Leveraging Artificial Intelligence to Advance Immuno-oncology Drug Development Using Functional Ex Vivo 3D-tumor Organoid Platforms of Fresh Patient Samples

Vijayendra Agrawal Ph.D.[1], Mibel Pabon, Ph.D.[1], Tina Pastoor, M.S[1] , Jenny Kreahling, Ph.D.[1] and Soner Altiok, M.D., Ph.D.[1]

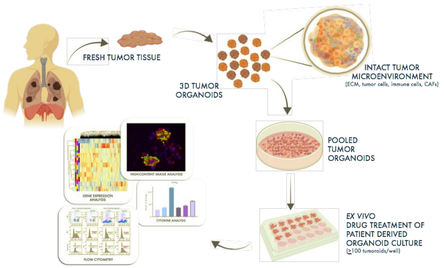
[1] Nilogen Oncosystems Tampa FL 33612

Introduction

Traditionally, omics data analysis of biologic samples has been conducted in a single-level manner. However, integrative analysis offers an effective way to harness strength across multi-level omics data and can be more powerful than single level analysis. In recent years we have developed complementary 3D *ex vivo* platforms utilizing unpropagated fresh patient tumor organoids with intact microenvironment to interrogate the efficacy of immuno-oncology drug and drug combinations. Here we aimed to develop a multiomic data integration and informatics platform to better understand the cellular and molecular mechanisms of pembrolizumab (Keytruda) and its mode of action.

Materials and Methods

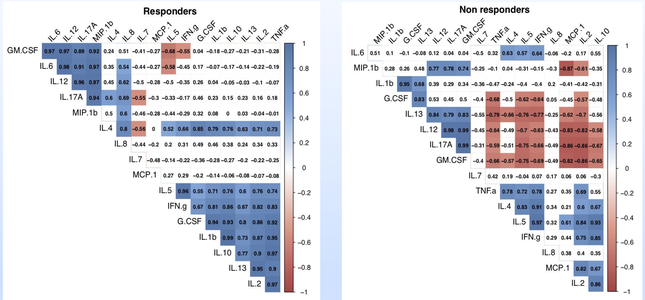
For the 3D-EX platform, tumor organoids were processed from fresh tumor tissue from NSCLC cancer patients obtained with IRB consent. Tumor organoids were then treated with pembrolizumab ex-vivo and treatment-mediated changes in tumor immune microenvironment were analyzed using multicolor flow cytometric analysis, multiplex cytokine assay as well as gene expression profiling by NanoString's 770 gene Immune Panel.



Results

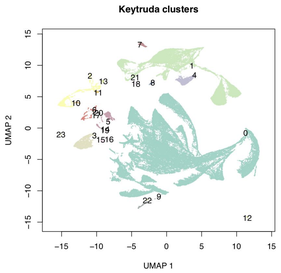
Here we present the results from the integrative analyses of multiomic data generated from the ex vivo treatment of 11 fresh tumor samples in the ex vivo assays. We probed multiple biological layers in parallel, including transcriptome, proteome, and phenome profiling.

Revealing correlations of cytokines in pembrolizumab responders vs non-responders



Pearson correlations of cytokines concentrations, across treatment and control samples, in pembrolizumab responders (left) and non-responders (right). Blue: significant positive correlation. Red: significant negative correlations. White: non-significant correlations. Some cytokines are highly mutually correlated; for example GM-CSF, IL12, IL17A and MIP-1β are highly correlated in both sets.

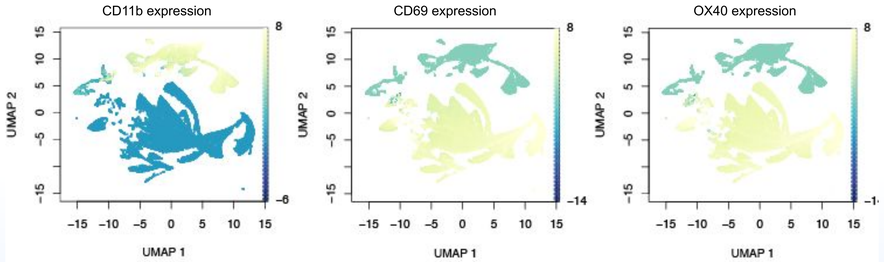
Flow cytometry suggests candidate markers of pembrolizumab response vs non-response



Our panel of flow markers identifies different cell populations in pembrolizumab-treated samples with distinct expression profiles. This is a two dimensional projection of a high dimensional space in which each cell is represented by a single point, and clusters of cells represent cells with similar expression profiles across the panel of flow markers we used. Clusters were identified with a k-nearest neighbors approach employing post-clustering to identify modules / communities, which represent the final cell-to-cluster assignments. Marker enrichment per cluster was then determined by selecting the top and bottom 5% from a scaled expression range of -1 to +1.

Marker	AUC	% cells responders	% cells non-responders
CD69	0.80	99	40
ICOS	0.78	99	40
CD11b	0.22	0	58
OX40	0.77	99	40

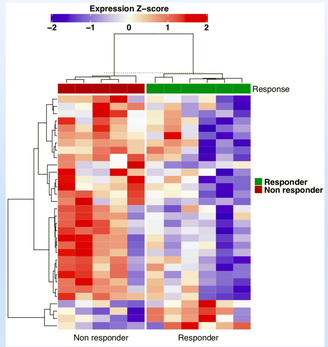
Pembrolizumab responders express higher levels of CD69, OX40, and ICOS—and lower levels of CD11b—than non-responders. ROC analysis was conducted by comparing responders versus non-responders in drug-treated cells.



