Engineering patient-derived tumoroids to enable high-throughput screening: Immuno-oncology workflows

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Introduction

In recent years, cancer immunotherapy has become one of the fastest growing areas in cancer research. Selecting suitable and cost-effective experimental models for cancer therapeutic development and validation is one of the major obstacles researchers face today. To overcome this, patient-derived tumor models are of increasing interest because they can better recapitulate many of the properties and the heterogeneity exhibited by the tumor microenvironment at a relatively low cost. Hereby, we propose a next-generation screening platform for an effective and efficient evaluation of cancer immunotherapies in patient-derived tumor models.

Materials and methods

Establishing a Tumoroid Reporter Pool

Colorectal adenocarcinoma dissociated tumor cells were obtained from Discovery Life Science and cultured using the Gibco™ OncoPro™ Tumoroid Culture Medium Kit. Lentivirus was developed by transfecting the EF-1a _GFP_Blasticidin plasmid into HEK293-derived virus production cells using the Gibco™ LV-MAX™ Lentiviral Production System. Tumoroids were transduced at a MOI of 50 and subjected to Blasticidin selection to generate a reporter tumoroid cell pool.

Validation of Reporter Pool

Gene expression of the transduced reporter cell pool was characterized in both bulk population and single-cell profiles using the Ion AmpliSeq™ Transcriptome Human Gene Expression Kit to ensure the retention of the gene expression profile. Gene quantification and correlation analysis were performed using the Torrent™ suite software.

Preparation of Effector Cells

NK-92 (Natural Killer) cell line was purchased from ATCC and cultured in Gibco™ RPMI 1640 medium supplemented with 10% FBS, 10% horse serum and 500 U/mL rhIL-2. Human leukopaks were purchased from the San Diego Blood Bank and processed using the CTS™ Rotea™ Counterflow Centrifugation System. Primary NK cells were then isolated using the Dynabeads™ Untouched™ Human NK Cells Kit and expanded in the Gibco™ CTS™ NK-Xpander™ Medium supplemented with 15% hAB serum and 200 U/mL rhIL-2. Human induced pluripotent stem cell (hiPSC) differentiated NK cells were generated using a previously published method (Ref). These hiPSC differentiated NK cells were further expanded in the Gibco™ CTS™ NK-Xpander™ Medium supplemented with 10% hAB serum and 200 U/mL rhIL-2.

Effector-target Conjugation

Tumor reporter cells were seeded into 96-well microcavity plates 3 days prior to treatment to allow tumoroid formation. NK cells at different effector-to-target ratio were added to each well in a 1:1 mixture of NK cell media and tumoroid media containing 500 U/mL of rhIL-2 and 10μM Invitrogen™ CellEvent™ Caspase-3/7 Red Detection Reagent.

Cytotoxicity Assessment

The cytotoxicity of NK cells against tumor cells was accessed through green fluorescence protein (GFP) signal and the activation of Caspase-3/7 from the tumor reporter pool. Cytotoxicity was monitored over 72 hours using an Incucyte Live-Cell Analysis System. Cytokine release profiles were analyzed from the supernatant at 72 hours using the Immune Monitoring 65-Plex Human ProcartaPlex™ Panel.
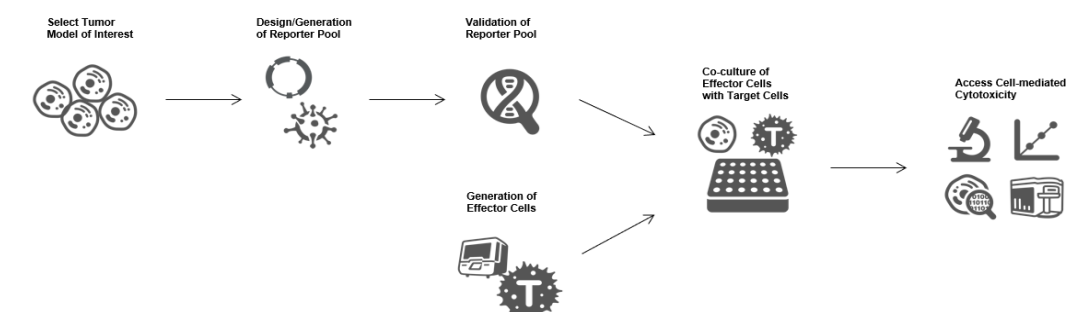

