

Feasibility of establishing and drug screening patient-derived rectal organoid models from pretreatment rectal cancer biopsies

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Background:

Response to neoadjuvant chemotherapy and radiation therapy in the treatment of locally advanced rectal cancer is heterogenous and prognostic of clinical outcomes, necessitating the need for predictive biomarkers to guide personalized treatment recommendations. Sensitivity to a given chemotherapy in patient-derived organoids predicts patient response to that chemotherapy, establishing it as a promising model for efforts to ascertain predictive biomarkers and personalize treatment decisions. This study assessed the feasibility of obtaining patient-derived rectal organoids from standard of care pre-treatment proctoscopy biopsies.

Methods:

In this clinical trial (NCT04371198), biopsies were obtained from patients with stage II rectal adenocarcinoma prior to receipt of neoadjuvant therapy. Tissue samples were mechanically and enzymatically dissociated to obtain a single cell suspension. Cells were then mixed with matrigel at a ratio of 2,000 cells:5 μ L Matrigel in a 50 μ L dome and plated on a 24-well tissue culture plate with colorectal cancer organoid media at 37°C/5% CO₂. Established patient-derived organoids were then used to perform drug screens with clinically-applicable chemotherapeutics including oxaliplatin, irinotecan, and 5-FU, followed by high throughput drug screen using our recently published MicroOrganoSpheres platform using the NCI Approved Oncology Drugs Set VI library.

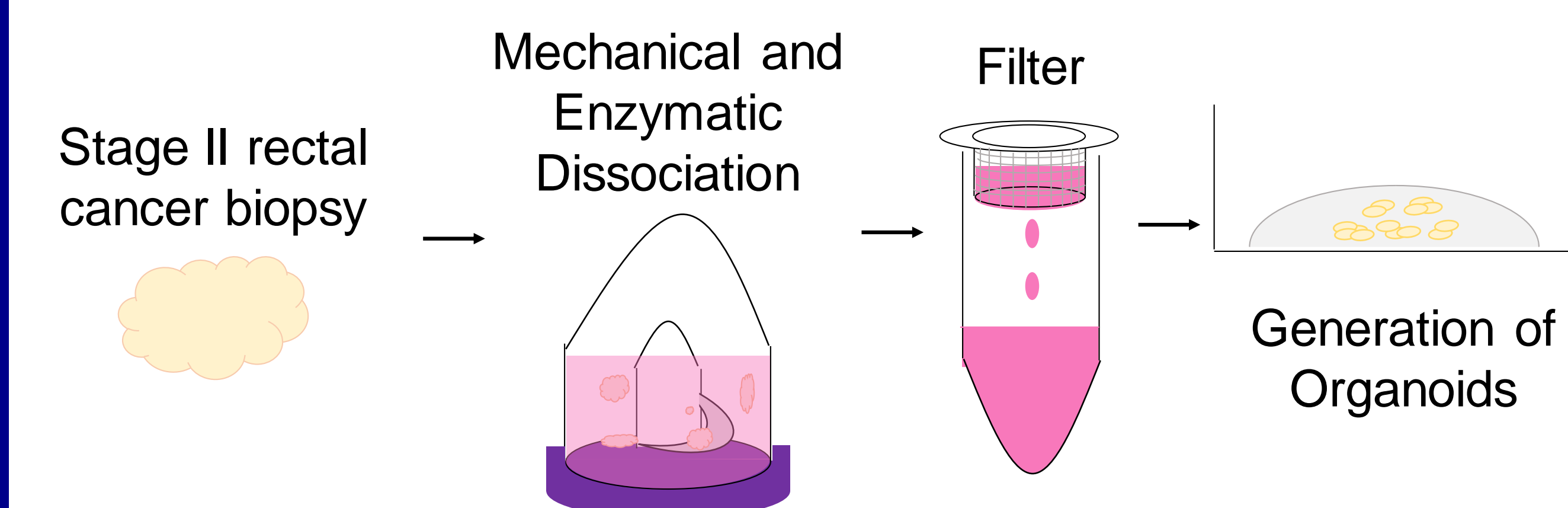
Results:

Of the 20 patients enrolled, 17 (85%) patient-derived organoids were created from pre-treatment specimens. 15 (88%) of these samples were successfully established as defined by the ability to passage organoids for at least two passages. All established samples were used to perform standard of care drug screens and high throughout drug screens, which demonstrated differences in drug sensitivities among the samples. Moreover, within two weeks of receiving the sample, four established quickly enough to complete drug screening with oxaliplatin, SN38, and 5-Fluorouracil.

