Development of enLIGHTEN™ Alpha-201 herpes simplex viral vectors encoding payloads targeting the tumor microenvironment

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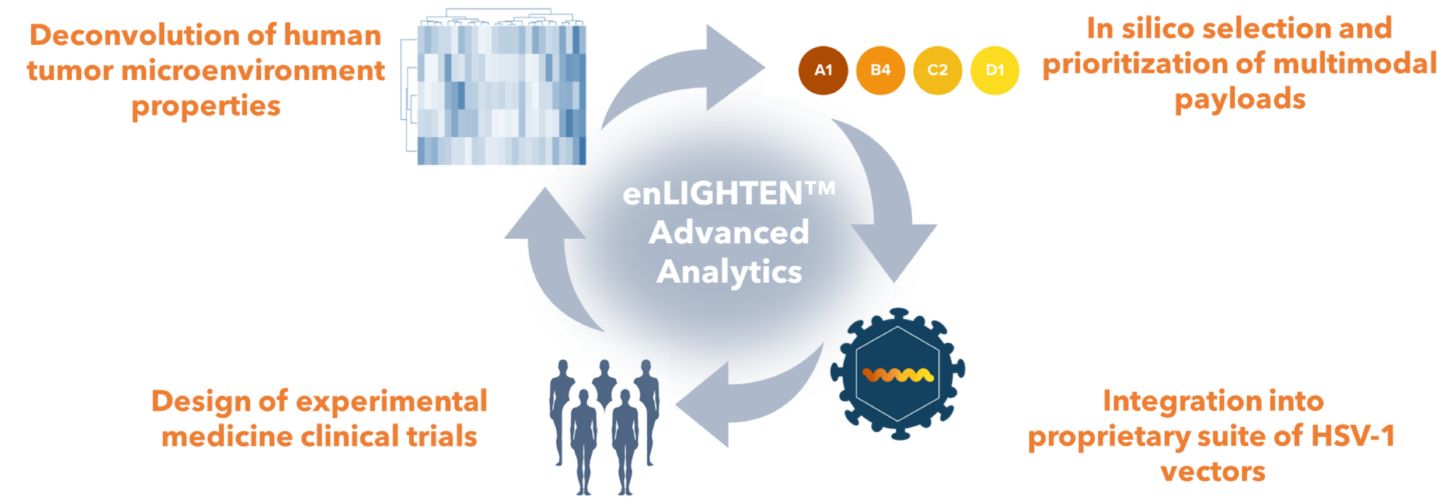
Overview of the enLIGHTEN™ Discovery Platform

- Failure to respond to conventional immunotherapies arises from heterogeneous mechanisms, present in the tumor microenvironments (TME), that drive resistance in non-responding patients.
- Candel's enLIGHTEN™ Discovery Platform applies advanced analytics to generate in silico prediction of multi-gene payload combinations with potential therapeutic benefit for specific solid tumor indications.
- Selected payloads, screened through ex vivo and in vivo multiplex assays, are then integrated into a viral chassis selected from Candel's modified herpes simplex virus-1 (HSV-1) vector suite resulting in a multimodal, programmable treatment combined in a single therapeutic.



Deconvolution of TME properties and in silico predication of payload combinations

The enLIGHTEN™ Discovery Platform was applied to immune checkpoint inhibitor (ICI)-treated patient datasets, to identify, in silico potential gene payload combinations aimed to overcome mechanisms underlying lack of response to ICI (Fig. 1).