Figure 1. Standard high-throughput screening workflow. Schematic illustration of proposed Immuno-oncology screening workflow
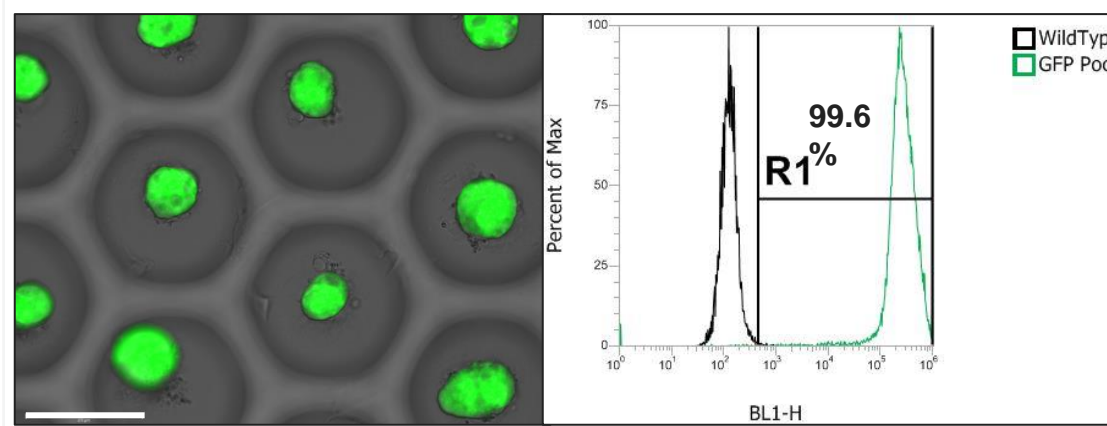
Results

Establishing a Tumoroid Reporter Pool

Figure 2. FACS analysis of antibiotic resistant stable pool. Transduced cells were analyzed on an Attune™ NxT flow cytometer. GFP signal was captured using the BL1 channel laser (excitation: 488nm, emission: 530/30nm). R1 represents GFP-positive cells. Scale bar represents 275μm.

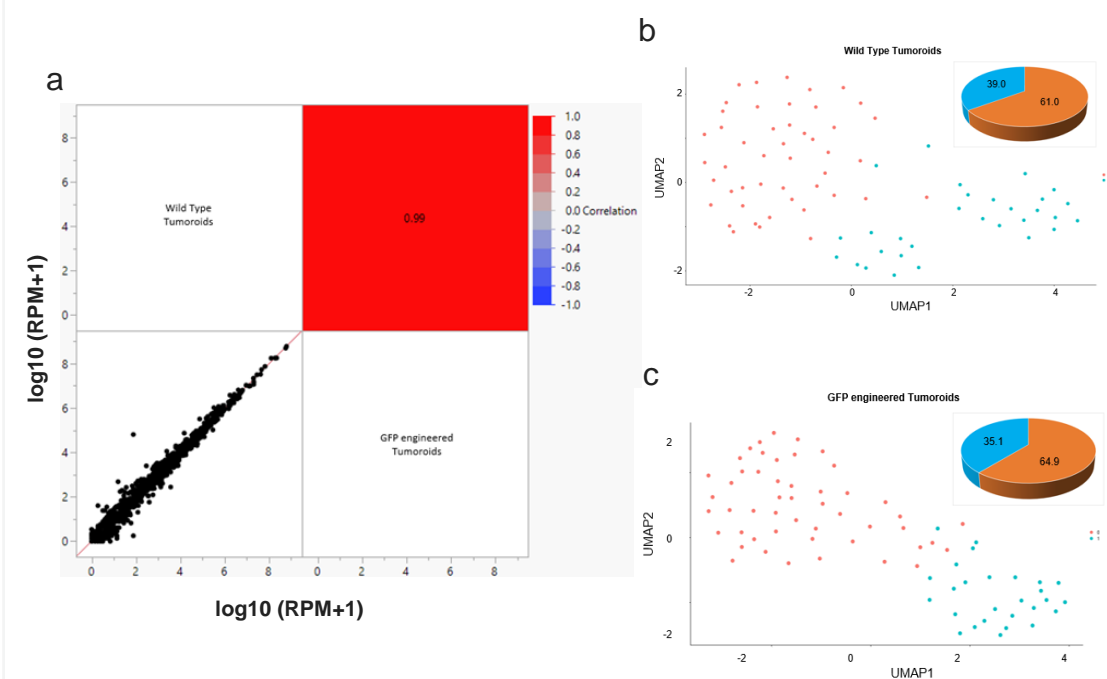### Transcriptome Profiling of the Tumoroid Reporter Pool

