Leveraging single-cell RNA sequencing data for the discovery and prioritization of chimeric aptamer-siRNA SeekR™ targets.

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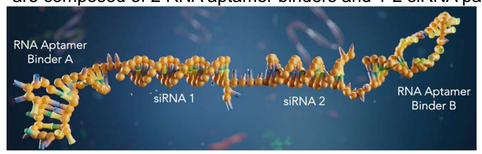
Abstract

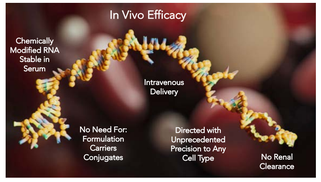
SeekR™ chimeric molecules consist of cancer-targeted aptamers directing a siRNA payload to a tumor. Aptamers are nucleic acid molecules that act much like antibodies, in that they can bind proteins in a sequence-specific manner. Combining RNA aptamers with siRNAs thus enables targeted delivery of the siRNAs to tumor cells, wherein it directs sequence-specific knockdown of cancer targets. To identify and prioritize SeekR™ targets, we have taken advantage of single-cell (sc) sequencing data. By analyzing scRNA-seq data, we can identify specific cell populations within tumors to co-target with an aptamer and siRNA. We collected publicly available colon cancer scRNA data sets for this analysis. A custom analysis pipeline was applied to these data to identify specific cell types within tumor samples. We then profiled specific cancer targets and determined percentages of target co-expression within a single tumor as compared to normal cells. This provides knowledge for tumor cell-type-specific targeting to reduce potential toxicity within non-tumor tissue. In addition to aptamer targeting, we performed ligand-receptor analyses to prioritize specific siRNA targets in specific cell types within the tumor environment. In summary, scRNA seq data provides a rich resource of data to direct SeekR™ development and optimization.

SeekR™ Therapeutic Platform

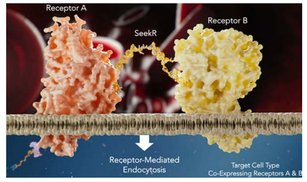
SeekR™ are composed of 2 RNA aptamer binders and 1-2 siRNA payloads



Unique Benefits and Properties of SeekR™ Therapeutics



SeekR™ Binding and Endocytosis



Initial SeekR™ Therapeutic Constructs

CANCER CELL DIRECT LETHALITY
COLON CANCER
LUNG/BREAST CANCER

T-CELL DIRECTED
CANCER IMMUNO-THERAPY
CLINICAL GOAL – OVERCOME IO RESISTANCE

SeekR™ RNA Aptamer Targets and Affinity

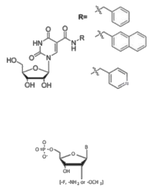
Cancer Cell Directed

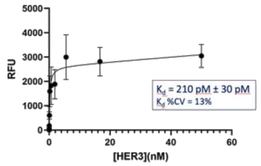
HER2/3
TROP2
PSMA
EpCAM

T-cell Directed

PD-1
CTLA4
CD73
LAG3

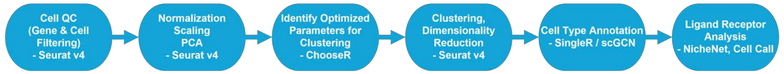
Targets First 4 of 12	Preliminary K_d Data High-performance RNA Aptamer Affinity
HER3	210pM
PSMA	5.7nM
TROP2	550pM
CD73	400pM





Single Cell Sequencing Workflow

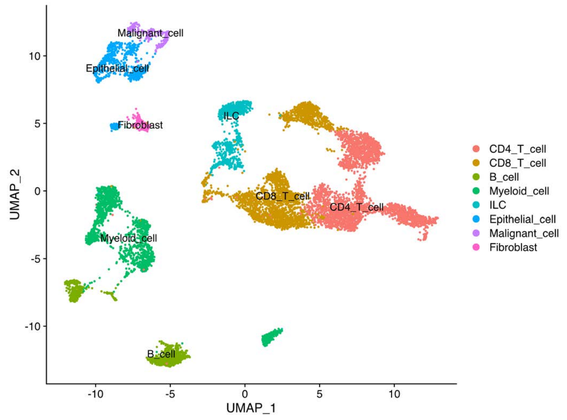
Methods and Steps in Single Cell Sequencing Analysis



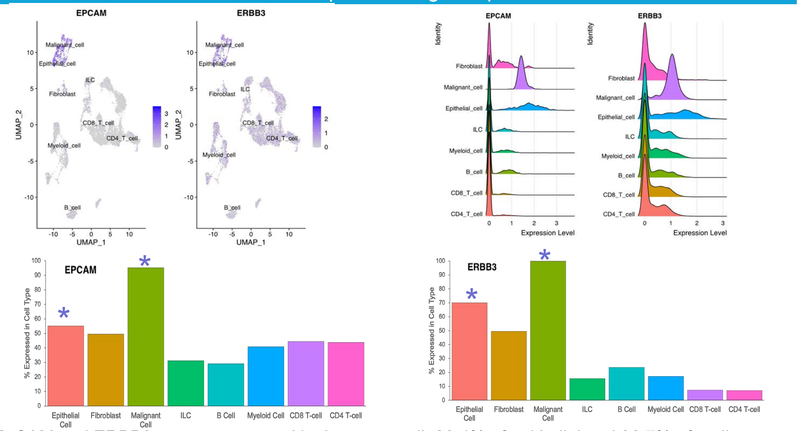
Colon cancer single-cell data set GSE146771 was used in this analysis

Zhang L, Li Z, Skrzypczynska KM, Fang Q, Zhang W, O'Brien SA, et al. Single-Cell Analyses Inform Mechanisms of Myeloid-Targeted Therapies in Colon Cancer. Cell. 2020;181:442-459.e29.

Colon Cancer Cell Type Annotation

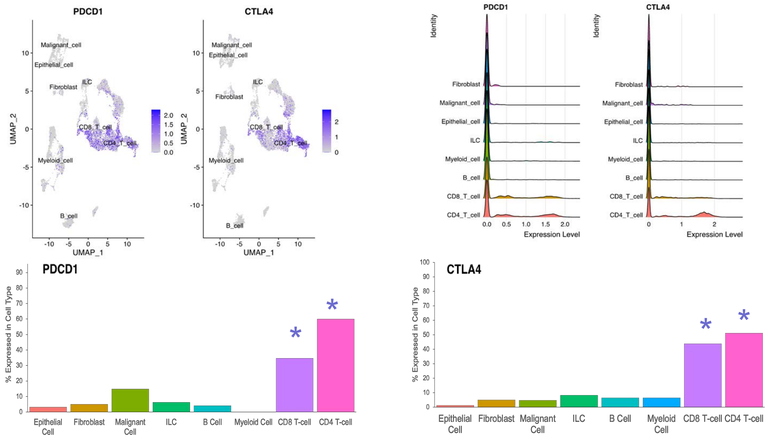


Cancer Cell Aptamer Target Expression



EpCAM and ERBB3 are co-expressed in the same cell. 39.4% of epithelial and 96.7% of malignant cells.

T-cell Directed Aptamer Target Expression



PDCD1 and CTLA4 are expressed in CD8+ and CD4+ T-cells within the tumor but at low levels.

Future Work & Conclusions Ligand Receptor Analysis

- Ligand Receptor analysis is being performed to identify prioritized surface targets on specified cell types in tumors.
- scRNA sequencing data can be used:
  - characterize aptamer target expression within specific cell types within a tumor.
  - optimize selection of aptamer targets for SeekR™ with high selectivity and specificity