A first-in-class multimodal immunotherapy for enhanced immune activation in the tumor microenvironment as a novel therapeutic strategy for solid tumors

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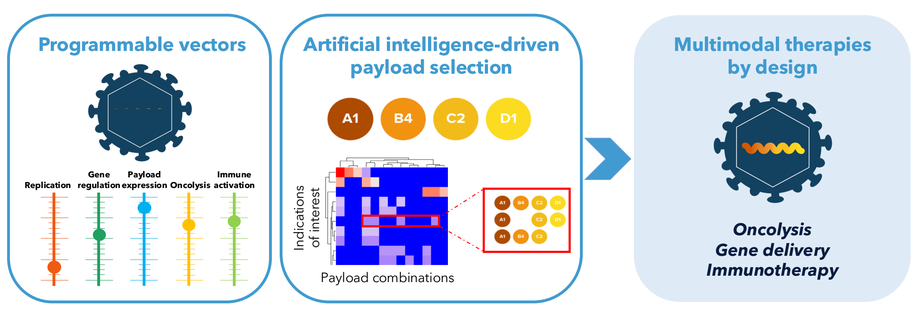
Abstract# PP0006

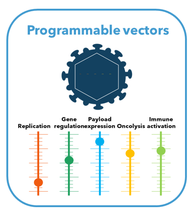
Overview of the enLIGHTEN™ Discovery Platform

- The enLIGHTEN™ Discovery Platform allows for the design of multimodal immunotherapeutics leveraging human biology and advanced analytics to select multigene payloads to be delivered by herpes simplex virus-1 (HSV-1) vectors to the tumor microenvironment (TME).
- Programmable vectors are engineered with specific features through the combinations of vector genome deletion/disruption and insertion of synthetic, non-native encodable elements.
- Optimal payload permutations are predicted in silico and tested in proprietary multiplex assays.



Schematic representation of the enLIGHTEN™ Discovery Platform

Alpha-201: a programmable vector for immune activation



Alpha-201, a replication-defective vector, was selected as the programmable vector for its properties of **enhanced immuno-stimulation** (Fig. 1, 2), **sustained payload expression, and regulated oncolysis** (see Fig. 4, section below).

Figure 1

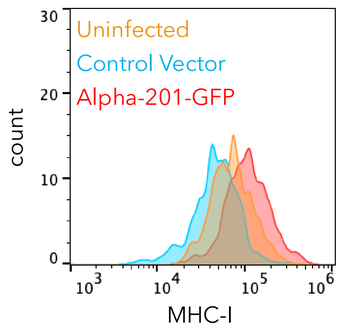


Figure 1: Hs578T cells were infected with 10 PFU/cell Alpha-201 or Control Vector (ICP27mutant). Cell-surface expression of MHC class I, an indicator of antigen presentation, was assessed by flow cytometry 24 h after infection.

Figure 2

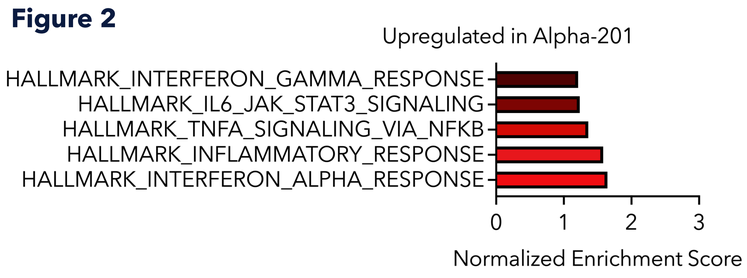
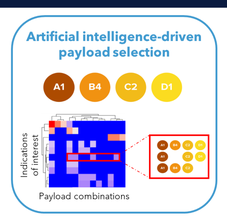


Figure 2: Gene Set Enrichment Analysis (GSEA) was performed on RNAseq data, and top pathways enriched in cells infected with Alpha-201 compared with Control Vector are shown.

In silico prediction of optimal payload combinations



Here, we applied enLIGHTEN™ to the large patient datasets [1-6] to identify potential gene payload combinations to enhance immune activation in the TME.

