Multi-Core Experiments: Synergistic Outcomes, or Just Squared Degree of Difficulty?

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Acknowledgments

Rosie Sears

Models / 3D Printing
- John Muschler
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scRNA-seq
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- Kevin MacPherson

Cyclic IF
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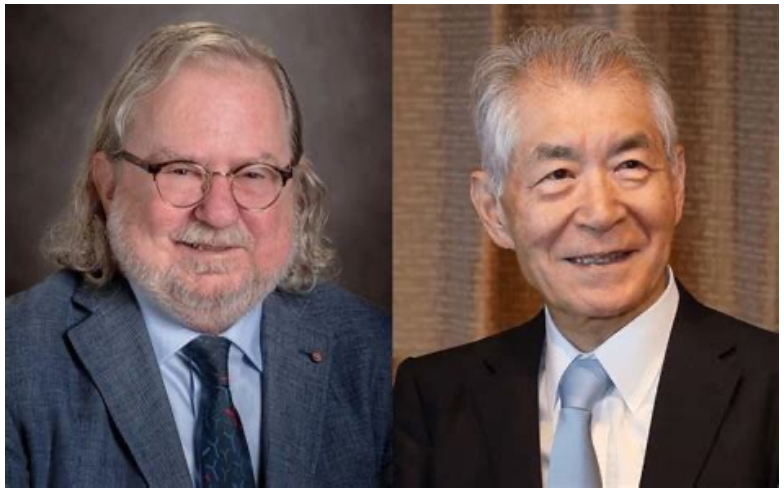
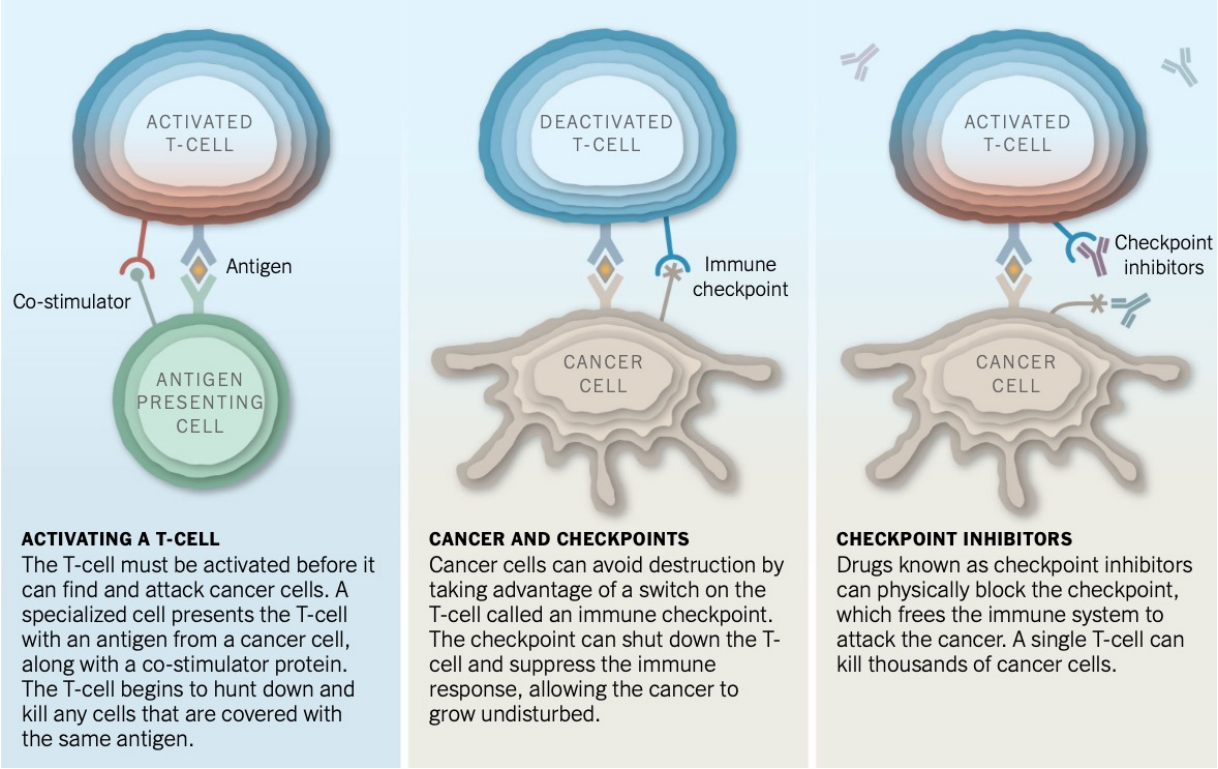
- Syber Haverlack (CEDAR)
- Jenny Eng
- Alex Smith

2016 Nobel Prize

For Cancer Therapy by Inhibition of Negative Immune Regulation

Jim Allison and Tasuku Honjo





Jimmy Carter's Melanoma Appears to Respond to Immunotherapy

THE FORMER PRESIDENT, WHO ANNOUNCED IN AUGUST 2015 HE HAD METASTATIC MELANOMA, RECENTLY SAID HE WAS CANCER-FREE AFTER TREATMENT WITH PEMBROLIZUMAB.

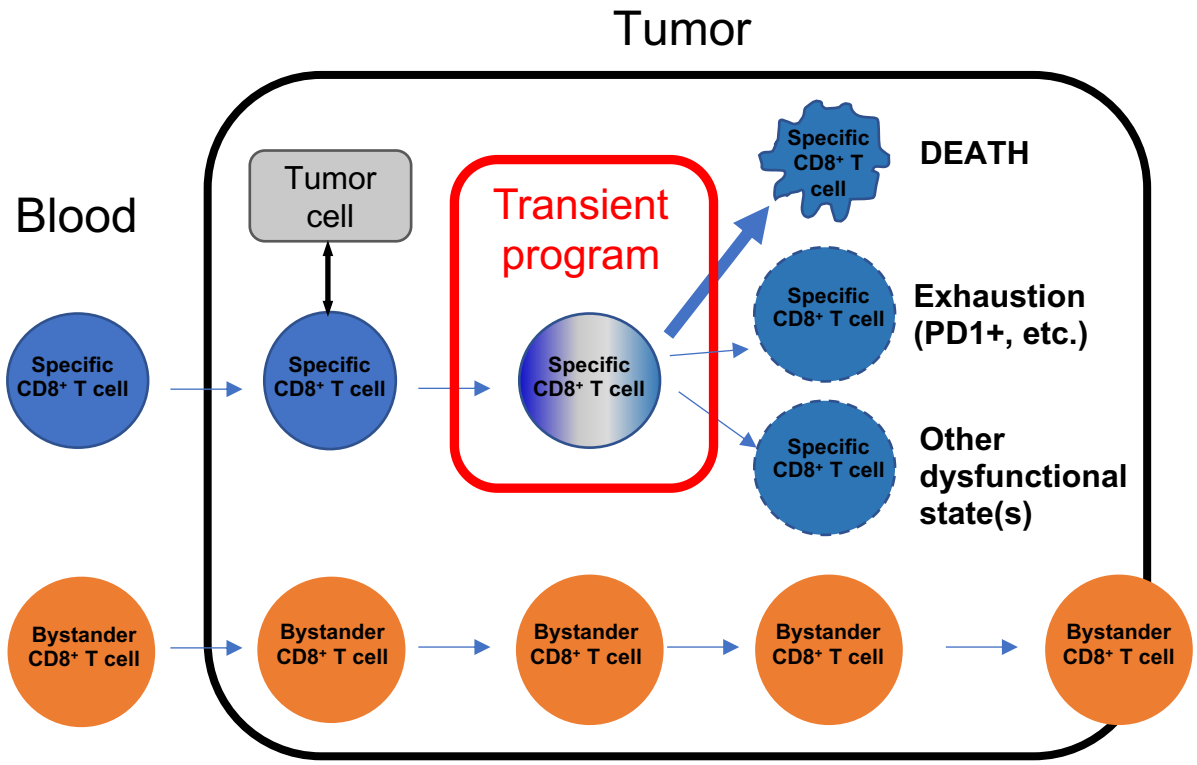
Just four months after former President Jimmy Carter announced he had metastatic melanoma that had spread to his liver and brain, the nonagenarian said he is cancer-free following radiation therapy and treatment with a cancer immunotherapy.

