Single Cell Analysis Shows That Reovirus Immune Priming Changes the Tumor Immune Micro Environment in Multiple Myeloma

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BACKGROUND

- Multiple myeloma (MM) is an incurable cancer characterized by clonal plasma cell proliferation in the bone marrow, accounting for approximately 10% of all hematologic malignancies.
- Recently, patients with relapsed or refractory disease have been treated with a combination of the oncolytic reovirus Pelareorep, bortezomib, and dexamethasone, which was well-tolerated and led to prolonged progression free survival of over 3 years in a subset of patients.
- To understand the complex tumor immune microenvironment (TIME) and immune response in patients before and after this treatment, we used imaging mass cytometry (IMC) to perform highly multiplexed, subcellular analysis of these patients' bone marrow samples.

METHODS

- We comprehensively characterized the changes in the MM TIME in pre and post bone marrow biopsy specimens taken from patients treated on a Phase 1b study with a combination of Pelareorep, bortezomib, and dexamethasone.
- For analysis with IMC, a marker panel of 35 antibodies was assembled to interrogate the various immune subsets of the bone marrow biopsies; each of these antibodies were conjugated to a unique metal isotope.
- The panel was validated with various positive control tissues including bone marrow, tonsil, lymph nodes, and spleen. Image visualization was performed using MCD Viewer from Fluidigm.
- After validation, the antibody cocktail was used to stain the bone marrow biopsies. MCD Viewer was used again to check the quality of the staining before moving on to the downstream analysis.
- Pixel-based classification was performed in Ilastik to generate the cell probability mask for each of the samples. These probability masks are then processed in Cellprofiler. The segmented samples were then imported into HistoCAT where the unsupervised clustering algorithm, PhenoGraph, was run to identify the unique phenotypes in the cohort.
- The data was then exported into Rstudio to perform more complex analyses such as: expression normalization, sub-phenotyping, t-stochastic neighborhood embedding (tSNE) plot generation, and nearest neighbor analyses. ImaCytE was used to generate representative images that highlight the phenotypes on the probability mask in addition to further spatial analysis.

RESULTS

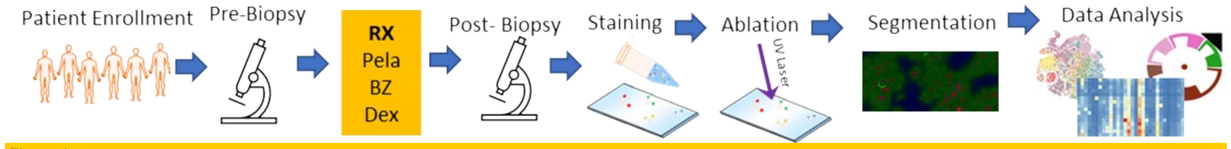

Figure 1

Study treatment and analysis schema. Prior to treatment, bone marrow biopsies are collected from each patient. Patients are then treated with a combination of Pelareorep, bortezomib, and dexamethasone. Post-treatment biopsies are then collected from each patient. Each bone marrow biopsy is stained with the antibody cocktail and ablated using the Hyperion™ Imaging System. The raw data is then visualized with HistoCAT++ and segmented in Ilastik using pixel-classification. Segmented data is then used in downstream analysis with HistoCAT and Rstudio.

NK Cells and T Cells		
Nd143	NKG2A	NK Cells
Nd146	CD16	NK / Myeloid Cells
Er166	NKp46	NK Cells
Nd148	NKG2D	NK Cells
Gd156	CD4	T Helper Cells
Dy162	CD8a	Cytotoxic T Cells
Er167	Granzyme	NK / T Cells
Er170	CD3	T Cells
Myeloma		
Pr141	MUM1	MM
Gd160	Reolysin	Oncolytic Virus
Eu151	CD138	MM, NK, & MDSCs
Other Immune		
Sm149	CD11b	MDSC
Nd144	CD14	Monocytes
Nd150	CD68	Macrophages
Gd155	CD163	M2 Macrophages
Dy161	CD31	Vascular, Megakaryocytes
Dy164	CD15	Granulocyte
Sm147	CD20	B Cells

Other		
Er168	Ki-67	Proliferation
Yb171	Histone3	Nucleus
Lu175	Caspase-3	Apoptosis
Ir191	Nucleic Acid	
Ir193	Nucleic Acid	
Checkpoint/Immune Regulators		
Eu153	LAG3	Checkpoint
Sm154	Tim-3	Checkpoint
Tb159	PD-L1	Checkpoint
Ho165	PD-1	Checkpoint
Yb172	HLA-ABC	Immune Regulator
Yb173	IDO	Checkpoint
Yb174	HLA-DR	Immune Regulator
Sm152	HLA-E	Tumor
Yb176	ULBP256	NK Ligand

Figure 2

Custom IMC panel composed of 32 metal conjugated antibodies. This shows each of the antibodies in their general category as well as metal isotope they are conjugated to and their target.