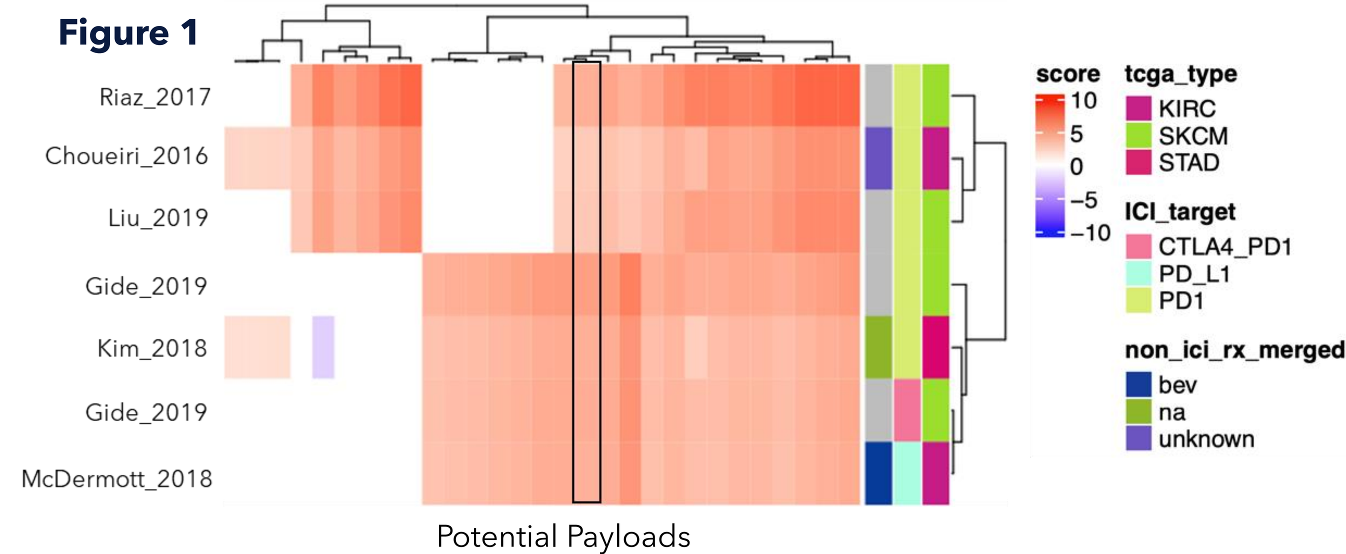


Figure 1: Deconvolution of TME properties and in silico predication of payload combinations from ICI-treated patient datasets. Harmonized bulk RNAseq data available was used to define statistical associations between TME properties/survival and TME properties/expression of each gene payload. A score was then calculated by combining the TME-to-survival and TME-to-payload p values as a -log10 meta p value. Columns: payloads, rows: ICI-treated patient populations. IFNγ is highlighted in a box.

Viral chassis Alpha-201 regulates pathways associated with responses to ICI

Alpha-201 is a viral chassis engineered for enhanced immunostimulatory activity coupled with sustained payload expression and regulated oncolysis. Cellular responses to viral chassis infection were profiled by RNAseq analysis (Fig. 2). The Alpha-201 viral chassis was selected for delivery of therapeutic payloads based on its ability to induce MYC and E2F targets and G2M cell cycle checkpoint responses, pathways associated with effective anti-tumor immune responses to ICI (Fig. 3). The Alpha-201 viral chassis also had a modest anti-tumor effect itself in a syngeneic tumor model similar to that achieved with anti-PD-1 antibody therapy (Fig. 4).

