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Discovery of predictive biomarkers of response to T cell-targeting biologics using *ex vivo* single-cell profiling coupled with TCR clonotype characterization

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HiFiBiO THERAPEUTICS

SUMMARY

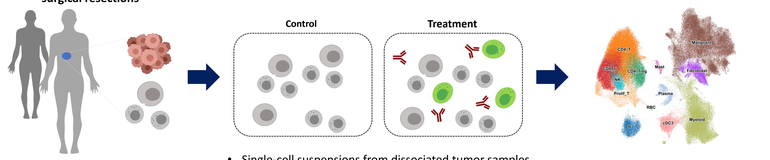
The discovery of predictive biomarkers of drug response is critical for forecasting patient benefit from novel immune-modulatory therapeutics. However, such discovery is challenging due to the heterogenous nature of the tumor microenvironment (TME) as well as the lack of an approach to analyze drug-responsive immune cells that impact tumor progression.

We developed a novel Drug Intelligent Science (DIS™) approach to define biomarkers of response by subjecting dissociated human tumor tissues to drug treatments, followed by single-cell transcriptomic profiling and TCR clonotype characterization. Responding T cells are identified as those showing shifts in gene expression consistent with known T cell activation signatures. TCR clonotypes can be used to match responding T cells in treatment conditions to their sister clones in the baseline state. Comparing the baseline gene expression profiles between T cell clonotypes that responded to the treatment and those that did not makes possible the discovery of gene expression signatures that predict response (Lee et al.).

We processed 17 tumor samples obtained from cancer patients and treated them *ex vivo* with two novel biologics currently under clinical development – HFB301001, a potentially best-in-class 2nd generation OX40 agonist, and HFB200301, a first-in-class TNFR2 agonist. We applied our biomarker discovery strategy to the integrated scRNA-seq and scTCR-seq data from these samples to define predictive signatures of response to these drugs. To further validate this strategy, we also generated single-cell data with our *ex vivo* culture system to characterize response to anti-PD-1 treatment. In the anti-PD-1 predictive response signature, we identified genes involved in inflammatory response and genes in the pathway of other co-inhibitory checkpoints. Application of the anti-PD-1 predictive response signature to bulk transcriptomic data from clinical studies with checkpoint inhibitors successfully stratified patients into two groups with significantly different risks of progression. The predictive response signatures for the two novel agonistic antibodies shared genes involved in inflammatory pathway with the anti-PD-1 signature, but also contained other distinct gene sets.

Ex vivo single-cell profiling coupled with TCR clonotype characterization enables the discovery of predictive response signatures that informs patient selection strategies for the early clinical development of novel therapeutics. These results suggest the potential for additional patient populations that may respond to these treatments.

EXPERIMENTAL SETUP



- Primary tumor samples obtained from Discovery Life Sciences and the Cooperative Human Tissue Network.
- A total of 17 tumor samples (8 RCC, 7 NSCLC, 1 MEL, and 1 UC) were selected to represent key indications for HFB200301 and HFB301001

- Single-cell suspensions from dissociated tumor samples
- Baseline flow cytometry characterization of immune cell composition and target expression
- *Ex vivo* culture of all cells, including tumor cells, without exogenous TCR cross-linking
- 24h *ex vivo* treatment with controls (isotype, αCD3, αPD1) and drug candidates (HFB200301 and HFB301001)

- HiFiBiO DIS™ scRNA-seq platform: 156,522 single cells

COMPUTATIONAL ANALYSIS WORKFLOW

Data preprocessing, batch correction, cell type identification

- Pipeline for read mapping and clonotype calling
- Data integration and batch correction using variational autoencoder (Lopez et al.)
- Automatic cell type classification based on transfer learning (Lotfollahi et al.) from internal reference atlas

Identification of responding TCR clonotypes

- Unbiased clustering of cell populations (Xu et al.)
- Characterize activation state of individual T cells based on published activation signatures (Szabo et al.)
- Identify cell clusters enriched in activated/responding T cells
- TCR clonotype barcode analysis: Identify responsive (R) versus non-responsive (NR) T cell clonotypes at baseline based on whether their sister clones were activated/responding in post-treatment samples.

