Multiscale Imaging of Tumor Architecture and Dynamics – concept. A strategic direction for the FNL

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Opportunity, Promise, and Open Questions

- Tumor Initiating Cell
- Organoid
- Imaging
- Computation

Tumorigenic initiation and differentiation programs

Leveraging Frederick National Laboratory: Launch coherent program for a systematic attack on fundamental open questions in tumor dynamics

Frederick National Laboratory for Cancer Research
NCI Provocative Questions that provoked us!

- **PQB – 2** What molecular and cellular events in the tumor microenvironment determine if a tumor at the earliest stages of malignant transformation is eliminated, stimulated for further development, or made indolent?

- **PQB – 4** What methods can be devised to characterize the functional state of individual cells within a solid tumor?

- **PQC – 4** What in vivo imaging methods can be developed to portray the "cytotype" of a tumor — defined as the identity, quantity, and location of each of the different cell types that make up a tumor and its microenvironment?

http://provocativequestions.nci.nih.gov/rfa
Opportunity, Promise, and contributing role of emerging 3D models

Which tumorigenic behaviors in emerging 3D models are relevant to drug resistance and metastatic recurrence? How to Optimize this relevance?

Toward 3D Tumor-Equivalent Models of the Earliest Stages

Tumorigenic initiation and differentiation programs
Seed for in vitro and in vivo: Tumor Initiating Cells, Root of Resistance and Metastasis

- **Need to be able to identify the TICs and expand them**
  - Many current markers are under investigation
  - Significant progress with more physiologically robust examples of architecturally and functionally tantalizing organoids through 3D culture
  - Much more to be done to optimize 3D culture conditions with fundamental tumor biology questions in mind

- **Probe understanding of resistance mechanisms as tumor is de-bulked**
  - TICs are highly tumorigenic and resistant to therapy
  - CTCs are another route to 3D culture models of growing relevance – yet early
  - Viability and proliferation is not same as metastatic relevance

- **Potentially the seeds for metastasis**
  - Kills patients, can be idle for many years in the particular niche
  - Seek architectural, molecular, and dynamic characterization of leading edge 3D models with correlative studies in vivo by orthotopic implantation to gain TME
  - Human => 3D => mouse; and mouse => 3D => mouse
Launch the FNL program with study of selected 3D model systems and their implantation, as first projects.

Build and learn, to hone a national lab Multiscale Imaging Engine, including testing and validating high-performance molecular probe toolsets.

Open the Engine to biologically provocative 3D models and metastatic models in the Community.
Opportunity, Promise, from emerging 3D models

* Optimize/enrich/validate imaging probe toolsets as national resource

Molecular profiling, imaging, dynamics in 3D culture & relevance (EMT, drug resistance)

Orthotopic Implantation: tracking determinants of metastatic potential

Which tumorigenic behaviors in emerging 3D models are relevant to drug resistance and metastatic recurrence in vitro and in vivo?

How to Optimize this relevance?

Tumor Initiating Cell

Circulating Tumor Cell

Tumorigenic initiation and differentiation programs

Organoid

Advances in 3D Culture
Launch the FNL program with study of selected 3D model systems and their implantation. Build and learn, to hone a national lab Multiscale Imaging Engine, including high-performance molecular probe toolsets. Open the Engine to biologically provocative 3D models in the Community

1. Understand the architecture and dynamics of the Clevers’ organoid system and/or related state-of-the-art models
   - Normal stem cell derived, transformed via engineered mutations
   - Patient-derived malignant TICs (Lgr5 marker)
   - Potential inclusion of CTC derived 3D organoid cultures
   - Other ex-vivo routes with promise as tumorigenic model systems

2. Deploy and refine the MultiScale Imaging pipeline for systematic 3D culture, as an engine for comparative functional and architectural analysis of 3D tumor model dynamics and their in-vivo implantation

3. Open to the community subsequently as a National Lab platform for access and partnership
Align, develop this reference lab for candidate 3D models. Advance 3D culture conditions for mechanistic pathway biology, in an engine for comparative functional and architectural analysis.