In August 2015, after surgery for a mass on his liver, tests revealed melanoma and further tests found that the cancer had

‘Why T Cells Fail’ Project

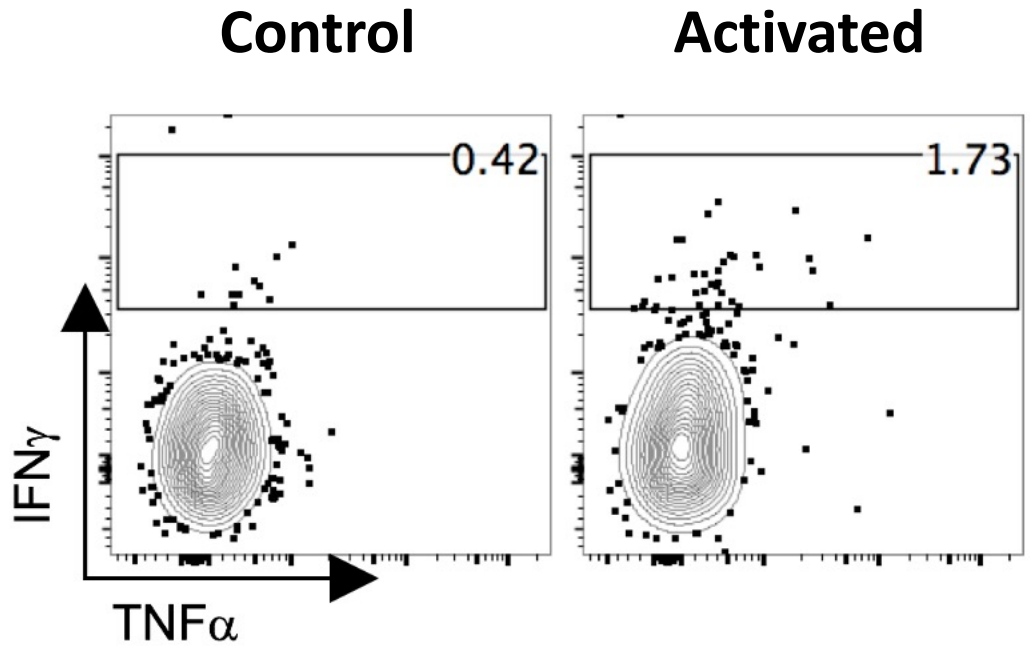
‘Seeing’ antigen in the Tumor Micro-Environment triggers dysfunction/death

Bystander cells just pass through.



- Overcoming **two obstacles** to understanding T cell dysfunction.

CD8 T Cells Within Tumors Make Anti-Tumor Cytokines

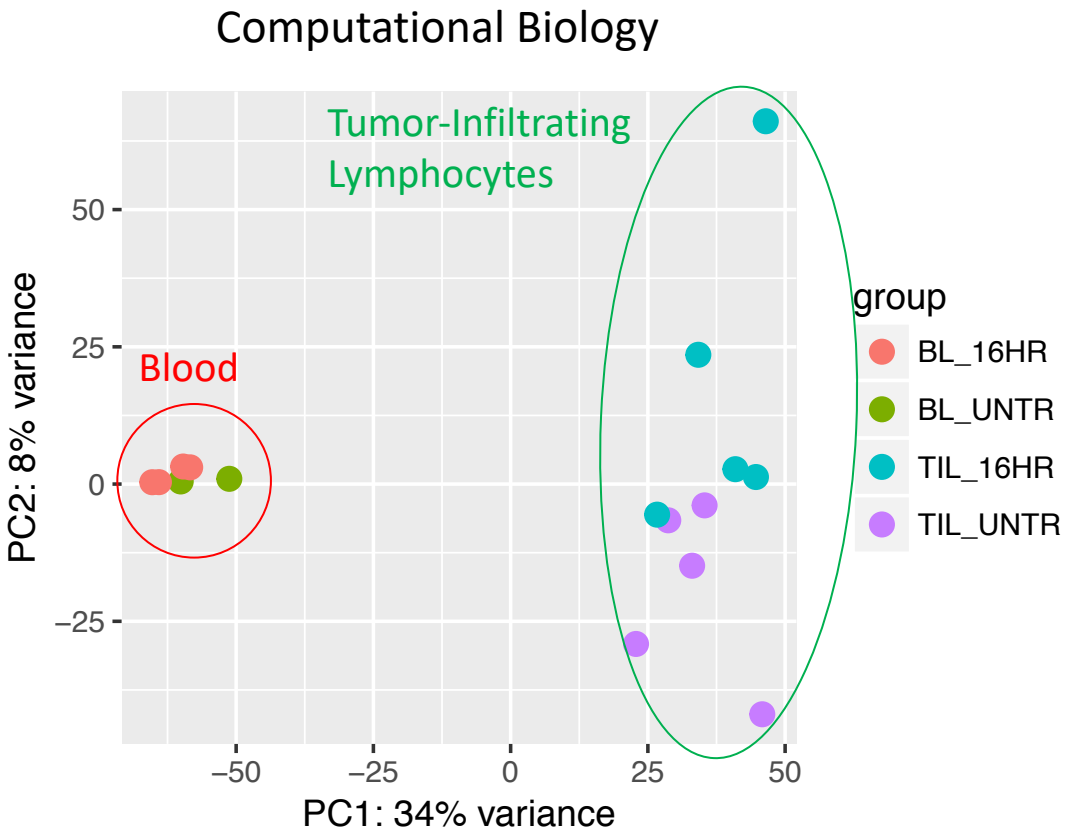


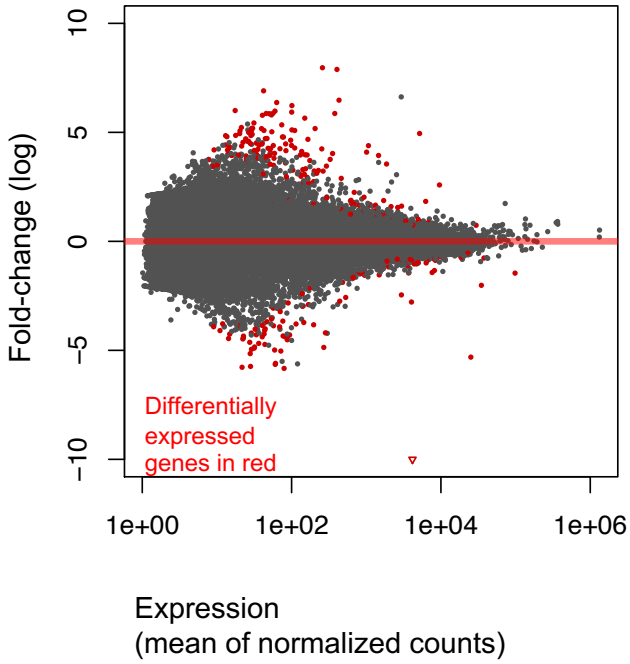
Flow Cytometry Core

Gene Profiling Core (RNA)

RNA-seq

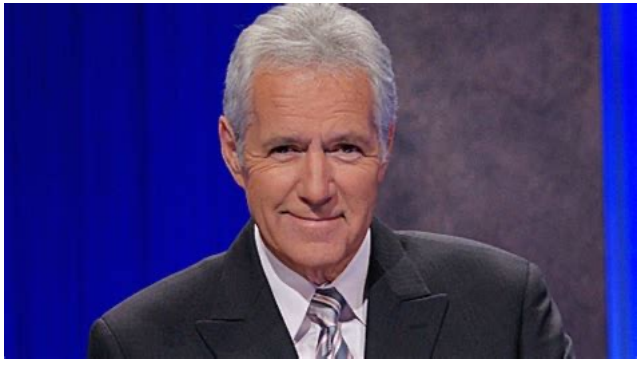
Identification Differentially-Expressed Genes





Brenden-Colson Center for Pancreatic Care

Oregon Pancreatic Tissue Registry



The New York Times

Neo

Reprogrammed Cells Attack and Tame Deadly Cancer in One Woman

Another patient who had the same treatment did not survive. But the demonstration of the technique could help with other cancers.