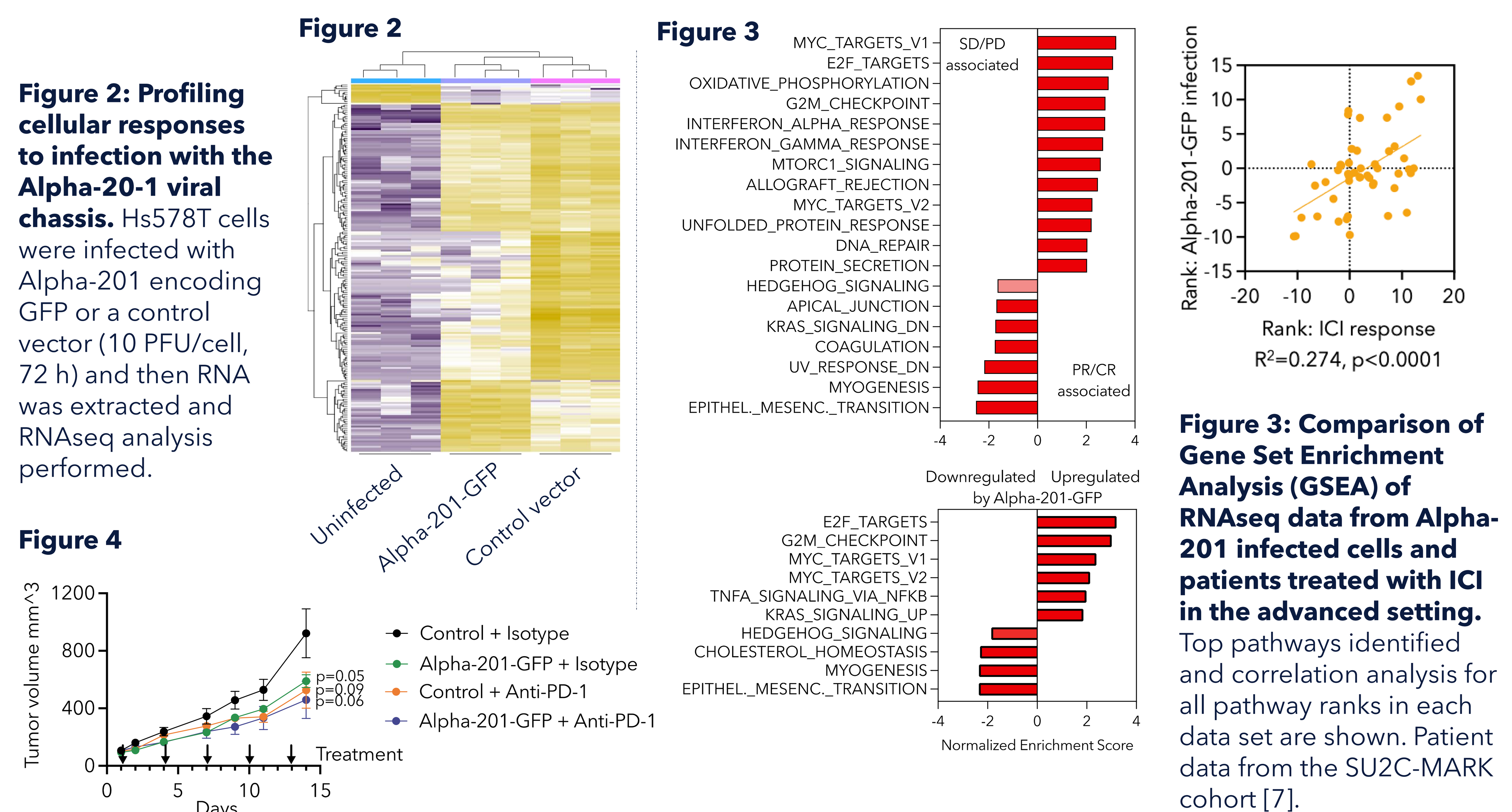


Figure 2: Profiling cellular responses to infection with the Alpha-20-1 viral chassis. Hs578T cells were infected with Alpha-201 encoding GFP or a control vector (10 PFU/cell, 72 h) and then RNA was extracted and RNAseq analysis performed.

Figure 3: Comparison of Gene Set Enrichment Analysis (GSEA) of RNAseq data from Alpha-201 infected cells and patients treated with ICI in the advanced setting. Top pathways identified and correlation analysis for all pathway ranks in each data set are shown. Patient data from the SU2C-MARK cohort [7].
Rank: NES*(-log10(p value)).
PR/CR: partial/complete response to ICI.
SD/PD: stable/progressive disease after ICI treatment.

Figure 4: Effect of Alpha-201 viral chassis ± anti-PD-1 antibody therapy on tumor growth in vivo. $5x10^5$ EMT6 cells were injected into the rear flank of Balb/c mice. Treatment ($3x10^7$ PFU Alpha-201-GFP, i.t. or 10 mg/kg, i.p. antibodies) began when tumors reached ~100 mm^3. N=5-8 mice per group. Anti-PD-1 clone: RMP1-14.