Define predictive gene expression signatures

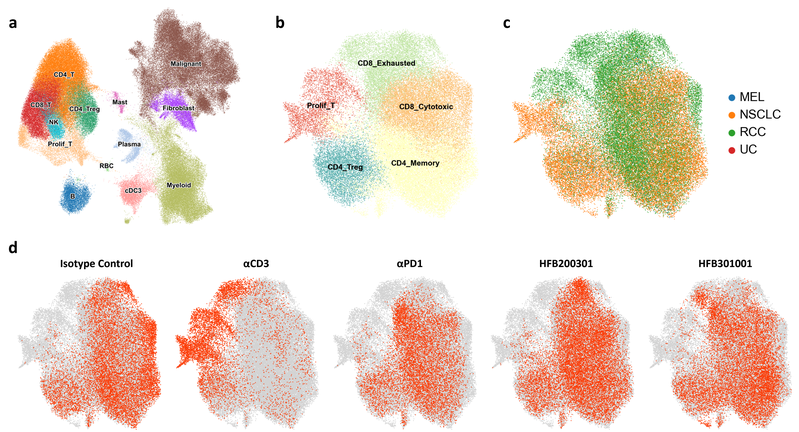
- Identify differentially expressed genes (Lopez et al.) between R and NR T cell clones at baseline
- Filter genes for specific expression in T cells for application to bulk RNA-seq data
- Compute logistic regression weights for each selected gene based on its z-scored gene expression

Validation against clinical data

- Project predictive signatures to published single-cell RNA-seq data with pre- and post-PD-1 treatment information
- Project predictive signatures to published bulk RNA-seq data from ICB clinical trials

RESULTS

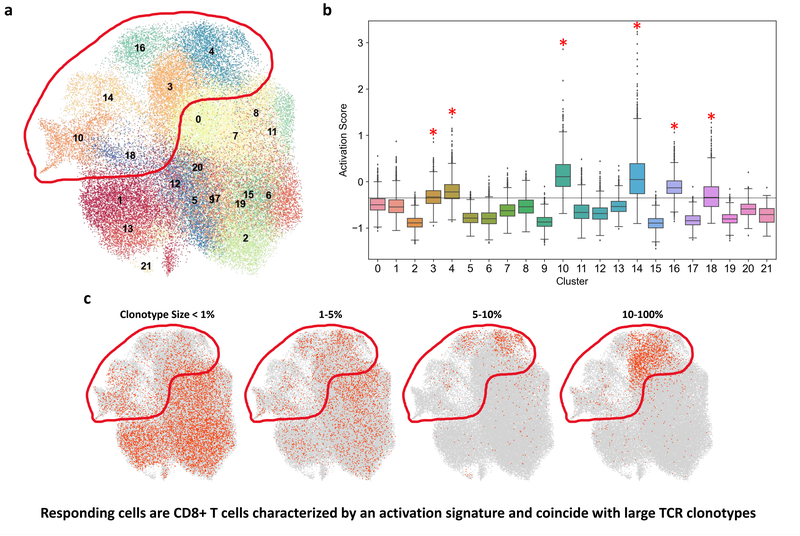
Single-cell profiling of T cells from *ex vivo* culture



Single-cell profiling from *ex vivo* culture captures T cell biology relevant to the TME and specific to treatment

Figure 1. Single-cell profiling of T cells from *ex vivo* culture. a, UMAP embedding of scRNA-seq transcriptomes of 156,522 cells that were treated with isotype control, αCD3, αPD1, HFB200301, or HFB301001 in *ex vivo* culture. Cell type label based on transfer learning from a scRNA-seq TME reference dataset (not shown) of 362,490 cells spanning 10 tumor indications. **b**, UMAP embedding of scRNA-seq transcriptomes of 54,215 T cells, a direct subset of the cells from **a**. Cell type identified. **c**, Identification of tumor indications on the same UMAP embedding as **b**. **d**, T cells from each treatment are colored red to show their relative distribution on the UMAP embedding.

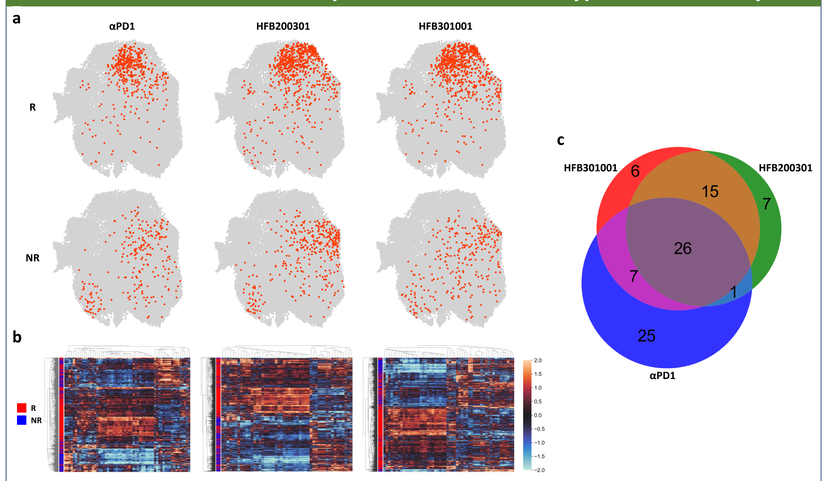
Response of single T cells to treatment



Responding cells are CD8+ T cells characterized by an activation signature and coincide with large TCR clonotypes