CLINICAL PROBLEM

Pancreatic ductal adenocarcinoma
notherapy, possibly because this ca
gen-reactive tumor-infiltrating lym
therapy with T cells bearing an alt
(TCR) gene targeting hot-spot mut
which are common in pancreatic c
a therapeutic alternative.

STUDY

A 71-year-old woman with progres
creatic adenocarcinoma received a
16.2×10^9 autologous T cells that ha
engineered to clonally express two
HLA-C*08:02–restricted TCRs targ
G12D expressed by the tumors. Fiv
sion, she received a single dose of
zumab (600 mg) to prevent cytokin
and on days 5 and 4 before infusio
travenous cyclophosphamide (30 m
body weight per day). At 18 hours
the patient began receiving intrave
interleukin-2 (600,000 IU per kilog
8 hours, five doses total) to suppo
the infused cells.

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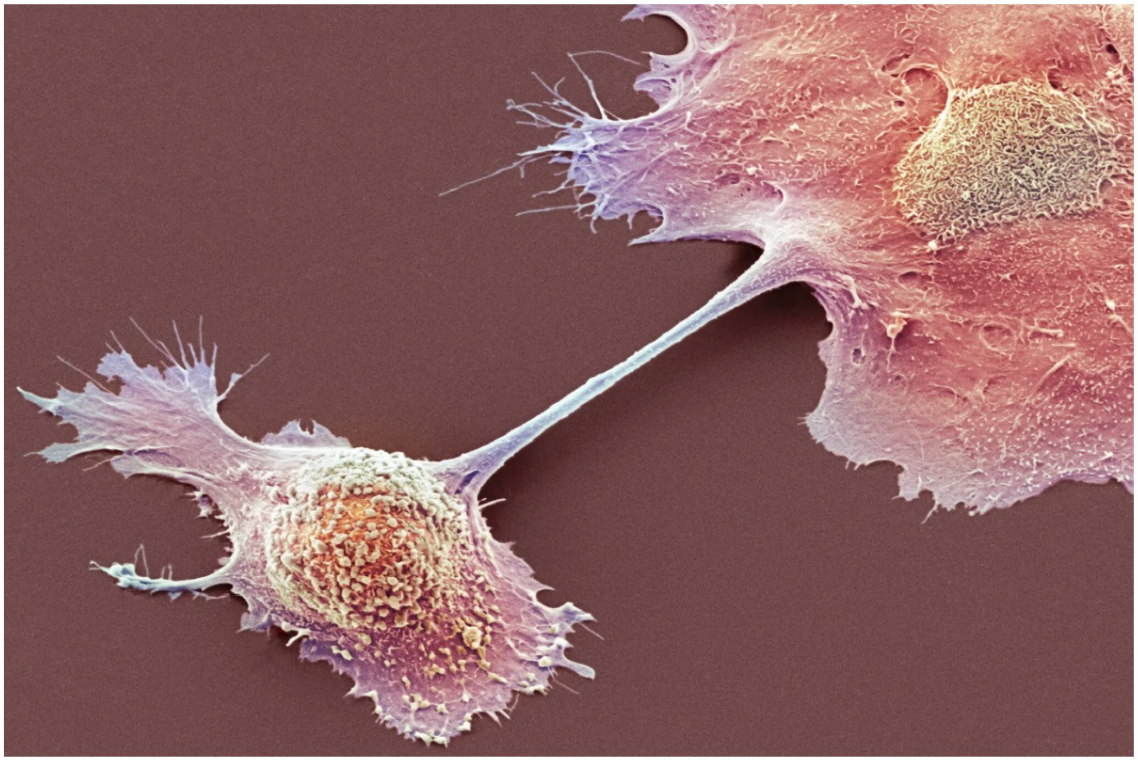
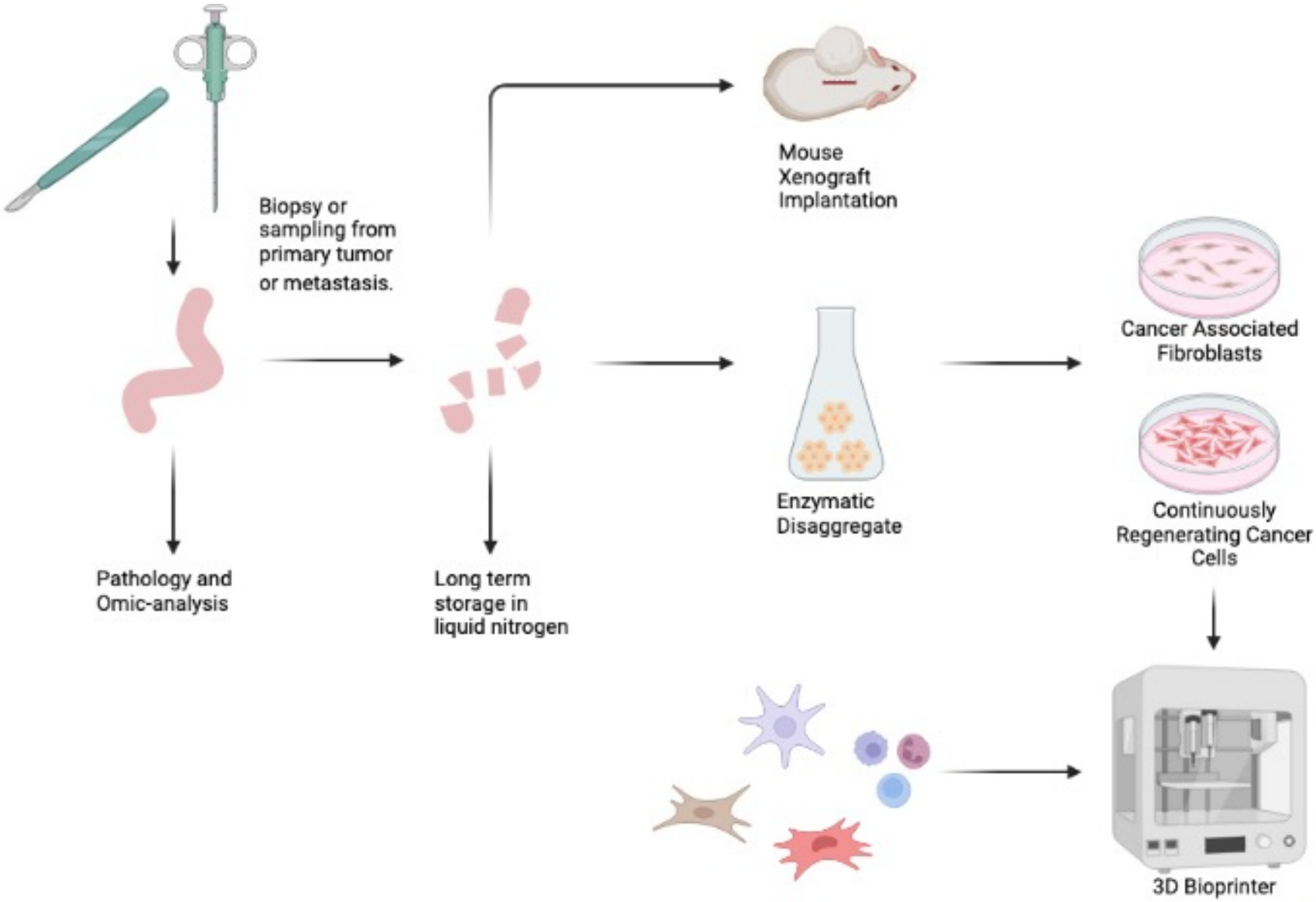
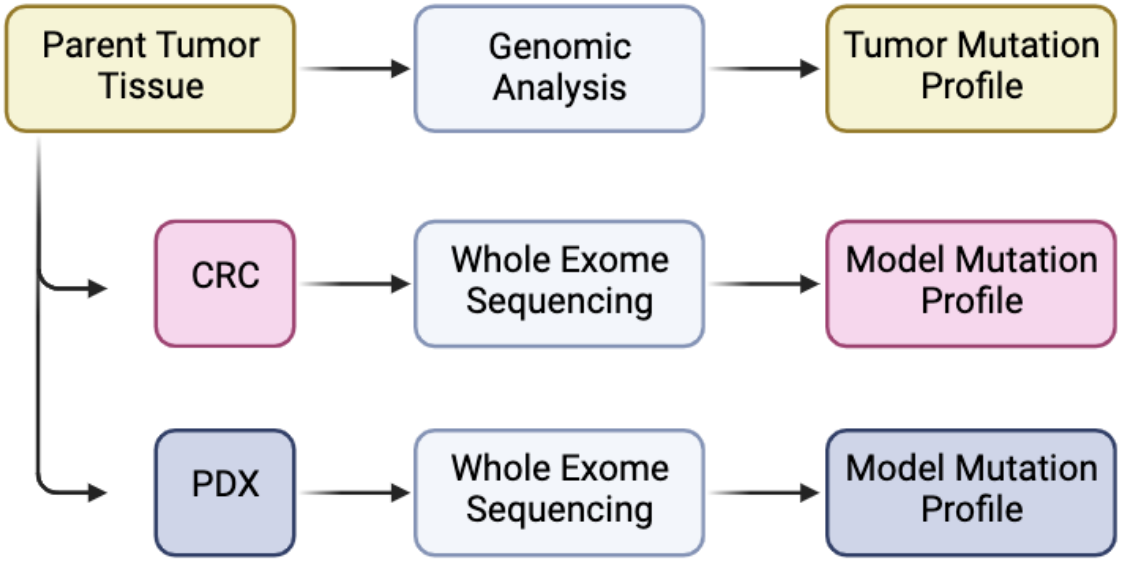
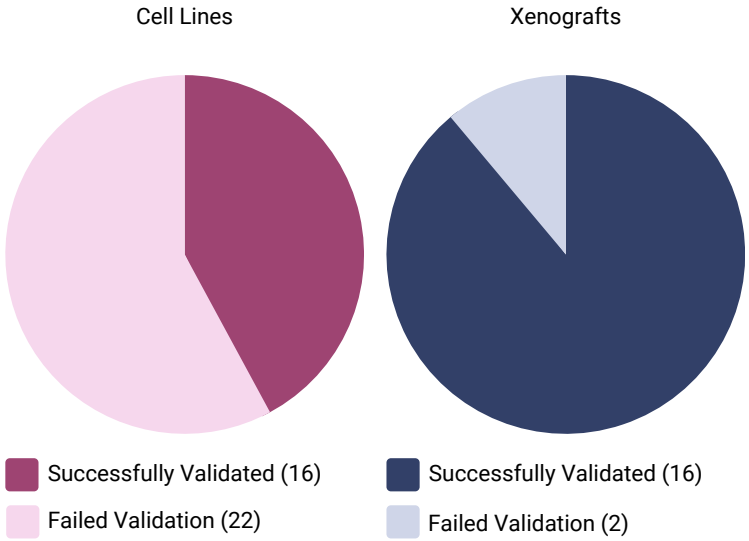