References:

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Alpha-201 encoding IFNγ enhances immune activation and effector function

Alpha-201 encoding IFNγ was characterized as an exemplar for payload-dependent responses. Oncolytic activity and payload expression were confirmed, and a strong IFNγ response gene signature was observed with Alpha-201-IFNγ infection (Fig. 5). Alpha-201 infection of cancer cells induced PBMC-mediated cancer cell killing and infection- and payload-dependent alterations in lymphoid and myeloid cell populations (Fig. 6). Dimensionality reduction (t-sne analysis) demonstrated robust phenotypic changes in several immune cell populations upon infection with Alpha-201 vectors (Fig. 7).

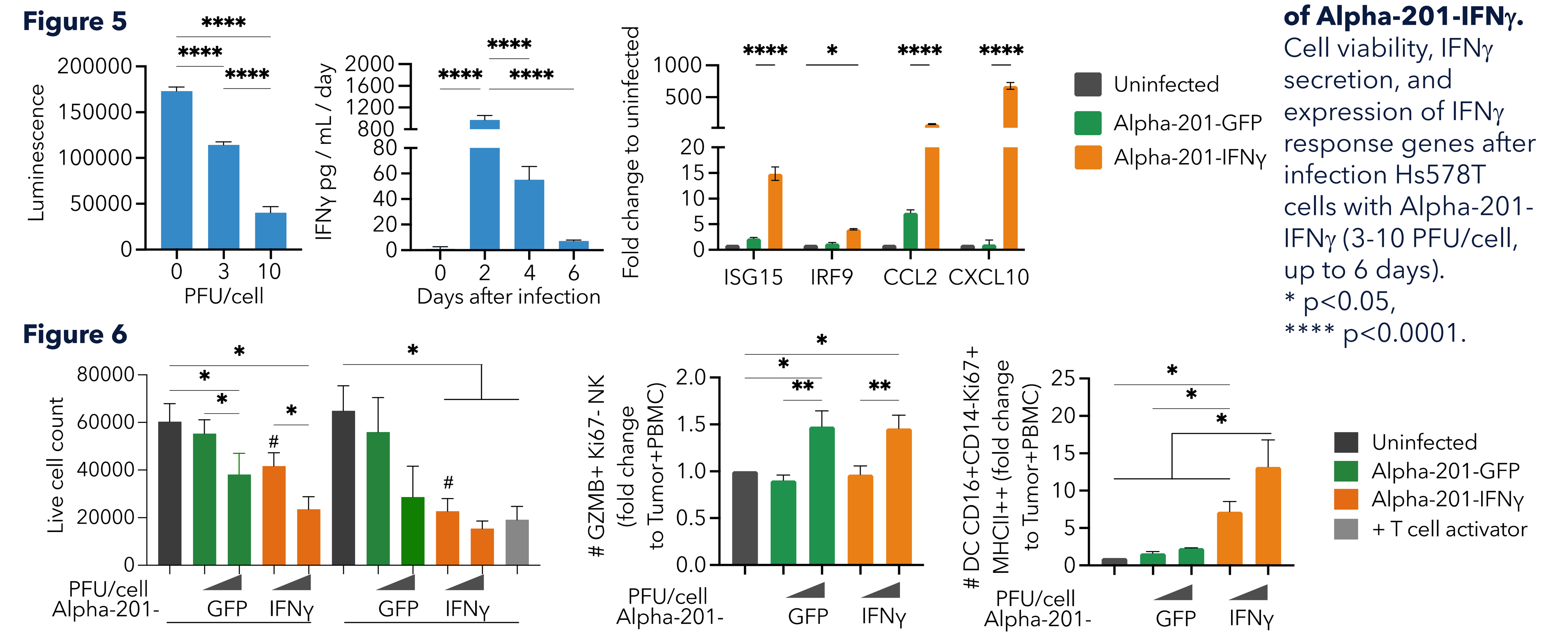

Figure 5: Validation of Alpha-201-IFNγ. Cell viability, IFNγ secretion, and expression of IFNγ response genes after infection Hs578T cells with Alpha-201-IFNγ (3-10 PFU/cell, up to 6 days).
* p<0.05,
**** p<0.0001.

