Case Study of Single-cell Protein Activity Based Drug Prediction for Precision Treatment of Cholangiocarcinoma

Aleksandar Obradovic[1,2,*], Lorenzo Tomassoni[2,*], Daoqi Yu[3,*], Elise Fraser[3], Susan Bates[4,5], Charles G. Drake[1,3,4,5,6,7], Yvonne Saenger[4,5], Filemon dela Cruz[3], Andrew Kung[3], Andrea Califano[2,4,5,8,9,10]

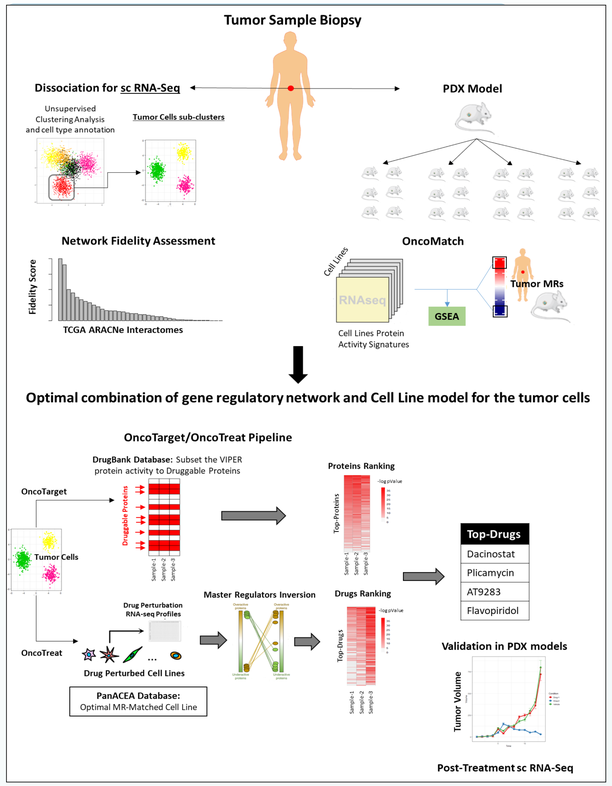
.*These authors contributed equally

1.Columbia Center for Translational Immunology, Irving Medical Center; 2.Department of Systems Biology, Columbia University Irving Medical Center; 3.Department of Pediatrics, Memorial Sloan Kettering Cancer Center; 4.Department of Medicine, Columbia University Irving Medical Center; 5.Herbert Irving Comprehensive Cancer Center, Columbia University Irving Medical Center; 6.Department of Urology, Columbia University Irving Medical Center; 7.Current address, Janssen Research and Development, Springhouse, PA, USA; 8.Department of Biochemistry & Molecular Biophysics, Columbia University Irving Medical Center; 9.Department of Biomedical Informatics, Columbia University Irving Medical Center; 10.J.P. Sulzberger Columbia Genome Center, Columbia University

Background and Aim

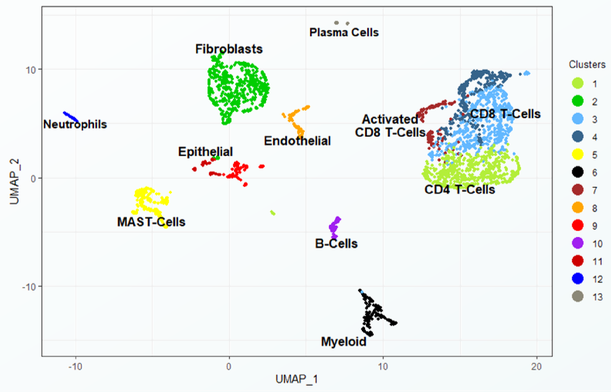
Cholangiocarcinoma (CCA) is an aggressive biliary adenocarcinoma with a median survival of only 12-37 months[1]. Since no therapeutic strategies have been successfully identified so far, we are proposing a novel and highly flexible computational precision medicine pipeline that leverages single-cell RNA-Seq (scRNA-Seq) data for the identification of effective drugs to treat rare heterogeneous tumor, like CCA.

Methodology

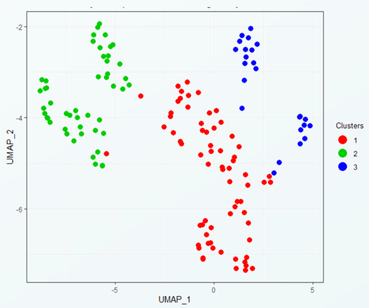


Results

1 The Cholangiocarcinoma Tumor Micro-Environment is highly Immune-infiltrated: VIPER-measured protein activity[2] of scRNA-Seq data from the tumor patient biopsy identified 13 different clusters. Tumor-Infiltrating T-cells comprise the largest cluster (54.3% of all cells). T-cells were further stratified into cytotoxic CD8 T-cells, CD4 T-cells and activated CD4 T-cells.

