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

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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

- Overcoming **two obstacles** to understanding T cell dysfunction.
CD8 T Cells Within Tumors Make Anti-Tumor Cytokines

Control

Activated

IFNγ

TNFα

0.42

1.73

Flow Cytometry Core

Gene Profiling Core (RNA)

RNA-seq
Identification Differentially-Expressed Genes

Computational Biology

Tumor-Infiltrating Lymphocytes

Blood

PC1: 34% variance
PC2: 8% variance

Differentially expressed genes in red

Expression (mean of normalized counts)

Fold-change (log)

mean of normalized counts

10

-10

0

5

1e+00

1e+02

1e+04

1e+06

Differentially expressed genes in red
Brenden-Colson Center for Pancreatic Care

Oregon Pancreatic Tissue Registry
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 therapy, possibly because this cigen-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 16.2×10⁶ autologous T cells that he engineered to clonally express two HLA-C*08:02–restricted TCRs targa G12D expressed by the tumors. Fusion, she received a single dose of zumab (600 mg) to prevent cytokine and on days 5 and 4 before infusio travenous cyclophosphamide (30 mg body weight per day). At 18 hours the patient began receiving intrave interleukin-2 (600,000 IU per kilo 8 hours, five doses total) to suppo the infused cells.
Surgical Samples Used to Create Cancer Cell Lines

1. Biopsy or sampling from primary tumor or metastasis.
2. Pathology and Omic-analysis.
3. Long term storage in liquid nitrogen.
4. Enzymatic Disaggregate.
5. Mouse Xenograft Implantation.
7. Cancer Associated Fibroblasts.
8. 3D Bioprinter.
Cell Line Validation: Tumor Mutation Profile

- Parent Tumor Tissue → Genomic Analysis → Tumor Mutation Profile
- CRC → Whole Exome Sequencing → Model Mutation Profile
- PDX → Whole Exome Sequencing → Model Mutation Profile

Cell Lines
- Successfully Validated (16)
- Failed Validation (22)

Xenografts
- Successfully Validated (16)
- Failed Validation (2)
3D Bioprinting

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

CLARITY
Cancer. Stroma.
Movie
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

1. Drug Sensitivity

2. Macrophage Polarization

3. T Cell Exclusion

Adapted from Langer et al, Yang et al., van der Woude et al.
<table>
<thead>
<tr>
<th>Potential for Success</th>
<th>Potential to Really Struggle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focused on big picture</td>
<td>Focused on the big picture</td>
</tr>
<tr>
<td>Attention to detail</td>
<td>Attention to detail</td>
</tr>
<tr>
<td>Persistence !!!</td>
<td></td>
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</tbody>
</table>
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.