Figure 6: Effect of Alpha-201 infection in an ex vivo cancer/peripheral blood mononuclear cell (PBMC) coculture system. Hs578T cells were infected with Alpha-201 encoding GFP or IFNγ (1-3 PFU/cell) and then cultured ± PBMC for 72 h. Cellular composition, function, and viability were then assessed by flow cytometry. * p < 0.05, ** p<0.01 compared to uninfected, # p < 0.05 in matched conditions with and without PBMC.

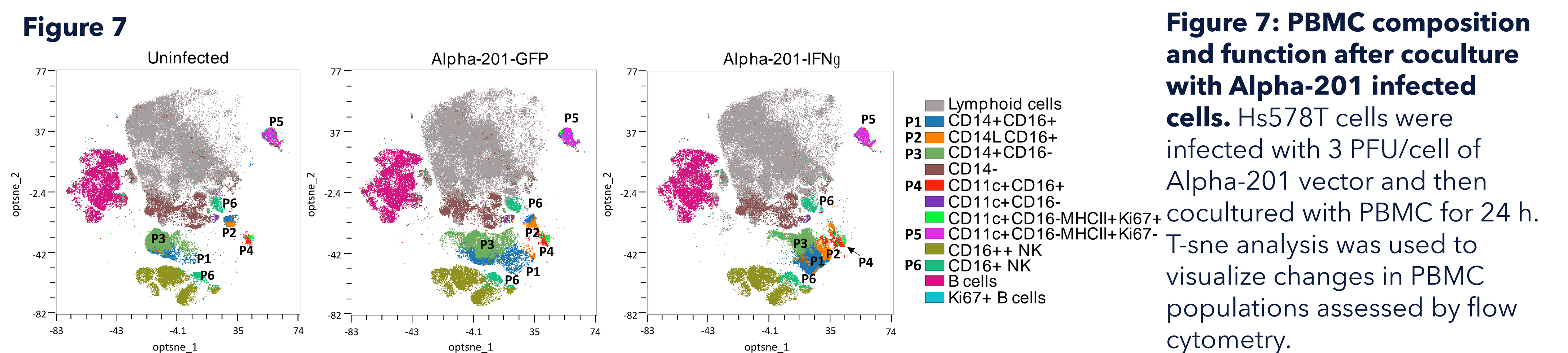

Figure 7: PBMC composition and function after coculture with Alpha-201 infected cells. Hs578T cells were infected with 3 PFU/cell of Alpha-201 vector and then cocultured with PBMC for 24 h. T-sne analysis was used to visualize changes in PBMC populations assessed by flow cytometry.

Multiplex evaluation enables rational payload prioritization

In silico identified payloads, encoded in Alpha-201, were then tested in vitro in multiplex assays (Fig. 8). PBMC-mediated cancer cell killing (Fig. 9) and immune activation (Fig. 10) were further enhanced in specific payload combination. Integrated analysis of the immune cell response and tumor cell killing to each payload combination was then used to support payload prioritization and selection (Fig. 11).