Figure 3

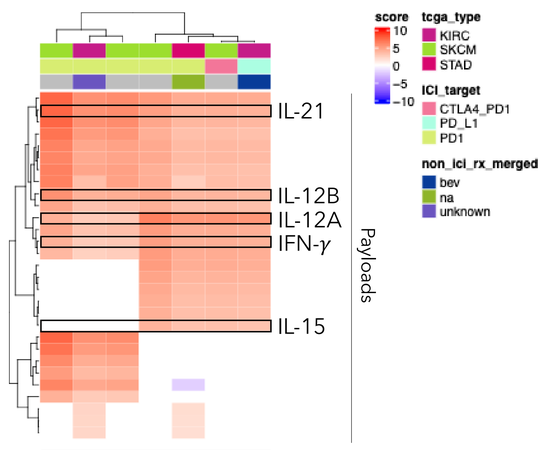


Figure 3: Deconvolution of TME properties and in silico prediction of payload combinations from patient datasets. Harmonized bulk RNAseq data available were used to define statistical associations between TME properties/survival and TME properties/expression of each gene payload. A score was then calculated by combining the TME-to-survival and TME-to-payload p values as a -log10 meta p value. Potential payloads chosen for combination are highlighted in boxes.

Validation of the Alpha-201 minivector library

A library of single gene encoding Alpha-201 vectors ("minivectors") was constructed, each expressing one of the in silico predicted payload components (e.g., IL-12, IFN-γ), and validated.

Figure 4

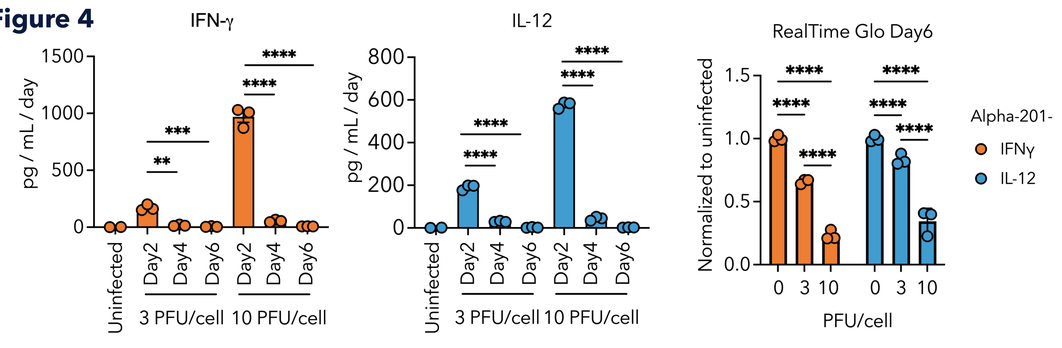


Figure 4: Hs578T cells were infected with Alpha-201 vectors (3 or 10 PFU/cell), and then conditioned medium was collected every two days for quantification of payload production rates. Cell viability was measured 6 days after infection. ANOVA with Tukey's correction, **p<0.01, ***p<0.001, ****p<0.0001.

References:
1. Gide T.N. et al. Cancer Cell 2019; 35(2):238-255.e6.
2. Hugo W. et al. Cell 2016;165(1):35-44.
3. Liu D. et al. Nat Med 2019;25(12):1916-1927.
4. Riaz N. et al. Cell 2017;171(4):934-949.e16.
5. Van Allen E.M. et al. Science 2015;350(6257):207-211.
6. McDermott D.F. et al. Nat Med 2018;24(6):749–757.

Ex vivo multiplex evaluation enables rational payload prioritization

Infection of cancer cells with in silico predicted payload combinations using single gene encoding Alpha-201 vectors induced PBMC-mediated cancer cell killing in an infection- and payload-dependent manner (Fig. 5, 6). Target cell killing was associated with significant changes in the composition and function of immune cells within the coculture (Fig. 7). Correlation analysis identified payload-mediated regulation of multiple immune cell populations correlated with effective PBMC-mediated cancer cell killing (Fig. 8). The combination of IL-12 and IL-15 was then prioritized for in vivo testing (Fig. 9).

Figure 5

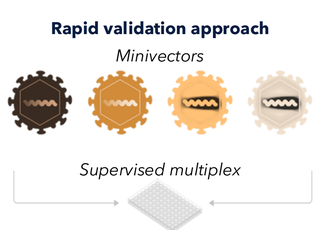


Figure 5: Schematic of rapid validation approach.

