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## Developing a physiologically relevant target validation platform for desmoid tumors

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The success of cancer precision medicine is contingent on the ability to determine an optimal therapeutic strategy given the molecular features of a patient's tumor. While desmoid tumors are relatively homogenous at the genomic level, we do not yet know to what extent this homogeneity induces dependency on a limited or an expansive number of cellular targets for survival. To address this gap, we aggregated over 40 short-term desmoid tumor cultures with the aim to molecularly characterize and expand the lines to a sufficient scale for genome-wide CRISPR and drug screening, and to share with the research community. We successfully generated two long-term cell lines harboring *CTNNB1* T41A and S45F mutations that have entered screening. However, we also observed outgrowth of non-malignant fibroblasts in most cultures, suggesting that current culturing conditions provide desmoid tumor cells with a growth disadvantage. We therefore hypothesize that extrinsic factors modulate the cellular composition of desmoid tumors. To test this, we developed a low-input workflow for measuring *CTNNB1* and *APC* mutations simultaneously in large numbers of combinatorial media conditions using multiplexed barcoded deep sequencing. After demonstrating feasibility and reproducibility, we then studied 32 conditions in 4 desmoid samples of variable starting tumor purity. Expectedly, most conditions showed non-malignant outgrowth as early as the first passage. Still, at least one tumor-rich condition was identified in each attempt. Additionally, media conditioned by long-term desmoid models exhibited a trend towards preservation of tumor cells, supporting our hypothesis.

To resolve relationships between media components and specific cell types during *ex vivo* propagation, we are also utilizing high-throughput targeted amplicon sequencing and transcriptomics in single cells to annotate gene expression with allelic information. In a preliminary experiment, we studied a mixture of *CTNNB1* T41A, S45F, and wildtype cells. Our method successfully resolved the genotype of individual cells and revealed differences in the relative abundance of transcriptional subpopulations between *CTNNB1* mutant and non-mutant mesenchymal cells. We aim to expand upon this preliminary study to produce a comprehensive single-cell genotype-annotated transcriptional landscape of desmoid tumor cells. The knowledge gained from this part of our work will help nominate *ex vivo* propagation strategies and support the discovery of biomarker-dependency relationships.

Our data to date demonstrate the feasibility of monitoring changes in tumor cell composition across multiple conditions. As we understand the cellular composition and timing of preservation of heterogeneity under specific media conditions, we see an opportunity to move the timing of drug and CRISPR-based perturbation within a few days of tumor sample acquisition, enabling a more physiologically relevant validation platform. Given the challenge of generating long-term desmoid tumor models, testing perturbations in early- or medium-term cultures for tumor-specific killing activity will also substantially expand the fraction of patient samples that can be interrogated for dependencies.