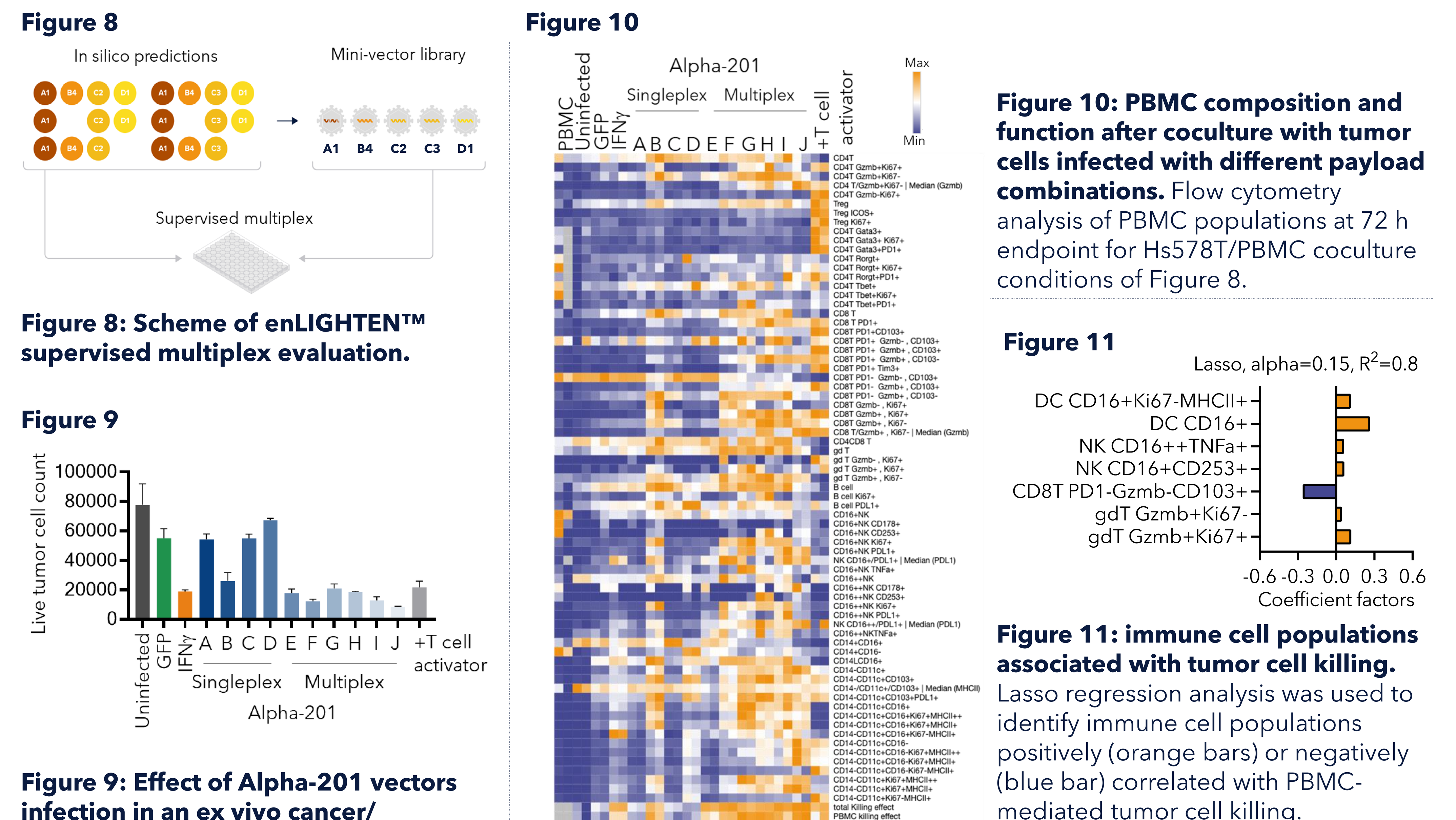

Figure 8: Scheme of enLIGHTEN™ supervised multiplex evaluation.

Figure 9: Effect of Alpha-201 vectors infection in an ex vivo cancer/ peripheral blood mononuclear cell (PBMC) coculture system. Hs578T cells were infected with Alpha-201 encoding GFP, IFNγ or other vectors in singleplex or multiplex (total 1 PFU/cell), then cultured ± PBMC for 72 h. Tumor cell viability was assessed by flow cytometry.

Figure 10: PBMC composition and function with tumor cells infected with different payload combinations. Flow cytometry analysis of PBMC populations at 72 h endpoint for Hs578T/PBMC coculture conditions of Figure 8.

Figure 11: immune cell populations associated with tumor cell killing. Lasso regression analysis was used to identify immune cell populations positively (orange bars) or negatively (blue bar) correlated with PBMC-mediated tumor cell killing.

Conclusions

These data validate enLIGHTEN™ in silico predictions of gene payload combinations and highlight the utility of the enLIGHTEN™ Discovery Platform to guide the development of novel viral immunotherapeutics to modify the TME by design.

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