Surgical Samples Used to Create Cancer Cell Lines



Cell Line Validation: Tumor Mutation Profile



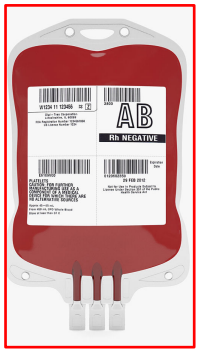


3D Bioprinting

Fibroblasts

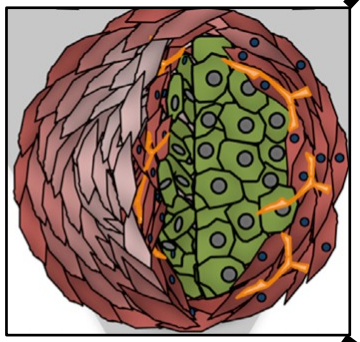
Cancer

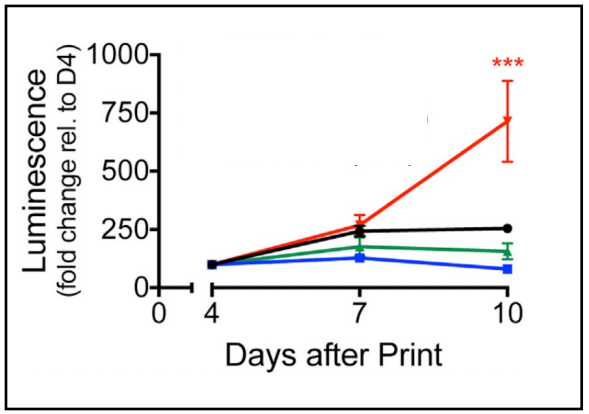
Endothelial



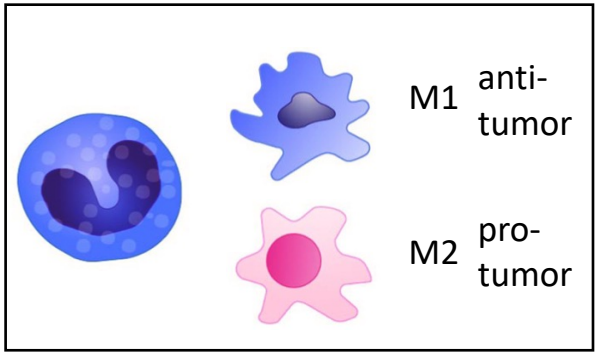
Monocytes

T cells

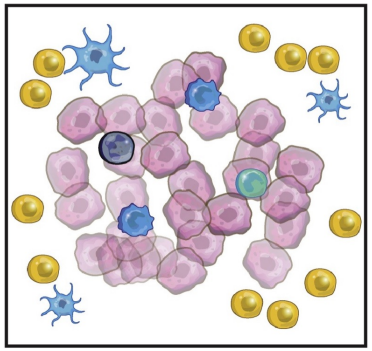




1. Drug Sensitivity



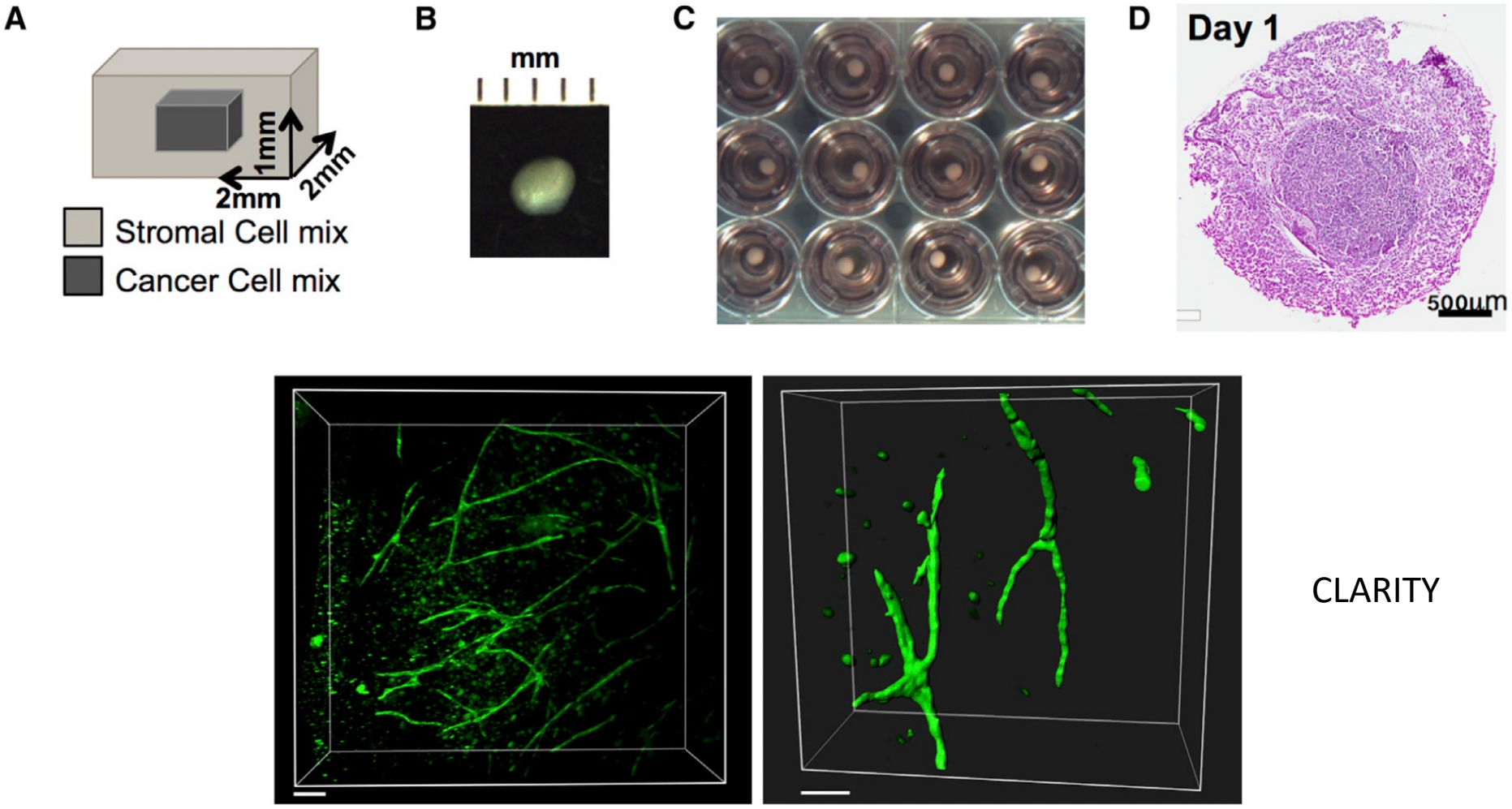
2. Macrophage Polarization



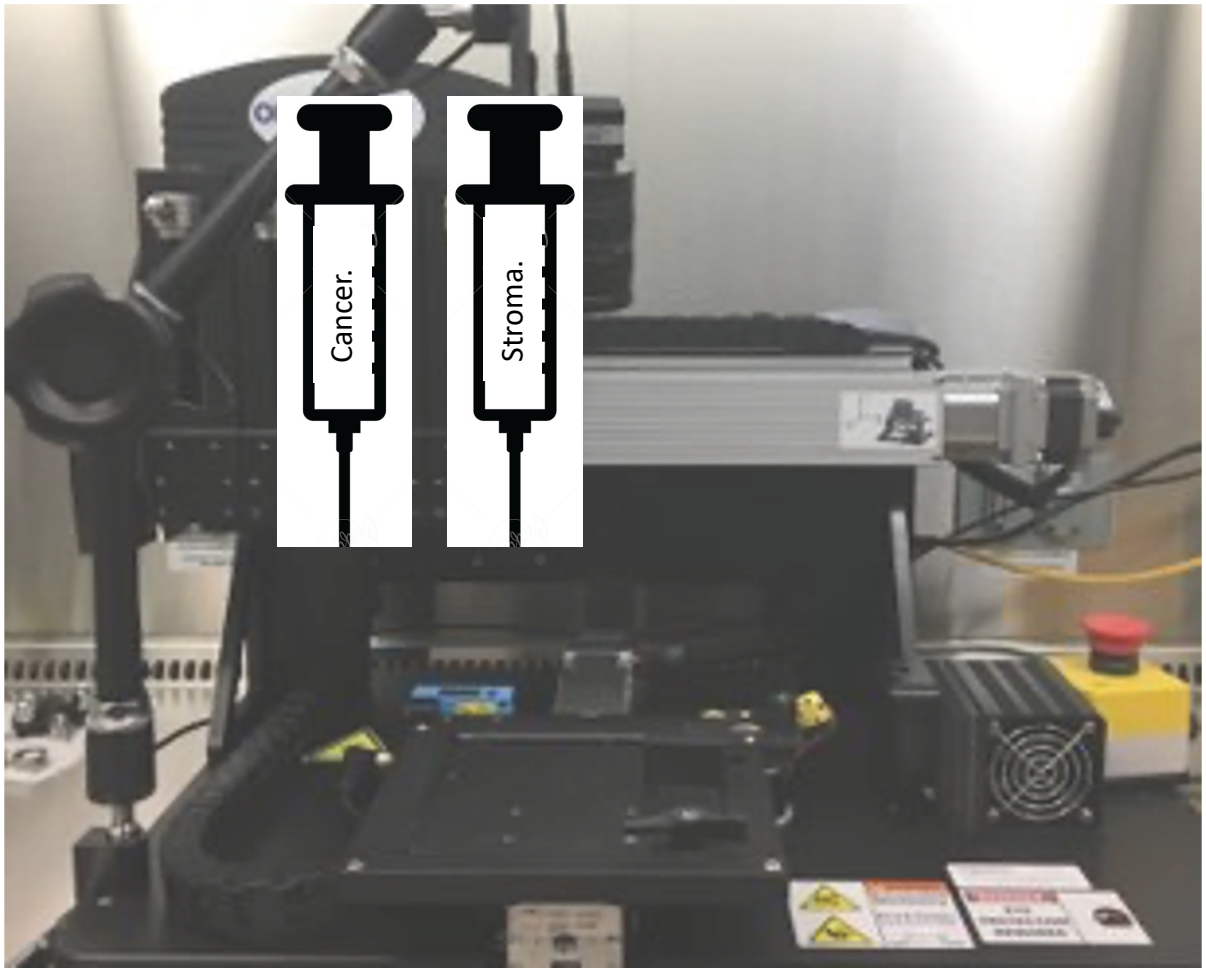
3. T Cell Exclusion

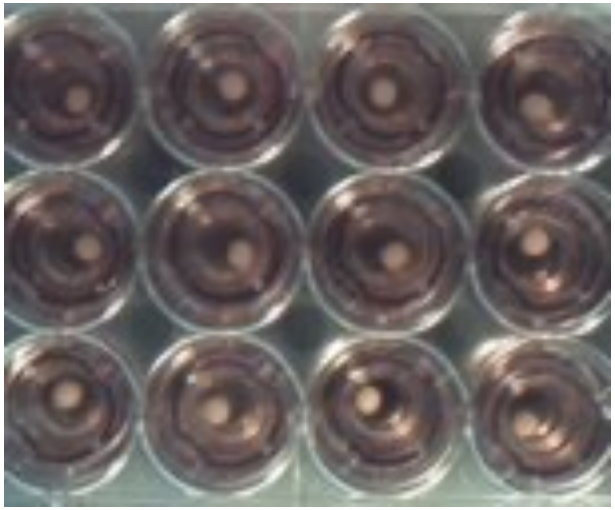
Adapted from Langer et al, Yang et al., van der Woude et al.