Figure 3. Bulk and single-cell transcriptomic profiling of transduced reporter pool. Gene expression in transduced and wild type tumoroids was measured using the Ion AmpliSeq™ Transcriptome Human Gene Expression Kit. a) Bulk gene expression and the correlation between transduced and wild type tumoroids were calculated using the Torrent™ suite software. Data are normalized and graphed in log10 (Reads per million +1) using JMP statistical software. b,c) Single-cell transcriptomes of transduced and wild type tumoroids are displayed by uniform manifold approximation and projection (UMAP) based on Seurat-based clustering. The pie chart shows the fraction of cells in the indicated cell subsets.
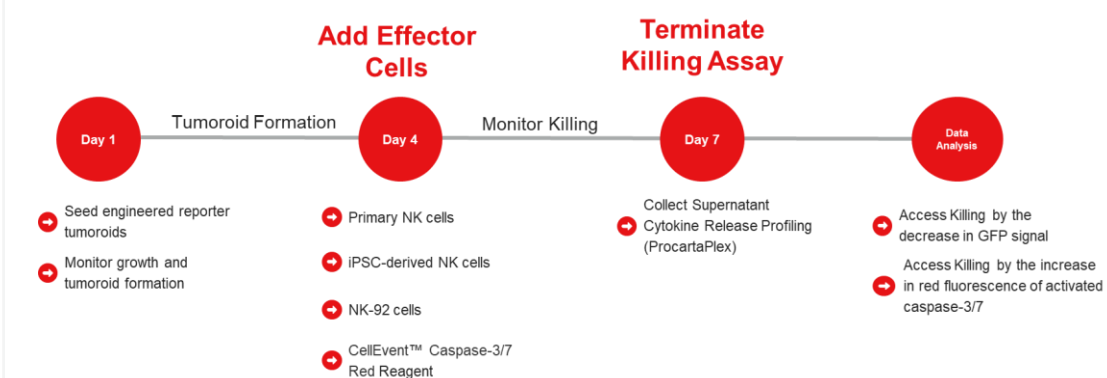
Killing Assay Workflow

Figure 4. Standard killing assay workflow using engineered reporter tumoroid pool.