Figure 2. Characterization of T cells that responded to treatment *ex vivo*. a, UMAP embedding of scRNA-seq transcriptomes from T cells as seen in Figure 1b. Leiden clusters identified. Clusters 3, 4, 10, 14, 16, and 18 correspond to activated T cells and are circled in red. **b**, Box plot showing the level of expression of an activation gene signature, computed as an activation score, for each Leiden cluster. The activation score is a composite of published signatures that define primary human T cells activated in culture by αCD3 and αCD28 (Szabo et al.). Clusters with median activation score above that of Cluster 18, a group of αCD3-activated T cells, are considered activated and marked by an asterisk. **c**, Same UMAP embedding as **a**. T cells from clonotypes of various sizes are colored red to show their relative distribution on the UMAP embedding. Clonotype size is represented as a percentage of the total number of T cells with TCR reads from the originating sample. Activated T cell clusters are circled in red and coincide with large TCR clonotypes.

Predictive biomarkers of response from TCR clonotype barcode analysis



TCR clonotype barcode analysis defines αPD1, HFB200301, and HFB301001 biomarkers

Figure 3. Predictive biomarkers of response to αPD1, HFB200301, and HFB301001. a, UMAP embedding of T cells as seen in Figure 1b. Responsive (R) and non-responsive (NR) T cells a baseline for αPD1, HFB200301, and HFB301001 are colored red to show their relative distribution on the UMAP embedding. αPD1-treated T cells: R = 451, NR = 240. HFB200301-treated T cells: R = 735, NR = 355. HFB301001-treated T cells: R = 740, NR = 291. **b**, Heatmaps showing the log-normalized and z-scored expression of genes (columns) from the αPD1, HFB200301, and HFB301001 biomarkers generated from differential gene expression analysis of R versus NR cells (rows). **c**, Venn diagram showing the number of genes shared between the three biomarkers.

Validation of predictive biomarker of response to αPD1

Signature	IMvigor210 Atezolizumab	POPLAR Atezolizumab	IMmotion150 Atezolizumab	IMmotion150 Atezolizumab + Bevacizumab	POPLAR Docetaxel	IMmotion150 Sunitinib
HiFiBiO DIS™ (10 genes)	0.77*	0.44***	0.58*	0.51**	0.69	1.01
Merck signature (16 genes)	0.83	0.47**	0.65	0.48**	0.9	1.08
Ayers et al. (18 genes)	0.83	0.67	0.71	0.56*	0.88	0.96
Wu et al. (23 genes)	0.77*	0.69	0.56*	0.54*	0.76	0.95
CD274 (PD-L1)	0.86	0.96	0.73	0.77	1.26	1.07
PDCD1 (PD-1)	0.77*	0.58*	0.74	0.48**	1.20	1.29
CD8A	0.84	0.56*	0.77	0.52**	0.86	1.28
CD4	0.93	0.71	0.96	0.77	0.62*	1.01
FOXP3	0.76*	0.64*	0.90	0.83	1.02	1.27

*** p < 0.001, ** p < 0.01, * p < 0.05

αPD1 biomarker derived from *ex vivo* culture predicts ICB-specific patient survival in clinical trials

Figure 4. Validation of predictive biomarker of response to αPD1. For the specified trial arms of IMvigor210, POPLAR, and IMmotion150, our αPD1 biomarker, along with other published αPD1 biomarker signatures, were used to dichotomize patients by the median signature score. PFS hazard ratios were computed by fitting a Cox proportional-hazards model to the censored PFS survival times using the dichotomized variable.

CONCLUSION

We demonstrate our unique DIS™ approach using *ex vivo* culture coupled with scRNA-seq and scTCR-seq profiling that captures T cell biology relevant to the TME. We use TCR clonotype barcode analysis to extract predictive biomarkers of response to αPD1, HFB200301, and HFB301001. We validate the predictiveness of the αPD1 biomarker with bulk RNA-seq profiles from IMvigor210, POPLAR, and IMmotion150. As next steps, we will apply these signatures to bulk transcriptomic data from TCGA in order to stratify patients by predicted likelihood of response to individual or combination treatments. The HFB200301 and HFB301001 biomarkers will be validated in our Phase I clinical trials.

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