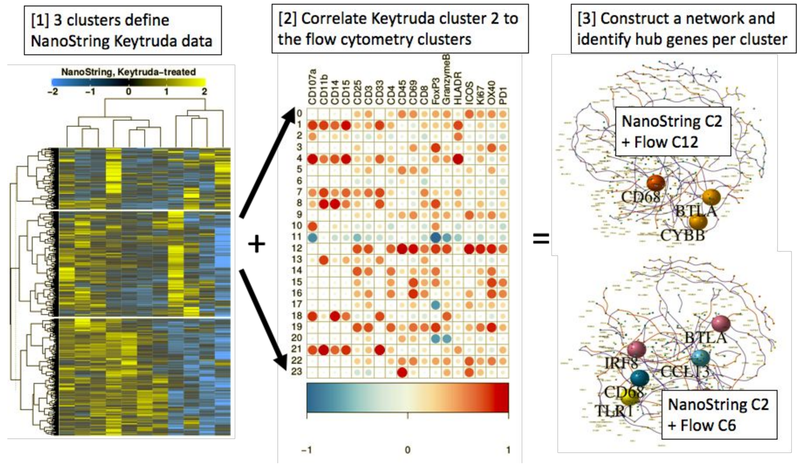
Markers from unbiased clustering of flow cytometry data in pembrolizumab-treated samples

Cluster	Number cells	Percent cells	Flow markers
0	64158	63.71	-
1	23789	23.62	ICOS+ CD11b-CD107a-CD33-CD15-
2	3908	3.88	CD15+ OX40-CD69-
3	2393	2.38	HLADR+ PD1-CD8-
4	1883	1.87	FoxP3+ CD11b-CD3-CD107a-CD14-CD33-CD15-
5	1329	1.32	CD107a+HLADR+ OX40-CD69-
6	1075	1.07	CD45+ CD11b-CD107a-CD33-CD15-FoxP3-
7	556	0.55	ICOS+CD45+ CD8-
8	296	0.29	CD11b+CD33+ OX40-CD69-
9	286	0.28	CD11b+CD14+ OX40-CD69-
10	241	0.24	ICOS+ CD11b-CD107a-CD33-CD15-
11	179	0.18	CD107a+ HLADR-PD1-CD4-
12	150	0.15	CD11b+CD45+ FoxP3-
13	123	0.12	CD45+ CD11b-CD107a-CD14-CD33-CD15-
14	105	0.10	CD11b+ OX40-CD69-
15	63	0.06	CD25+ CD11b-CD107a-CD33-CD15-
16	46	0.05	OX40+CD69+ CD3-
17	26	0.03	CD69+ CD11b-CD107a-CD14-CD33-CD15-
18	24	0.02	ICOS+ FoxP3-
19	24	0.02	CD14+ CD8-CD25-OX40-CD69-
20	14	0.01	FoxP3+OX40+ CD11b-CD107a-CD14-CD33-CD15-
21	12	0.01	CD45+ GranzymeB-FoxP3-
22	12	0.01	CD11b+CD33+ OX40-CD69-
23	10	0.01	ICOS+ CD11b-CD107a-CD33-CD15-

Gene expression differences between responders & non-responders in NanoString data



Network analysis of NanoString gene expression analysis + correlation with flow cytometry clusters reveals key hub genes as candidate regulators



Integrating NanoString with flow cytometry expression data reveals key hub genes that may mediate pembrolizumab activity. K-means clustering was performed on NanoString gene expression data from pembrolizumab-treated samples, specifying 3 centers. The resulting expression profile for each cluster was then correlated to each flow cytometry cluster identified also in pembrolizumab-treated samples. A co-expression network was constructed for each correlation and key hub genes identified by hub score > 0.4. CD68 and IRF8 dominate NanoString cluster 2 correlations, whereas clusters 1 and 3 are dominated by PLA2G2A+MAGEC2 and IFNGR2+ICAM1 hub genes, respectively.

Discussion

- Integrated OMICS analysis revealed distinct molecular signatures of pembrolizumab responsiveness at the gene expression and protein levels.
- Cytokine profiling demonstrated that GM-CSF, IL12, IL17A and MIP-1β are highly correlated in the ex vivo studies.
- CD68 was a hub gene in a gene coexpression network associated with flow cytometry cluster, enriched for CD69, ICOS, and OX40, the markers whose presence on flow cytometry best identified pembrolizumab responders
- Our data points towards the role of CD68+ macrophages and myeloid cells in response to pembrolizumab treatment

Conclusion

Our results demonstrated that multiple types of omics data obtained from Nilogen's comprehensive 3D ex vivo drug testing platforms can reveal cellular mechanisms that are active in individual tumors and may classify them into subtypes for response to drug treatment. As such, implementation of the functional 3D ex vivo assay combined with integrative multiomic data in clinical studies may enable us to ascertain which components of the tumor immune microenvironment to target within a patient. We will use these data to develop machine learning approaches to integrate complex data generated with Nilogen's 3D platforms to inform on drug mode of action, to develop rational drug combinations, and for biomarker discovery for patient stratification in clinical trials.