Advance molecular imaging probe toolsets as a national resource
Multiscale Imaging

- **Subcellular imaging**: Molecular Architecture of a single cell
  - EM
  - High Resolution Optical
- **Organoids**: Cellular Architecture
  - EM
  - Optical
- **Animal**: Added complexity
  - Optical
  - Ultrasound
  - Nuclear

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Strategy for Potential Launch

- **Leveraging Existing FNL Capabilities**
  - Molecular (e.g. Sequencing, Mass Spectrometry)
  - Imaging (EM, Optical, MRI, Ultra Sound, PET)
  - Computation (Image analysis, Data Warehousing, Pathway Modeling)
  - Animal Models (LASP, CAPR)

- **Expansion of FNL Capabilities**
  - Imaging, Single-Cell sequencing, increased sensitivity

- **Partnerships and Collaborations**
  - Novel Tumor Equivalent Models and Metastatic Models
  - Imaging Probes (Subcellular -> Clinical)
  - Strengthening existing core areas
EM Ultrastructural Imaging Platform for determination of Cellular architecture

- Traditional thin-sectioning TEM of tissues, cells, and virus pellets
- Negative stain analysis of protein complexes, viruses, nano-particles
- SEM for biological material

3D Spheroids formed by mesothelioma cells by thin-section EM and by SEM
Optical Imaging: Cellular Architecture and Dynamics

N-STORM super-resolution microscope: Applications include 3D imaging of cellular structures, single molecule imaging.

Image analysis software: Applications include analyzing individual cells in tissues and organoids, molecular colocalization analysis, quantification and modeling of F-actin redistribution in response to drugs.

Cell micropatterning technology.

*Individual cells grown on fibronectin micropatterns.*

*F-Actin fibers imaged with conventional microscopy*

*Super-resolution, D-STORM image. (Color represents height)*

*Cell tracking in a live mouse embryo. (In collaboration with Dr. Terry Yamaguchi, CCR)*
Integrated tools that can be used to determine the evolution of Cellular Architecture

Segmentation of individual cell nuclei in a fully grown MCF10A acinus (left), and segmentation of individual whole cells in a 3 day old mouse embryo (right)

OMAL System Architecture for High Throughput Image Analysis. Currently used for automatic gene positioning in breast cancer detection (In Collaboration with Dr. Tom Misteli, CCR). Visualization developed in collaboration with Dr. Yanling Liu (Imaging and Visualization Group, ABCC)
Light-sheet microscopy allows for less invasive analysis enabling the study of dynamics

1) Light Sheet Microscopy

2) Fluorescence Life-time Imaging addition to fluorescence correlation spectroscopy

3D rendering of a 2-day old living zebrafish heart. Carl Zeiss Inc.
Higher field Instruments for Animal Imaging will provide functional molecular imaging in longitudinal studies.

Dynamic contrast-enhanced MRI of orthotopic mouse model of pancreatic cancer. Inset shows time course of contrast agent concentration, which can be fit to models reflecting blood flow and vessel permeability. http://www.mriresearch.ubc.ca/content/facilities/7T/
FNLCR would be a hub for imaging probes

- There will be a need for optical, magnetic, ultrasound and radiolabeled probes used over all length scales.
- There are many groups developing probes in the extramural community, many of which are valuable, but the costs of sharing are too high, and there is limited commercial value.
- There is a SIGNIFICANT research value and the FNLCR can be the clearing house for the validated probes (NCL, ACL serve as models).
- For promising probes that can be used in clinical studies to validate the 3D models or for use in diagnostics, can work with partner in moving these studies forward (BDP, NCL, CLIA).
### Current Quantitative Analysis at FNLCR for measuring Tumor Architecture & Dynamics

#### FLUORESCENCE
- Cell Tracking
- Phenotyping
- Tracer Kinetics
- Imaging disease-related biomarkers and pathways
  - HER2
  - Angiogenesis
  - Apoptosis
  - Other probes available

#### ULTRASOUND
- Anatomical volumes
- Blood Volume
- Blood Flow (Doppler)
- Cardiac Function
- Tissue Doppler
- Image guided injections
- Imaging disease-related biomarkers and pathways
  - Perfusion (Untagged microbubbles)
  - Angiogenesis (VEGFR2 tagged microbubbles)

#### MRI
- Dynamic Contrast Enhanced (DCE)-MRI (Permeability)
- Dynamic Susceptibility Contrast (DSC)-MRI (Perfusion)
- Tracer Kinetics
- Anatomical volumes

#### NUCLEAR
- Imaging disease-related biomarkers and pathways
  - \[^{18}\text{F}]\text{FDG}: \text{Glucose Metabolism}
  - \[^{18}\text{F}]\text{FLT}: \text{Proliferation}
  - HER2 assay: \[^{111}\text{In}]\text{ and }[^{89}\text{Zr}]\text{ labeled Trastuzumab}
  - HER1 assay: \[^{111}\text{In}]\text{ and }[^{89}\text{Zr}]\text{ labeled Panitumumab}
  - Other probes available

#### BIOLUMINESCENCE
- Cell Tracking
- Tumor Growth
- Metastasis

#### PHOTO-ACOUSTICS
- Vascular Oxygen Saturation
- Ischemia
There are many probes that can be used to identify cells & measure changes in metabolism and RNA expression.