Figure 6

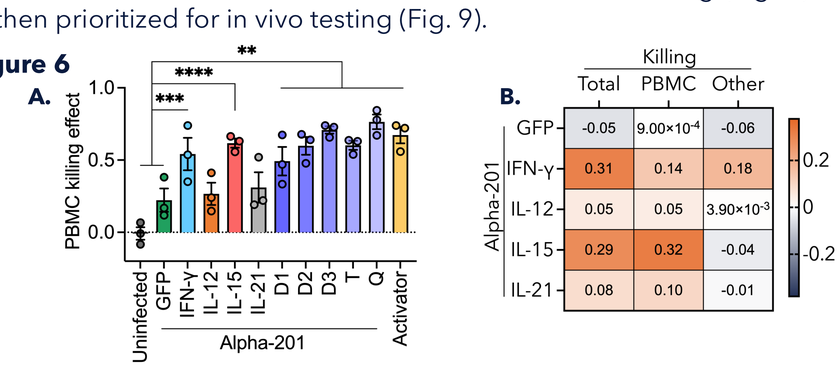


Figure 6: Hs578T cells were infected with Alpha-201 encoding GFP, IFN-γ, or other vectors individually or in multiplexes (total vector: 1 PFU/cell), then cultured ± PBMC for 72 h. D1: IFN-γ, IL-12; D2: IFN-γ, IL-15; D3: IL-12, IL-15; T: IFN-γ, IL-12, IL-15; Q: IFN-γ, IL-12, IL-15, IL-21 **A.** PBMC-mediated target cell killing was assessed by flow cytometry. **B.** Association of target cell killing with each payload was analyzed by OLS regression.

Figure 7

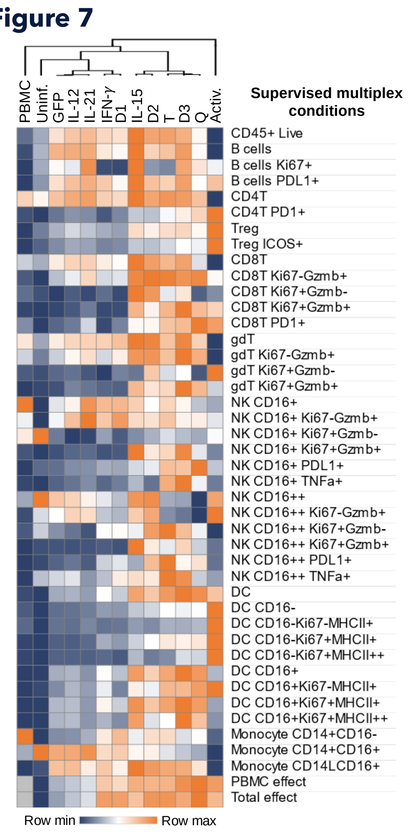


Figure 7: Flow cytometry analysis of PBMC composition and function after coculture with tumor cells infected with different minivector combinations.

Figure 8

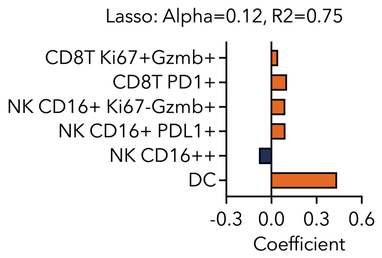


Figure 8: Lasso regression analysis was used to identify immune cell populations positively (orange bars) or negatively (blue bar) correlated with PBMC-mediated tumor cell killing.

Figure 9

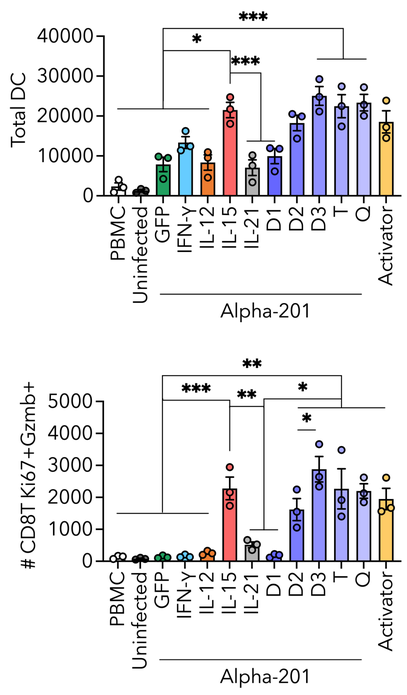


Figure 9: Total cell number by supervised multiplex condition for immune populations associated with PBMC-mediated tumor cell killing. N=3. ANOVA with Tukey's correction. *p<0.05, **p<0.01, ***p<0.001.