3D Tumor Bioprinting – Dense Tissues, Self-Organizing Endothelial Structures

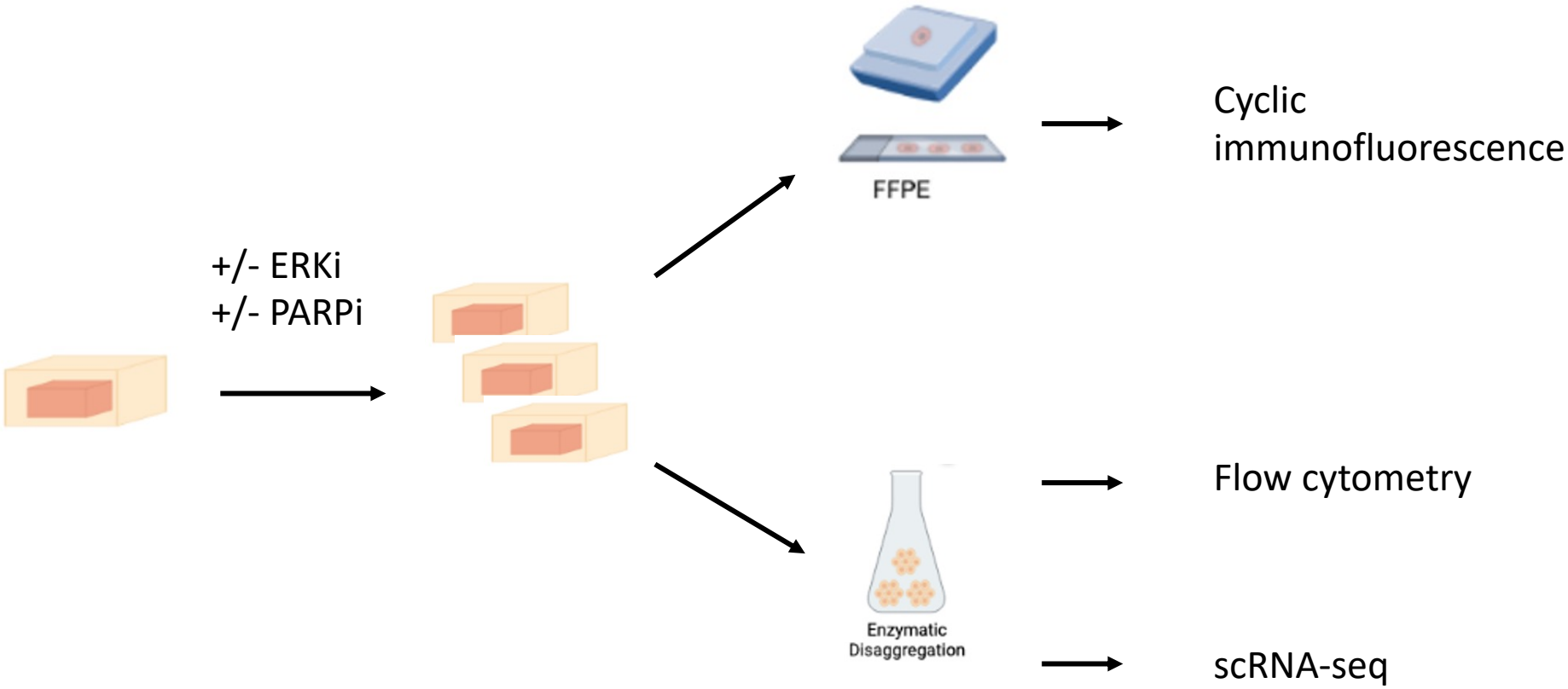


Langer et al, *Cell Reports*, 2019

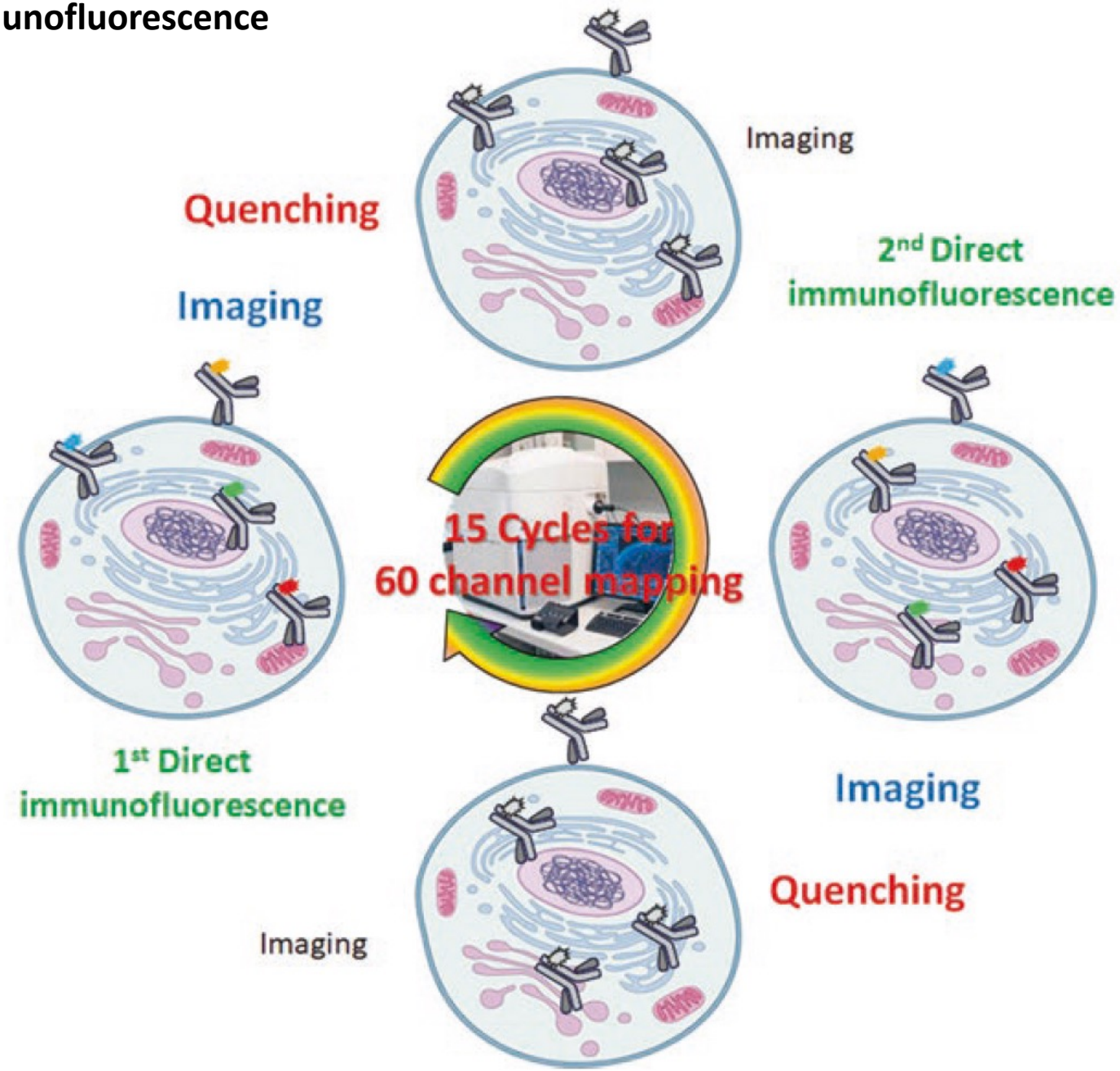

Cancer.
Stroma.