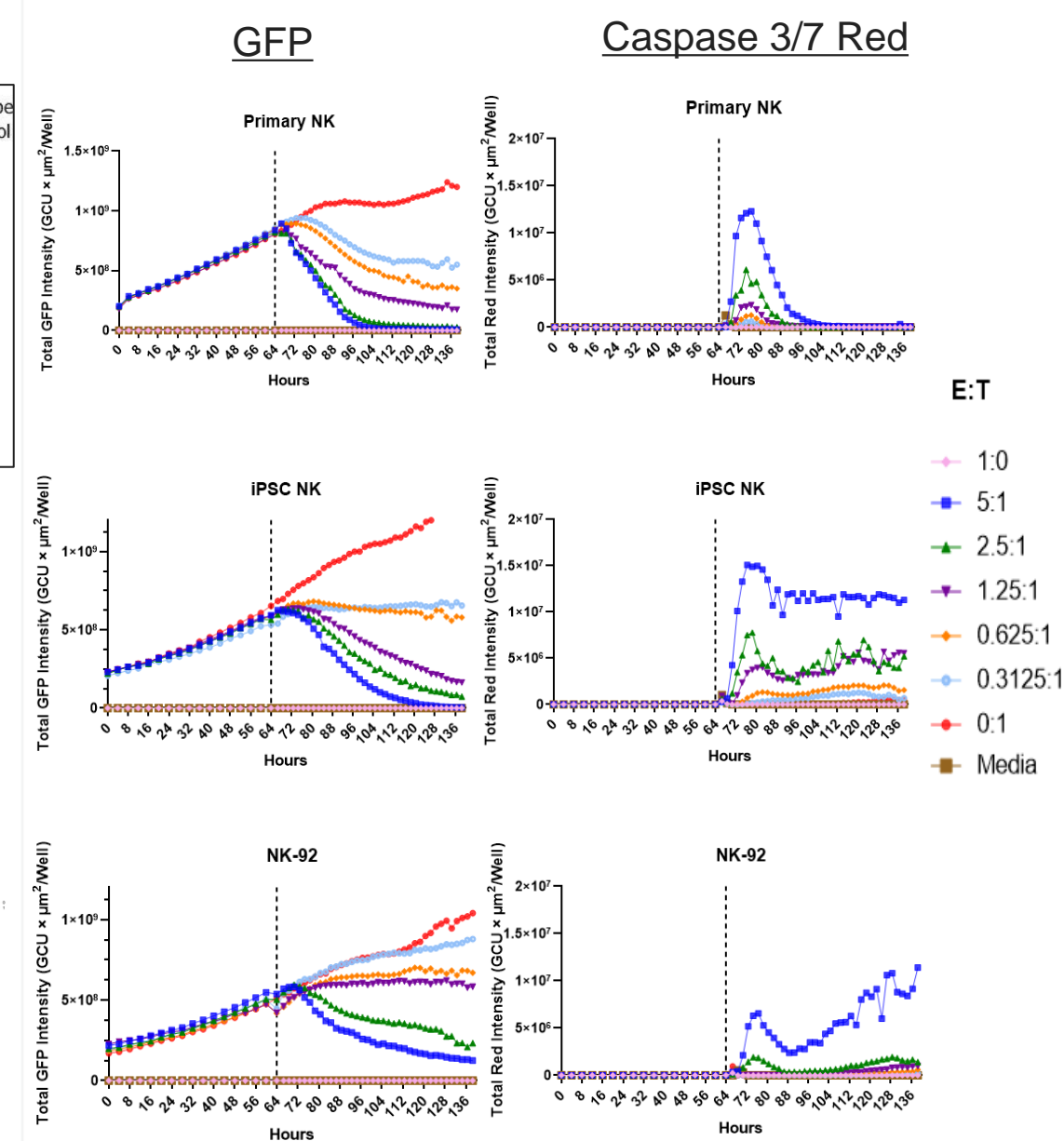### Real-time Cytotoxicity Assessment

Figure 5. NK-cell mediated cytotoxicity against engineered tumor reporter cells. Engineered tumor reporter cells were seeded into 96-well microcavity plates and monitored every 4 hours using the Incucyte. Three types of NK cells (Primary NK, iPSC-derived NK, and NK-92 cells) at different E to T ratio and 10μM CellEvent™ Caspase-3/7 Red Detection Reagent were added at day 4 upon tumoroid formation and monitored every 2 hours over 3 days. The intensity of GFP tumoroids and activated caspase3-7 red was captured and graphed as total intensity per well (GCU x μm² /well). Dotted line represents time of NK cell (effector cell) treatment.
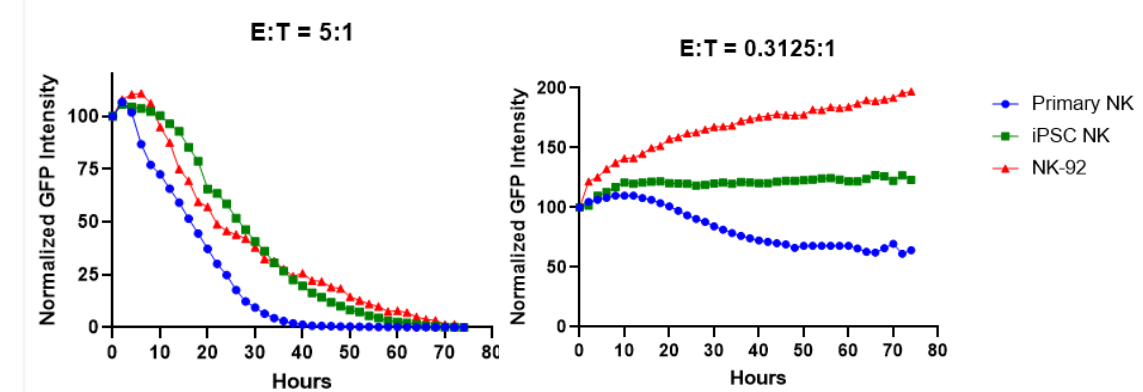

