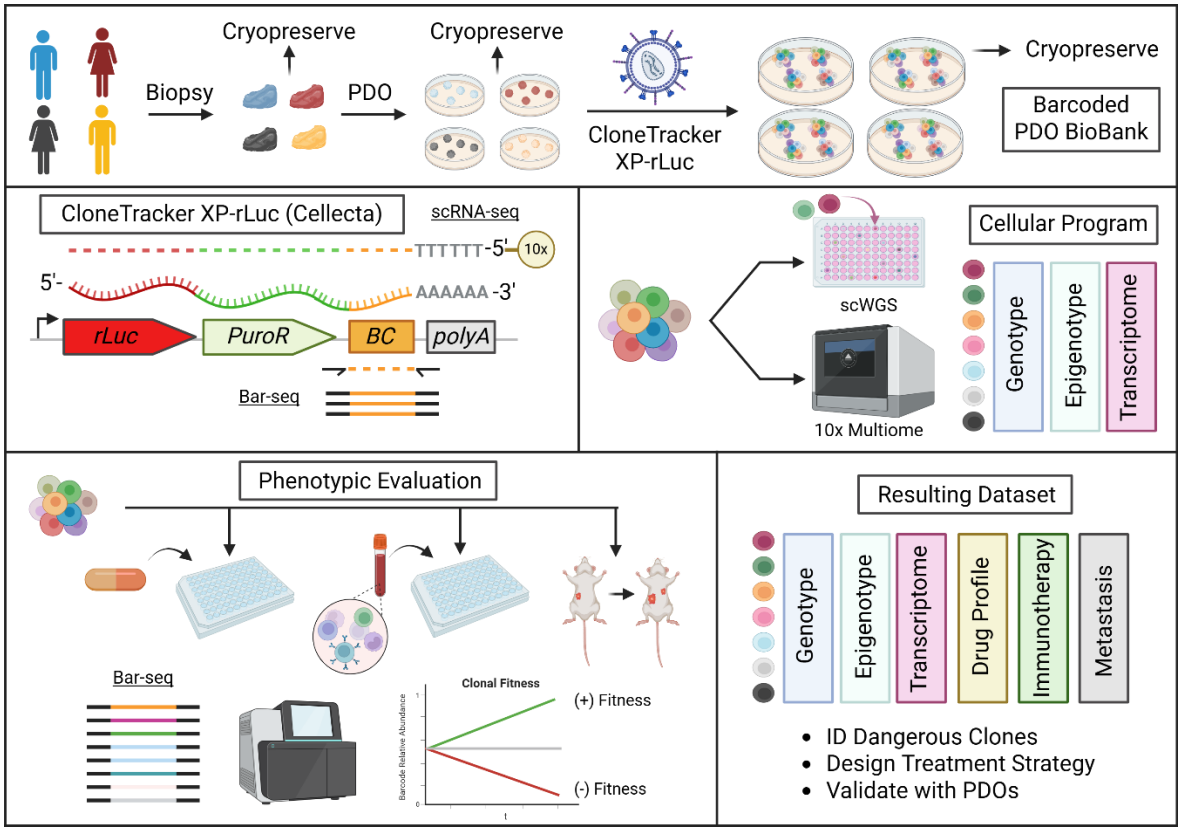
Targeting Distinctly Dangerous Subpopulations in Cancer

Cancer is a complex, living disease characterized by rapid growth and genome instability. This combination drives clonal evolution and intratumoral genetic, epigenetic and phenotypic heterogeneity. A tumor is thus a dynamic collage of clones, each of which has a distinct capacity for immune evasion, drug resistance and metastasis. Identifying and therapeutically targeting the most dangerous clones has the potential to improve patient outcomes.

This heterogeneity, combined with interpatient differences and both real and technical noise, makes finding these dangerous clones difficult. Therefore, we propose a systematic characterization of individual clones from several patient-derived organoids (PDOs). To do this, we will generate a barcoded PDO biobank and characterize each clone's genotype, epigenotype and gene expression profile. Then, we will assay each clones' treatment susceptibility profile and capacity for metastasis through barcode sequencing (Bar-seq). The resulting dataset will identify cellular programs (the interconnected layers of genotype, epigenotype and transcriptome) that lead to treatment failure and disease progression. Importantly, this dataset will allow us to rationally design treatment strategies against specific cellular programs and the PDO biobank will provide the means to develop and validate targeted therapies.



PDOs are 3D cultures derived from cancer stem cells (CSCs) that maintain the genetic, epigenetic and functional heterogeneity of the original tumor. PDOs are genetically stable in culture and have been shown to be highly predictive of treatment outcomes[1–5]. PDOs can be genetically modified[5], subjected to high-throughput drug screening[1–3], co-cultured with immune cells to assay immunotherapies[1,2] and engrafted in mice to study disease progression and metastasis[1,4].

Here, we will use hepatocellular carcinoma (HCC) as a proof of concept, but this approach can be amended for any carcinoma (and should be to identify cross-type mechanisms). HCC is a primary liver cancer that has both a high incidence and death rate, the latter in part due to a lack of known targetable mutations and late diagnosis. There are several studies that have successfully generated HCC-PDOs[6–8], but we will assume no PDOs are available to us.

First, tumor tissue will be dissociated to single cells and seeded on Matrigel, a basement membrane matrix that serves as 3D scaffolding, then cultured to a diameter of ~75 μm^3. A fraction will be cryopreserved, and the rest re-dissociated and transduced with lentivirus packaged with CloneTracker XP-rLuc (Cellecta) vector. These vectors tag cells with a unique barcode and a red-shifted luciferase (rLuc) reporter gene. The barcodes are expressed at the 3' end of the transcript, allowing them to be identified by single-cell RNAseq, and expression of rLuc can be visualized *in vivo*. As clones divide, the barcode is passed to daughter cells and because PDOs are populated by CSCs, we can track clonal differentiation lineages. After puromycin selection, aliquots of barcoded PDOs are cryopreserved and the rest are characterized by single-cell whole genome and 10x Multiome sequencing. This establishes each clone's cellular program: its genotype, epigenotype and gene expression profile.

The phenotypes of each clone can then be assayed through Bar-seq and aligned to cellular program. Aliquots of PDOs will be taken pre and post treatment for gDNA extraction and Bar-seq. The change in relative abundance of each barcode will equate to that clone's fitness under treatment. Fitness can be assayed under dozens of treatments simultaneously and adapted to immunotherapies by co-culturing PDOs with patient-matched PBMCs. Finally, disease progression and metastatic capacity can be assayed by engrafting barcoded PDOs *in vivo* and tracking barcode frequencies in metastases.

The resulting dataset including information regarding clonal genotype, epigenotype, gene expression profile, treatment susceptibility, immunotherapy susceptibility and metastatic capacity will be a gold mine for uncovering dangerous subpopulations in HCC. Not only will it identify the clones that are most likely to lead to treatment failure or metastasis, but it will also provide the data necessary to rationally develop treatment strategies to prevent these. Hits will be validated against published HCC datasets to ensure they are not technical artifacts. Ultimately, this platform could enable truly personalized therapies in which dangerous clones are targeted based on single-cell analysis of a patient's tumor.

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