MicroOrganoSpheres as a Clinically Applicable Precision Oncology Platform for the Discovery of Novel Therapies in Colorectal Cancer

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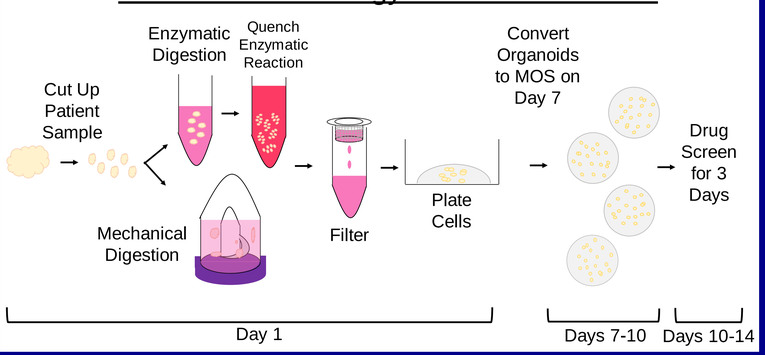
Background:
Patient derived models of cancer, such as cell lines, patient-derived organoids, and patient-derived xenografts, are useful models of patient response in the clinic. However, these models are often not clinically applicable within the time periods necessary to inform clinical decision-making, as they can take weeks to months to develop. An ideal platform using patient-derived models would be able to be generated from a core biopsy with a subsequent drug screen within 10-14 days to minimize delay in therapy. We recently reported the development of MicroOrganoSpheres (MOS) that can be used in drugs screens within 14 days of obtaining a biopsy. In the current study, we have now developed a MOS pipeline in colorectal cancer (CRC) that can be used as a precision oncology platform to identify new and novel therapies and to predict response to therapy.

Methods:
CRC patient tissue samples were collected under a Duke Institutional Review Board approved protocol (Pro00089222). Resections or biopsies were first mechanically and enzymatically digested to obtain a single cell suspension. Cells were then plated in Matrigel at a ratio of 20,000 cells:5 µL Matrigel to establish "mini-bulk" organoid cultures. After establishment for 5-7 days, cultures were harvested with subsequent generation of MOS at a ratio of 50 cells per MOS. After growing for 3-4d, MOS were used for dose-response curves using oxaliplatin, SN38, and 5-Fluorouracil (5-FU) as well as high-throughput drug screens with the NCI Approved Oncology Drugs Set VI library.

Results:
We first developed and optimized a MOS pipeline on over 50 CRC specimens, including 9 primary rectal, 35 primary CRC, 12 CRC liver metastasis, and 1 CRC lung metastasis lines with a success rate of 80% and an average of 10-21 days from biopsy to generation of MOS in 10-21days after obtaining a biopsy. The high success of generating CRC MOS in a clinically applicable time frame led to the next phase of the project where 10 CRC samples obtained were used in our MOS pipeline to generate MOS and perform a high throughput drug screen against standard of care chemotherapy agents used in CRC (oxaliplatin, irinotecan and 5-FU) as well as the NCI Approved Oncology Drugs Set VI within 14-21d. The range and average IC50 were found to be 100 nM - >50 uM with average of 10 uM for oxaliplatin, 50 nM-10 uM with average of 800 nM for SN38 and 400 nM – 20 uM with an average of 1 uM for 5-FU. The most sensitive drugs found in the Approved Oncology Drugs Set VI were Bortezomib, Romidepsin, and Panobinostat and the most resistant were Capecitabine , Carmustine, and Procarbazine hydrochloride.

Conclusion:
These results demonstrate that our MOS pipeline can be used as precision oncology platform within a clinically applicable time frame to potentially guide therapy. We are now in the process of correlating drug response to MOS to patient outcome data and these will be presented at the annual meeting.

A Precision Oncology Platform with MOS



Visible Drug Sensitivity with CRC MOS

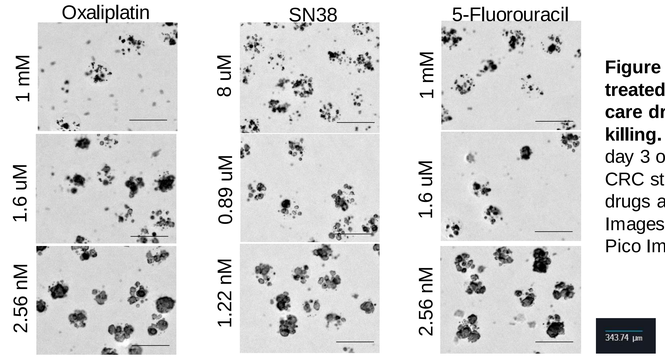


Figure 1: CRC MOS treated with standard of care drugs show visible killing. MOS imaged on day 3 of drug screens with CRC standard of care drugs at three doses. Images taken at 4X on Pico ImageXpress.