Figure 6. Comparison of the killing efficiency of different types of NK cells. Figure shows the normalized GFP intensity per well of the co-culture of NK cells (Primary NK, iPSC-derived NK, and NK-92 cells) and reporter tumoroids at E:T ratio of 5:1 and 0.3125:1.GFP intensity at each time point were normalized to the respective GFP intensity at time of NK cell treatment.
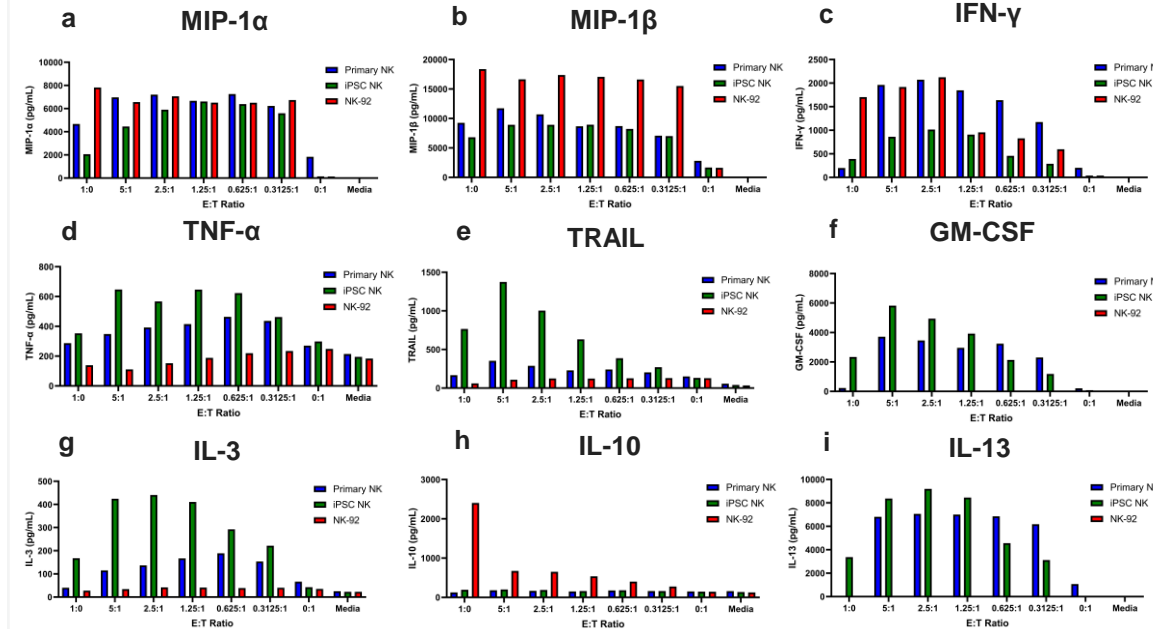
Cytokine Release Profiling

Figure 7a-i. Cytokine secretion accessed by Immune Monitoring 65-Plex Human ProcartaPlex™ Panel. Cytokine produced 3 days post NK cell-mediated tumor killing was detected using the Immune monitoring 65-plex Human ProcartaPlex™ Panel. Key cytokines involved in NK cell activation, suppression, and secretion at different effector to target ratios are listed.

Conclusion

- We have successfully established a next-generation cancer immunotherapeutic screening platform in patient-derived tumoroids. Utilizing this platform, researchers can design their own reporter tumoroid pool to meet their specific research needs

- The performance of the screening platform has been successfully demonstrated by the real-time assessment of three types of NK cell-mediated cytotoxicity in patient-derived dissociated colorectal adenocarcinoma tumor cells.

  - Target cell GFP intensity, activation of caspase-3/7 in apoptotic cells and cytokine profiling.

- Given the rapid progress cancer research, the proposed high-throughput screening platform will enable the development of more cancer therapeutics.

References/ Acknowledgement

1. Zhu, H., Kaufman, D.S. (2019). An Improved Method to Produce Clinical-Scale Natural Killer Cells from Human Pluripotent Stem Cells. In: Kaneko, S. (eds) In Vitro Differentiation of T-Cells. Methods in Molecular Biology, vol 2048. Humana, New York, NY. https://doi.org/10.1007/978-1-4939-9728-2_12

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