Robust in vivo anti-tumor efficacy upon delivery of payload combination

Intratumoral treatment with Alpha-201 vectors encoding IL-12 and IL-15 resulted in significant tumor growth inhibition in both tumor models tested (EMT6, LLC1; Fig. 10). Importantly, tumor regression was observed in 25% of EMT6 tumor-bearing mice (Fig. 10B). Immune activation, both within tumors and in the periphery, was associated with antitumor activity (Fig. 11).

Figure 10

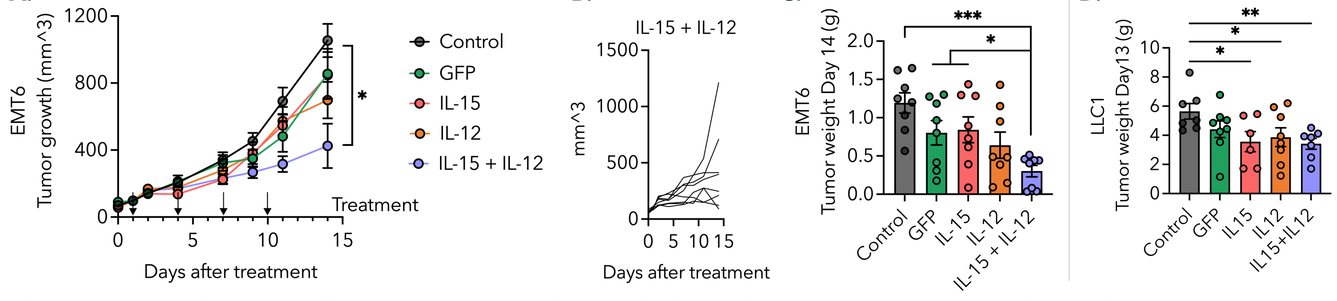


Figure 10: A. 5x10^5 EMT6 cells were injected into the rear flank of Balb/c mice. Treatment (3x10^7 PFU Alpha-201 minivector combinations, i.t.) began when tumors reached ~100 mm^3, and tumor growth was monitored over time. Treatment was well-tolerated based on body weight and animal observations. N=8 mice per group. **B.** Individual tumor growth curves are shown for the IL-15 + IL-12 treated mice; 3 mice had tumors regressing by the end of the study. **C.** Final tumor weight of EMT6 tumors. **D.** Minivector combinations were also tested in LLC1 tumor-bearing mice using the same experimental conditions. Final tumor weight of LLC1 tumors is shown. N=5–8 mice per group. *p<0.05, **p<0.01, ***p<0.001.

Figure 11

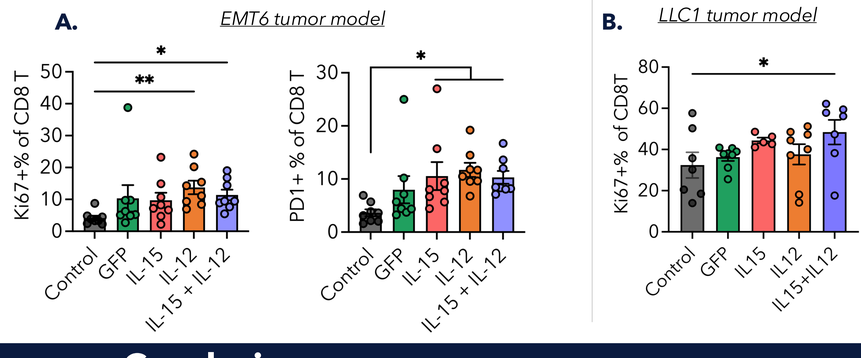


Figure 11: A. Flow cytometry analysis of PBMC isolated from Alpha-201 minivector treated EMT6 tumor-bearing mice. N=8 per group. **B.** Flow cytometry analysis of Alpha-201 minivector treated LLC1 tumors. N=5–8, *p<0.05, **p<0.01.

Conclusion

- Application of the enLIGHTEN™ Discovery Platform to predict optimal payload combinations in silico and test in proprietary multiplex assays enabled rational payload prioritization.
- Multiparameter decision matrix validated the synergistic effect of IL-12 and IL-15.
- In vivo anti-tumor efficacy and immune activation was observed upon Alpha-201-mediated delivery of IL-12 and IL-15, supporting the development of a first-in-class agent for enhanced immune activation in the TME of solid tumors.

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