Movie

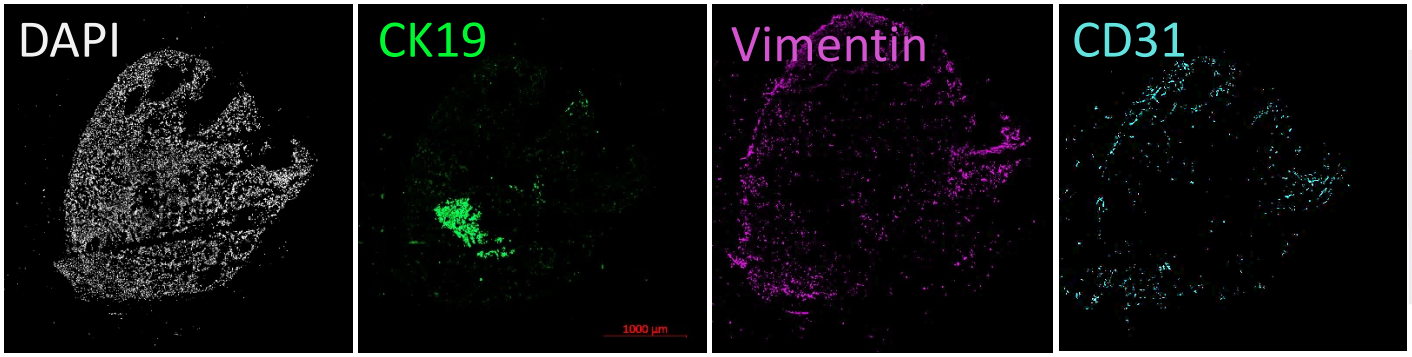

+/- ERKi
+/- PARPi
FFPE
Cyclic
immunofluorescence
Enzymatic
Disaggregation
Flow cytometry
scRNA-seq