Conclusions:

These results demonstrate the feasibility of establishing patient-derived rectal organoids from biopsy specimens obtained by proctoscopy, and reinforce the utility of patient-derived organoids as a tractable ex vivo platform to personalize rectal cancer treatment. Planned future directions include in vitro determination of radiation therapy sensitivity as well as systematic assessment of the correlation between individual patients and their organoid model.

Clinical Protocol and Methods



Goal: Establish patient derived organoids and perform drug screens within two weeks of obtaining rectal cancer biopsies

Rectal Cancer Organoids Established from Biopsies

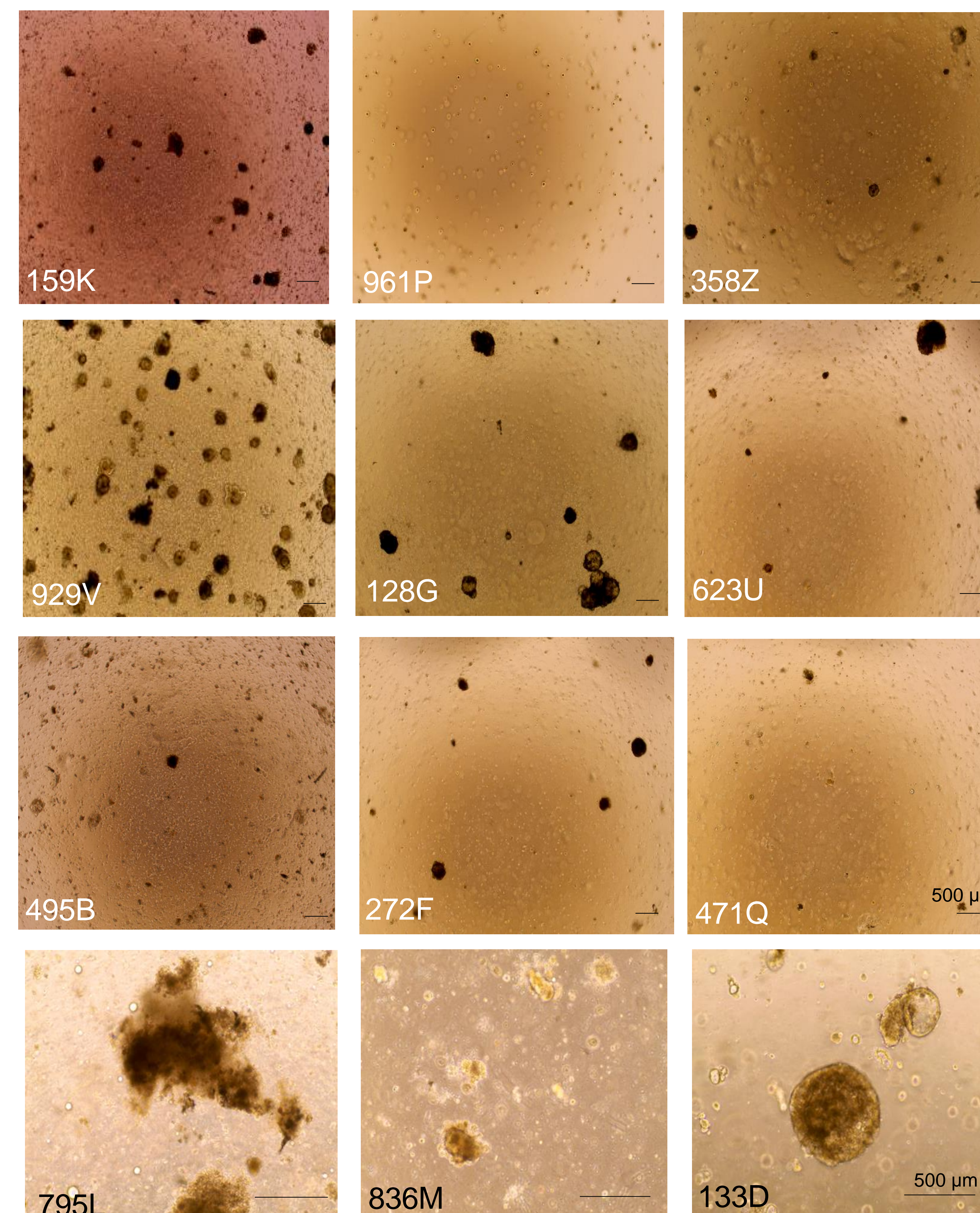


Figure 1: Establishment of rectal cancer organoids from biopsy samples. Representative images of rectal cancer organoids growing in 50 μ L Matrigel domes. Images taken at 4X (Top Three Row) or 10X (Bottom Row) on an EVOS microscope.