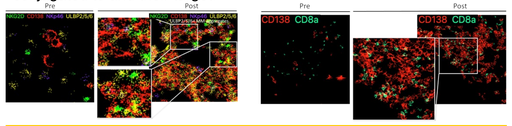

Figure 3

Visualization of raw IMC expression data for MM10 shows infiltration of NK cells and CTLs into tumor. Markers used in left panel: NKG2D/NKp46 to identify NK cells, CD138 to identify tumor, ULBP 2/5/6 to identify NK ligand expressed on tumor. Markers used in right panel: CD138 to identify tumor, CD8a to identify CTLs.

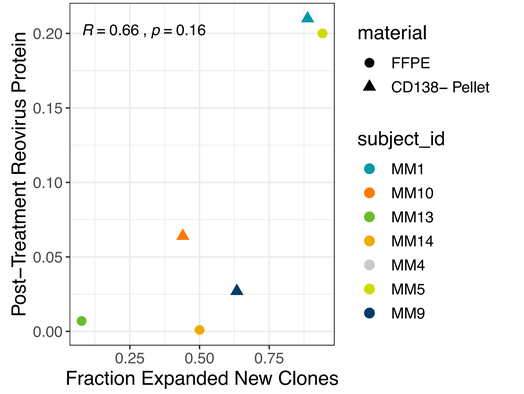

Figure 4

T cell clonality assay showing varying levels of clonal expansion across the cohort. Patient to note here is MM10.

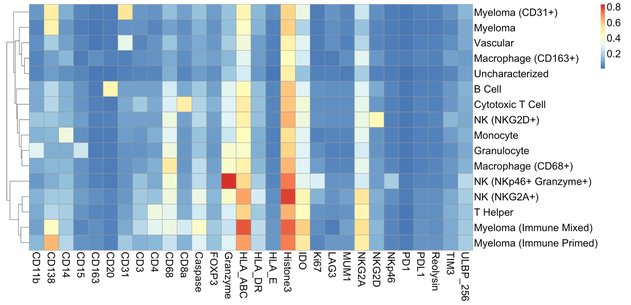

Figure 5

Expression heatmap of phenotypes identified across all samples after performing pixel-classification segmentation in Ilastik and running the PhenoGraph unsupervised clustering algorithm in HistoCAT.

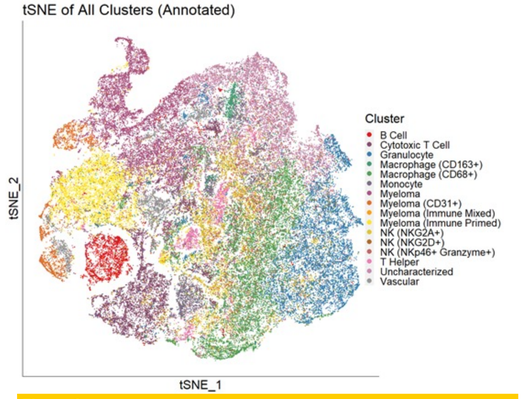

Figure 6

tSNE of all clusters generated by PhenoGraph

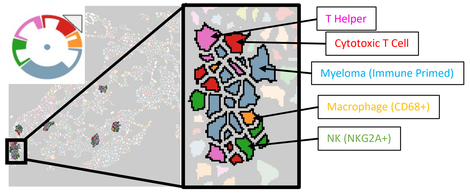

Figure 7

ImaCytE spatial analysis showing the various immune cells surrounding the immune primed myeloma

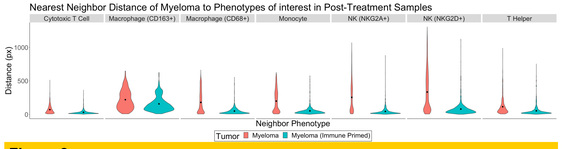

Figure 8

Nearest neighbor analysis performed in R showing immune phenotypes distributed more closely to immune primed MM than regular MM in post-treatment samples

CONCLUSIONS

- IMC allows us to analyze the potent immune response and cellular interactions in the tumor microenvironment in multiple myeloma treated with Pelareorep and Bortezomib.
- Characterization of these complex interactions allows for a deeper understanding of the key mechanisms of action of these treatments and planning of future combination studies.