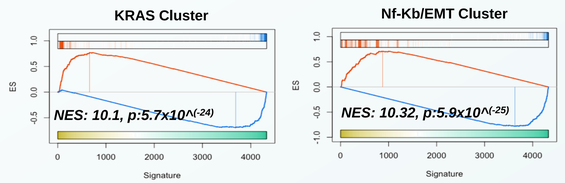


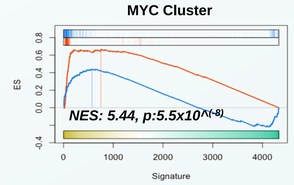
2 Protein activity identifies molecularly-distinct, coexisting tumor cell subpopulations: Activity-based analysis of tumor epithelial cells revealed three molecularly distinct subpopulations whose aberrantly activated proteins are enriched in different pathways.



Pathways	
C1	• Estrogen Response • KRAS Signaling
C2	• TNK-alpha Signaling via NF-kb • Epitheliel to Mesenchymal Transition
C3	• MYC Targets

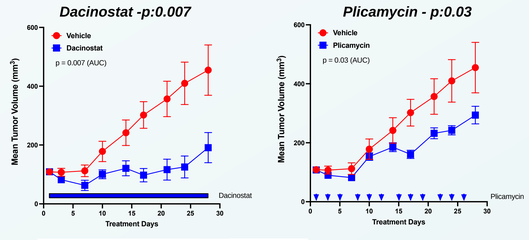
3 Tumor subpopulations are recapitulated by the PDX Master Regulators: VIPER analysis of PDX bulk-tissue RNA-Seq showed significant enrichment of the differentially activate proteins identified from the three single-cell tumor sub-clusters. As expected, enrichment was higher in the two larger clusters, C_1 and C_2, and lower for the smaller one, C_3

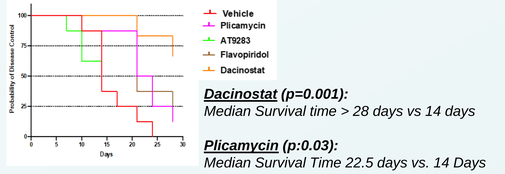




4 OncoTarget[3] and OncoTreat[4] drug prediction results: top druggable proteins predicted by OncoTarget and top-ranked drugs predicted by OncoTreat to revert tumor MRs. Most right column of both heatmaps show predictions on bulk PDX RNA-Seq data.

5 Drug Validation: Plicamycin, AT9283, Dacinostat and Flavopiridol were selected for *in vivo* validation and administered to low passage PDX models. Dacinostat and Plicamycin significantly reduced tumor growth rate, with Dacinostat stabilizing disease over 28 days of treatment. Both drugs also significantly extended overall survival, compared to vehicle control, based on Kaplan-Meier regression.

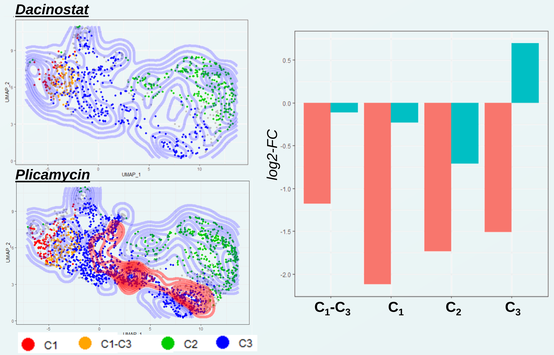




***Dacinostat* (p=0.001):**
Median Survival time > 28 days vs 14 days

***Plicamycin* (p:0.03):**
Median Survival Time 22.5 days vs. 14 Days

6 Post-Treatment scRNA-Seq: Coherently with drug sensitivities predicted by OncoTreat, sc analysis of Dacinostat-treated PDX confirmed the ability of the drug to deplete all tumor sub-populations. Plicamycin-treated tumor showed significant expansion of C_3



References

1. Dhanasekaran R, Hemming AW, Zendejas I, et al: Treatment outcomes and prognostic factors of intrahepatic cholangiocarcinoma. Oncology reports 29:1259-1267, 2013;
2. Alvarez MJ, Shen Y, Giorgi FM, et al: Functional characterization of somatic mutations in cancer using network-based inference of protein activity. Nature genetics 48:838-847, 2016;
3. Mundi, Prabhjot S., et al. "Pre-clinical validation of an RNA-based precision oncology platform for patient-therapy alignment in a diverse set of human malignancies resistant to standard treatments." *bioRxiv* (2021);
4. Alvarez, Mariano J., et al. "A precision oncology approach to the pharmacological targeting of mechanistic dependencies in neuroendocrine tumors." *Nature genetics* 50.7 (2018): 979-989.