Cyclic Immunofluorescence

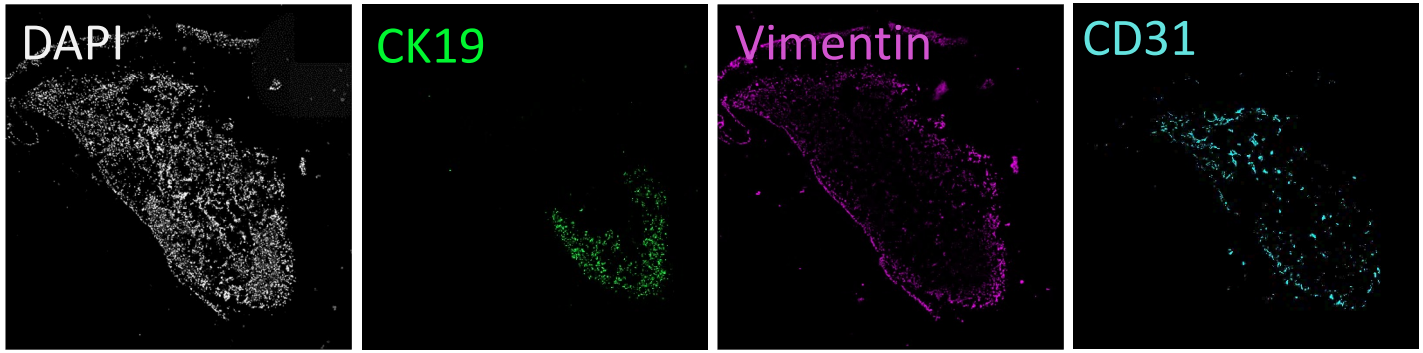


Eng et al., *Meth Mol Biol*, 2020

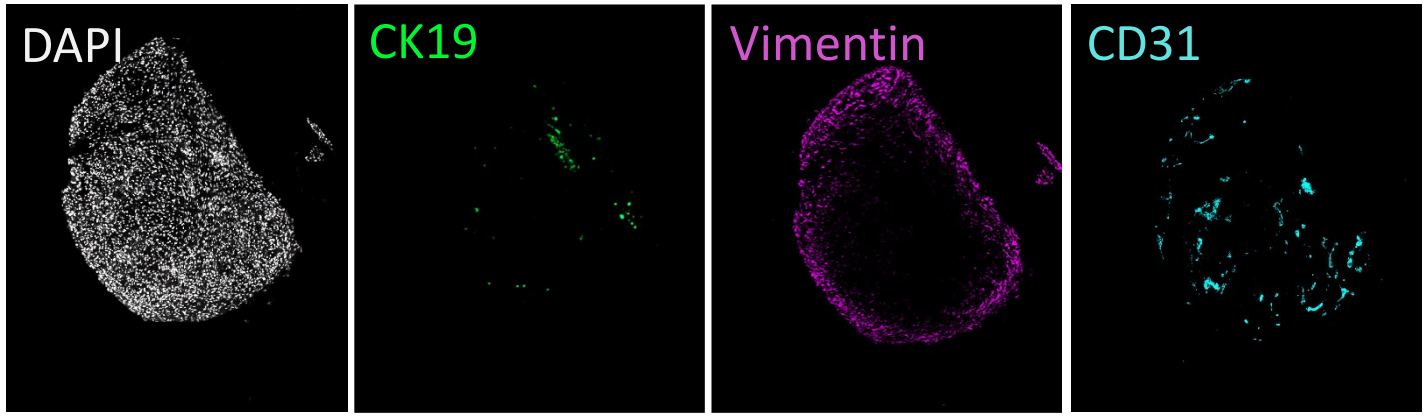
Day 5 - Untreated



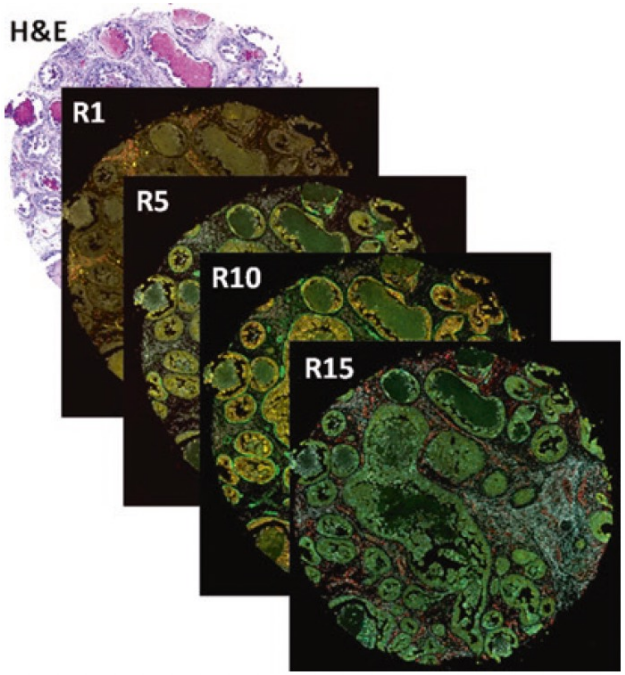
Day 9 - Untreated

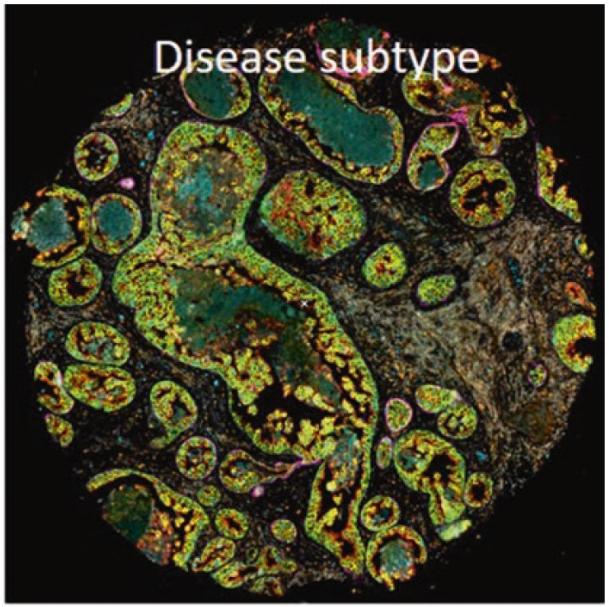


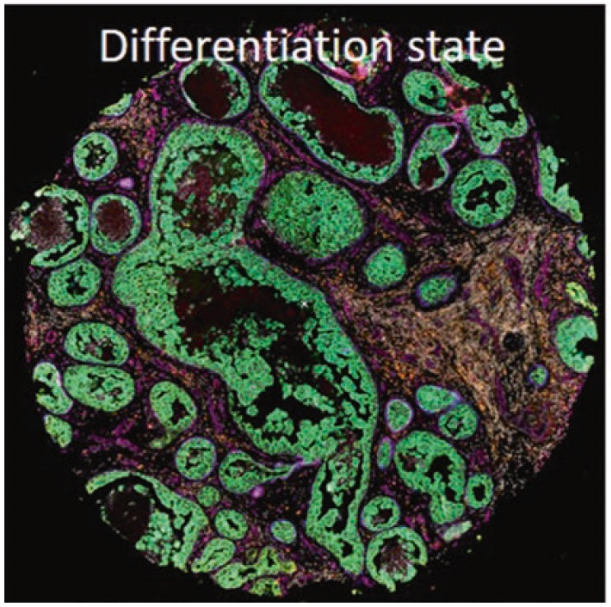
Day 9 - MEKi (cobimetinib)

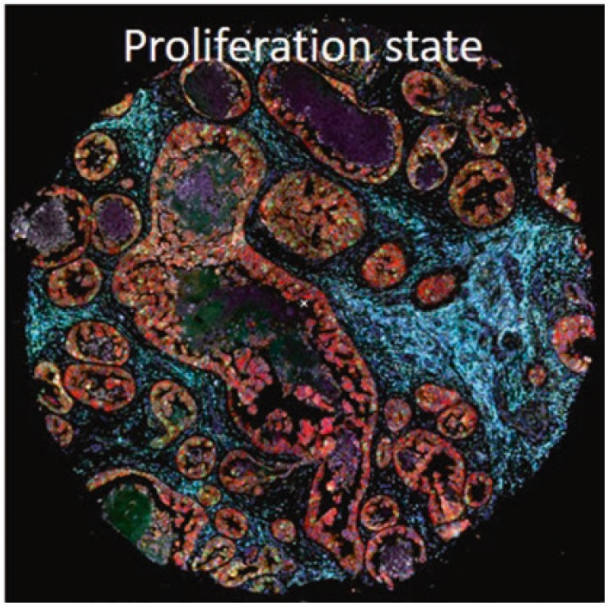


Cyclic Immunofluorescence - Multiple Uses









Eng et al., *Meth Mol Biol*, 2020

What Can We Learn from 3D Bioprinting?

Hypothesis 1: Tumor cells and stromal cells, within a 3D bioprint, will be much more similar to cells within a tumor, compared to cells cultured in 2D.

Hypothesis 2: Tumor cells that are growing within a 3D bioprint, when treated with cobimetinib (MEKi) and/or olaparib (PARPi), will best model the drug response occurring in treated patients (compared to 2D cultures or PDX mice).

Hypothesis 3: Innate immune cells (monocytes), when incorporated into a 3D bioprint, will be useful for identifying mechanisms of macrophage polarization.

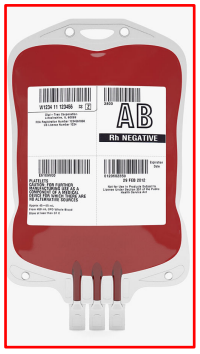
Hypothesis 4: T cells from a syngeneic donor will be useful for determining mechanisms that prevent T cell infiltration, and determining mechanisms of T cell dysfunction.

3D Bioprinting

Fibroblasts