High Throughput Drug Screens on Rectal MOS

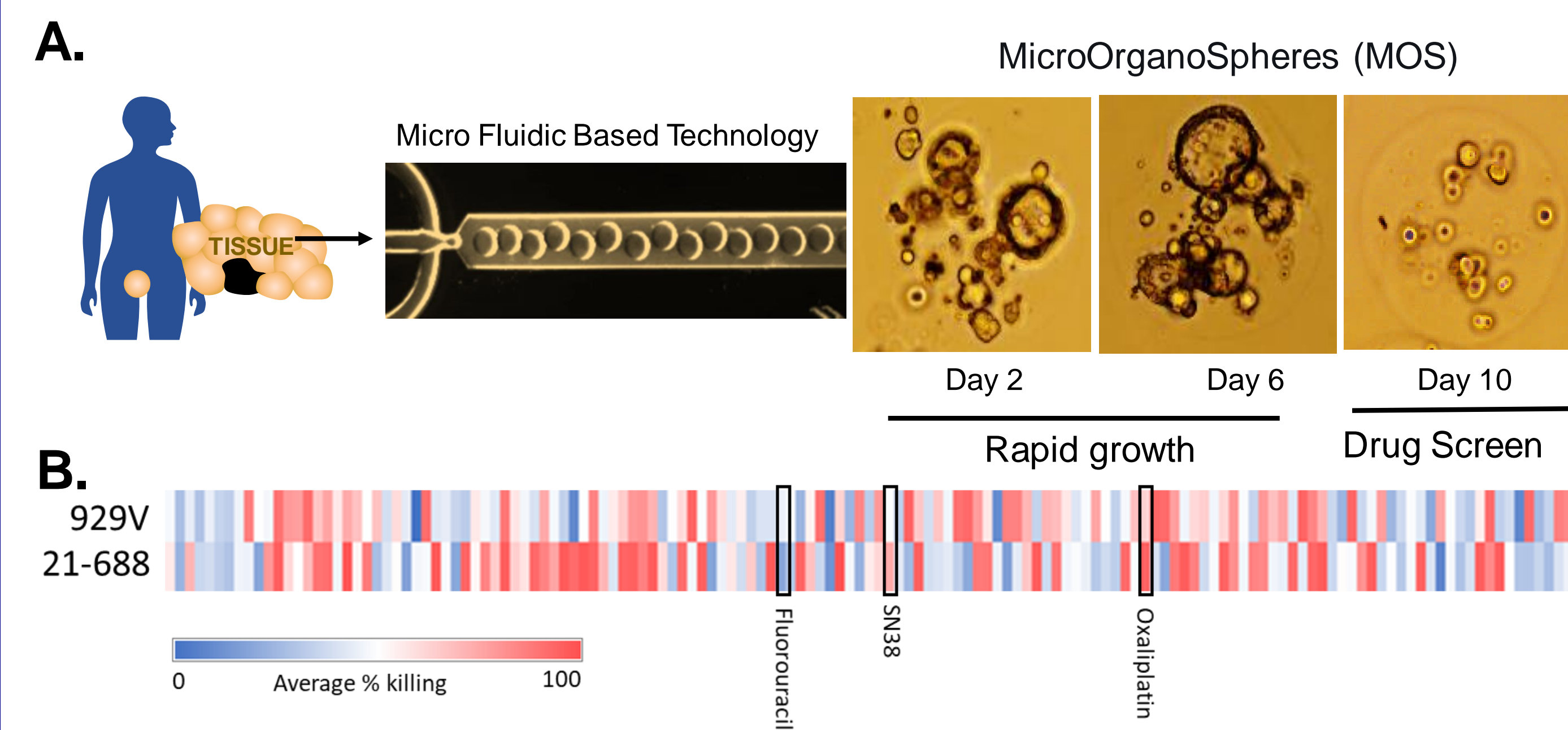


Figure 3: MicroOrganoSpheres (MOS) facilitates Drug Screen. A) Using microfluidic based technology, MOS can be generated in 10d for drug screens. B) High throughput drug screen of rectal MicroOrganoSphere (MOS) across 147 FDA approved cancer drugs reveals differing drug sensitivity

