelled therapeutic agents are introduced to the system, allowing for real-time tracking and measurement of therapeutic efficacy. The labelled molecules bind specifically to target cells or pathways, enabling visualization and quantification. **B)** Representative images showing labelled therapeutic binding to RCC tumoroids. **(C)** Bar graph showing therapeutic bound to the cells in different RCCs and their relation to the therapeutic concentration. **(D)** Bar graph showing therapeutic bound to the cells in different RCCs and their relation to the therapeutic concentration and time of incubation.

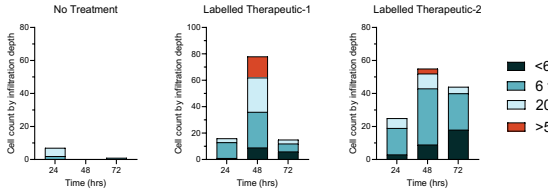

Fig. 8: Kinetics of therapeutic internalization in RCC tumoroids. RCC tumoroids were either left untreated or treated with two distinct labeled therapeutics to assess the rate of therapeutic internalization. The data is presented as the number of cells bound to the therapeutic at varying infiltration depths within the tumoroids, providing insight into the penetration and binding efficiency of the treatments over time.

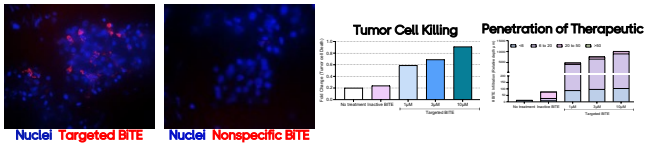

Fig 9: Internalization of targeted vs. nonspecific BiTEs in RCC tumoroids.
Left panel: Fluorescence microscopy images show nuclei in blue and BiTEs in red. The use of labeled therapeutics allowed visualization of BiTE internalization. The targeted BiTE (left) shows strong internalization, while the nonspecific BiTE (right) shows minimal red signal, indicating weak internalization. **Right panel:** Bar graphs quantify the number of cells bound to the BiTEs at different depths.

Summary

Evaluating Drug Mechanisms Using Labelled Therapeutics

Efficacy
- Labelled therapeutics enable precise measurement of drug efficacy by tracking the therapeutic agent's activity in real time.
- This allows researchers to assess how effectively the drug achieves its intended biological response within target tissues.

Binding:
- The labelled markers help in visualizing the binding process between a drug and its specific target, such as receptors or proteins.
- This provides insights into binding affinity and helps in understanding the specificity of drug-target interactions.

Internalization
- By using labelled agents, the process of drug internalization into cells can be monitored, revealing how a therapeutic enters the cellular environment.
- This information is crucial for drugs that need to act within cells to be effective, such as targeted therapies.

Penetration
- Labelled therapeutics allow the study of drug penetration through barriers like cell membranes or tissue layers.
- This helps in evaluating the drug's ability to reach its target site within the body and contributes to optimizing formulation and delivery strategies.

Interaction
- The approach provides detailed data on how drugs interact with cellular components, such as enzymes, receptors, or other molecules.
- It helps to delineate off-target effects and elucidates the molecular pathways involved in the drug's mechanism of action.

Mechanism of Action Evaluation
- Labelled therapeutics allow direct observation of drug interactions with target cells, tissues, or receptors.
- They help in mapping the distribution and localization of drugs within the body, providing insights into pharmacokinetics and biodistribution.
- This approach enables visualization of drug-receptor binding events, cellular uptake, and intracellular pathways.

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