Cancer

Endothelial



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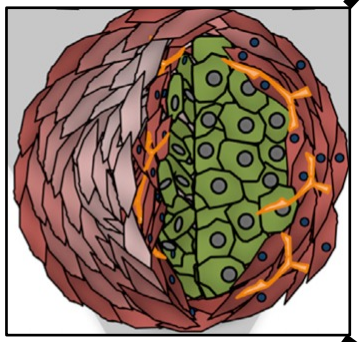
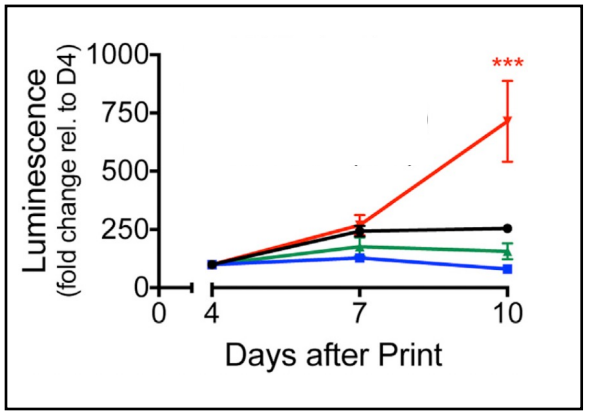
T cells





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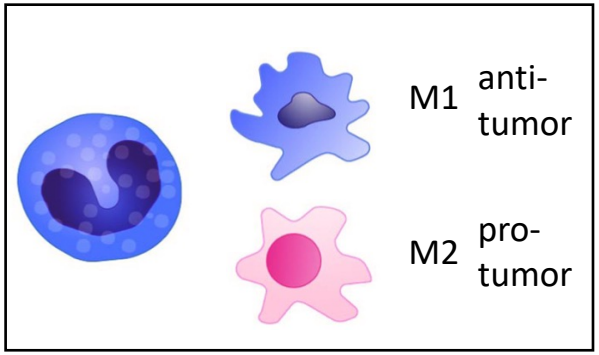

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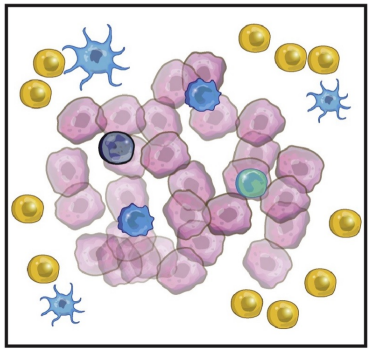

3. T Cell Exclusion

Adapted from Langer et al, Yang et al., van der Woude et al.

Potential for Success	**Potential to Really Struggle**
Focused on big picture	Focused on the big picture
Attention to detail	Attention to detail
Persistence !!!	

Cross-Core Projects – Lessons Learned

Coordinating schedules is *really* difficult.

Practice the individual steps (once or twice).

- Think about the protocol in modules (mouse, flow, RNA, etc.)
- How long should this module take?
- What yields are likely?

But you won't figure out **all** the problems until you **go for it**.

- Ahh, the media becomes alkaline while sitting during sort.

Assume it's not going to work until the 2nd or 3rd try.

Create off-ramps (save $).

Who is going to analyze the data??? What will the figure look like?

- Tell everyone what you expect to happen.
- They may **know** where the failure points are.

Chris Harrington is *really* patient.