Single-Agent Drug Screens of Rectal Organoids

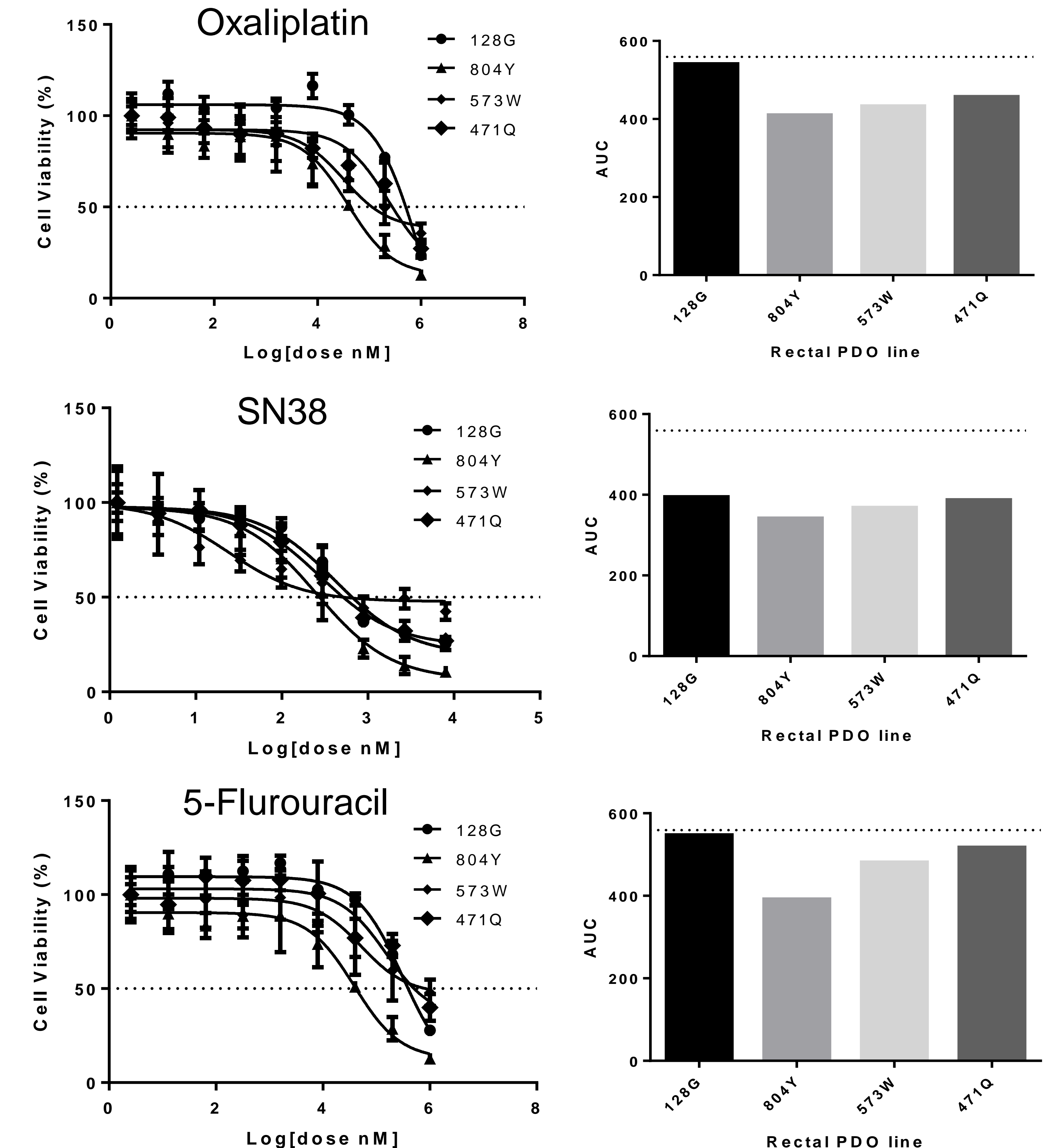


Figure 2: Single agent drug screens for oxaliplatin, SN38, and 5-Fluorouracil within two weeks of receiving biopsy samples. Drug screens were completed with nine concentrations per drug and five replicates per concentration. Organoids were incubated with drug for three days before adding Cell Titer Glo as a measure of cell viability.

Future Directions

- Continue high-throughput drug screens on established rectal organoids using the NIH147 panel of FDA-approved drugs
- *in vitro* and *in vivo* determination of radiation therapy sensitivity
- Systematic assessment of the correlation between individual patients and their organoid model.

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