Multiscale Application – Potential collaboration with The HUB Foundation for Organoid Technology’
Multiscale Scenario: Tumorigenic and Tumor Dynamics

- Potential Collaboration of FNL with Clevers’ Organoid Hub
- Other Innovative sources can join in first wave of projects into Engine
- Organoid model scenario:
  - Earliest Stage in Tumor Dev: Response to external stimuli
  - Molecular profiling within architecture: Genomic, Expression (RNAseq), Metabolites, Cell-surface (FACS), super-resolution optical
  - Determine molecular/cellular features that may be correlated with metastatic potential in vitro and in vivo (molecular probes & imaging)
  - Monitor dynamics of organoid as it changes from in vitro to in vivo upon orthotopic implantation, of tumor and of stromal and immune cells
- Reference organoid system has been validated as TIC/engineered, and TIC-PDAC, TIC-CRC formats, and with reference behavior of organoids arising from culture of normal stem cells
Establishment of Pancreas Organoids from normal Pancreatic Ducts or Cancers

Work presented in this slide kindly provided by Prof. Hans Clevers, Hubrecht Institute, Netherlands
Human Pancreas Organoids mimic the original tissue - demonstrating the importance of measuring Architecture

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Orthotopic xenografts of T1 and T5 samples metastasize to distant sites

PDAC#T1

Pancreas

Lymph node

PDAC#T5

Pancreas

Spleen

CAM5.2

Human specific ab

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FNLCR today and tomorrow

- Large-scale team science; Systematic reproducible protocols for 3D culture and molecular imaging; Expansion of probe toolkit for \textit{in vitro} and \textit{in vivo} mechanistic imaging
- MITAD as Engine for comparative functional and architectural analysis of candidate 3D tumor models and their dynamics
- Field of Opportunity: advances in 3D culture of TIC-hu (tumor/engineered), CTC-hu, TIC-mouse, chip-based tissue systems...

\textbf{MITAD enables future comparative characterization projects:} Access for the National Cancer R&D Community, NCI intramural, extramural, academic, pharma, non-profit

\textbf{Envisage strategic technology partnerships} on leading-edge instrumentation and molecular \textit{in vitro} / \textit{in vivo} imaging probe technology, and entrepreneurial activity linked to the FNL hub

Frederick National Laboratory for Cancer Research
MITAD to enable reference platform for leading-edge 3D tumor models

Align, develop this reference lab for candidate 3D models.
Advance 3D culture conditions for mechanistic pathway biology, in an engine for comparative functional and architectural analysis.

Advance molecular imaging probe toolsets as a national resource

Frederick National Laboratory for Cancer Research
Current SAIP Instrumentation for Multiscale Imaging

3T MRI
(Philips Medical Systems)

- Ultrasound
(Vevo 2100; VisualSonics, Inc.)
- Imaging angiogenesis using microbubbles tagged with VEGFR2 antibodies.

NUCLEAR

- NanoSPECT/CT
(Bioscan, Inc.)
- Inveon \(\mu\)PET/CT
(Siemens Medical)
- \[^{111}\text{In}]\text{Panitumumab}
(Fused SPECT (Color)/CT (grey))
- Animal Model: athymic nudes MDA-MB-231 cells

- \[^{89}\text{Zr}]\text{-Panitumumab HER1 probe (PET/CT)}

OPTICAL

- Bioluminescence
Xenogen IVIS SPECTRUM
(Caliper Life Sciences)

- Cell Trafficking
Maestro-GNIR (Cambridge Research Institute); FMT 2500
(Tomographic Fluorescence)
(PerkinElmer/VisEn Medical)

Photo-Acoustic
(Vevo LAZR; VisualSonics, Inc.)

- Imaging Oxygen saturation \((\text{SO}_2)\).

Archive

- Image Analysis
High-end workstations
Remote access to analysis programs

Autoradiography & Biodistribution

- A protease activatable fluorescent \textit{in vivo} imaging agent (ProSense 750) in a murine GEM model for pancreatic adenocarcinoma.
Imaging and Visualization Group (IVG):
Focused on algorithms, software, and workflows for rapid and reproducible analysis of biological images.

- Automated algorithms and workflows to facilitate reproducible analyses and increase throughput for quantitative analyses of tumor volumes, metastases, and measured properties.

- Designed for ease of use by SMEs rather than computer scientists.

- Algorithms and workflows for both 2D sections and 3D volumes.

FNL/ISP/ABCC  Jack Collins, Yanling Liu