High Throughput Drug Screens on CRC MOS

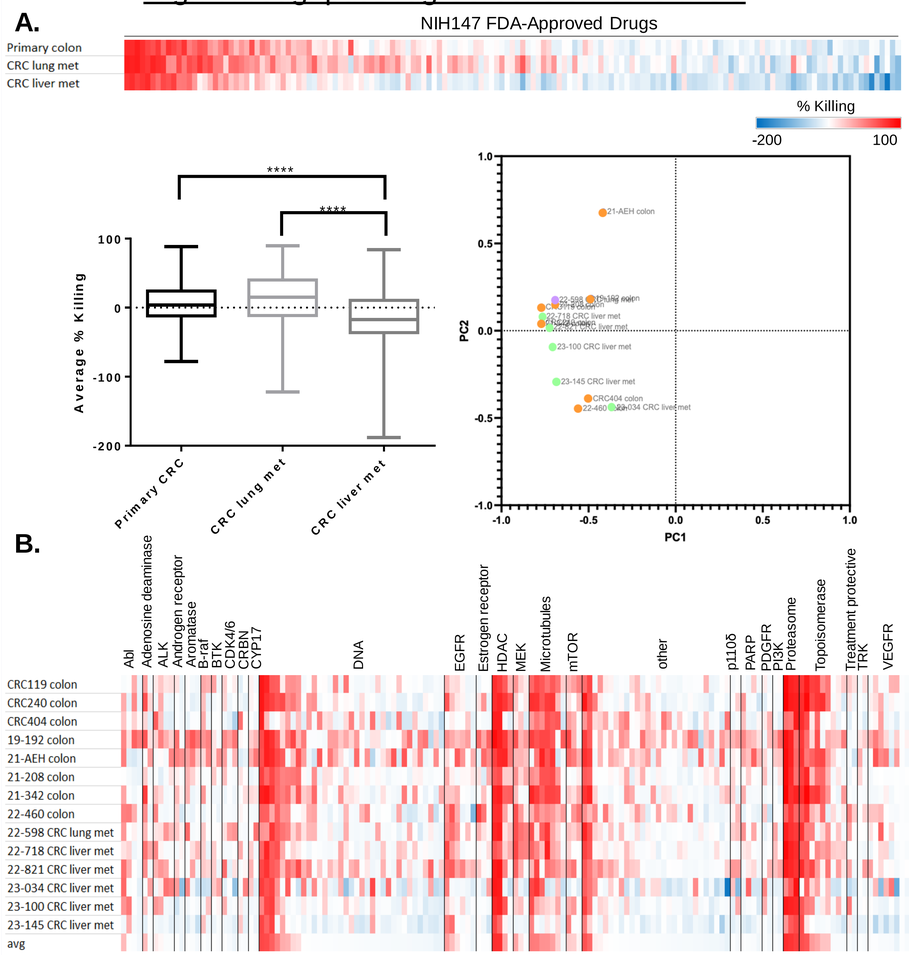


Figure 2: CRC Primary and liver metastasis lines have differing sensitivities to FDA-approved drugs. A) Heat map of high throughput drug screens on averages for all CRC primary and met lines. High average percent killing is depicted in red with low average percent killing in blue (top). Boxplots of average percent killing in CRC primary and met lines (left). ****: $p < 0.0001$. Principle Component Analysis of all 14 organoid lines (right) **B)** Heat map of high throughput drug screens on MOS from 14 CRC primary and met organoid lines. Drugs are classified from high throughput screen based on target. High average percent killing is depicted in red with low average percent killing in blue. Each group is sorted from highest to lowest average percent killing.

Standard of Care Drug Screens on CRC MOS

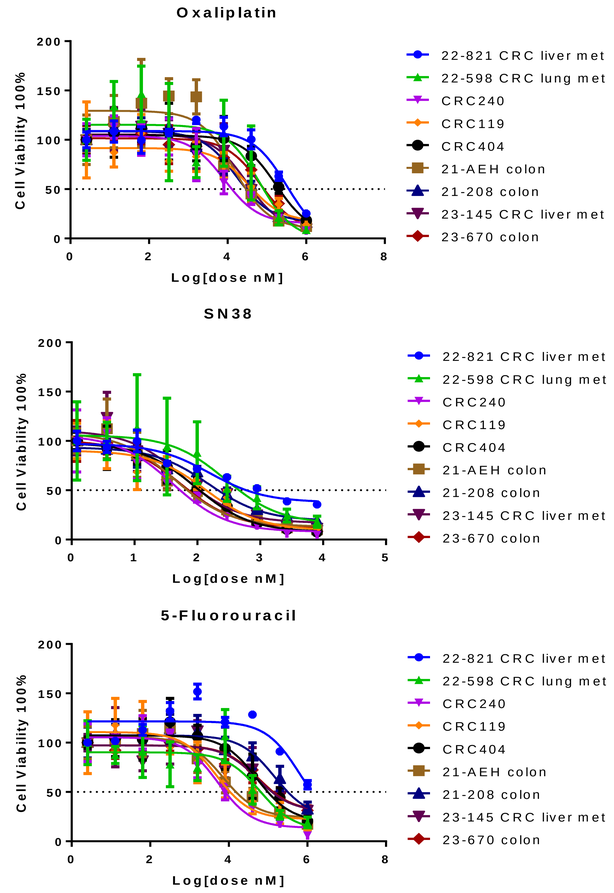


Figure 3: Single agent drug screens for oxaliplatin, SN38, and 5-Fluorouracil. Drug screens were completed with nine concentrations per drug and five replicates per concentration. MOS were incubated with drug for three days before adding Cell Titer Glo as a measure of cell viability.

Future Directions

- Systematic correlation of drug response in MOS from dose response curves for standard of care therapies to patient outcome
- Continue high-throughput drug screens on CRC MOS using the NIH147 panel of FDA-approved drugs

References

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