Cytokine-Driven Divergence Between Allogeneic CAR-T Platforms

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INTRODUCTION

- Cytokine signaling is a key mechanistic driver of T-cell differentiation, expansion, and long-term functional persistence.
- In one allogeneic CAR-T platform (Platform 1), modifying the signaling environment promotes less differentiated memory states and supports robust CAR-mediated expansion.
- In a second platform (Platform 2), analogous signaling modifications produce a markedly different biological response.
- These findings suggest platform-specific cytokine dependencies and differentiation-program sensitivities.

METHODS

- Platform-specific responses to controlled signaling environment modifications were evaluated.
- A design of experiments (DOE) guided by quality-by-design (QbD) principles was applied in the scale-down model (SDM) to assess factor influence and interactions.
- Assessed parameters include:
  - Growth metrics and cumulative population doublings (PDL)
  - Genetic engineering efficiency
  - Memory subset composition
  - Short-and long-term functional activity

RESULTS

Manufacturing Platform for Allogeneic CAR-T Cells

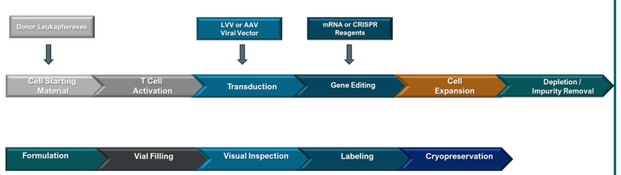

Figure 1. Allogene's Manufacturing Platform.
A standardized, platform-based manufacturing workflow composed of discrete unit operations enables rapid process optimization and flexibility across programs. The modular design supports efficient incorporation of new pipelines and accelerates platform-specific optimization while maintaining a consistent manufacturing backbone.

Cytokine-Driven Growth Responses are Platform Dependent

Figure 2. CAR T cells cultured in two cytokine mixtures exhibit platform-specific responses during manufacturing.
(A) In Platform 1, cytokine mixture A and cytokine mixture B result in distinct viability and cell diameter trajectories over time, although end-of-process viability is comparable. The cytokine conditions yield a modest but reproducible difference in cumulative PDL by the end of the process (n=4).
(B) In contrast, the same cytokine mixtures elicit minimal differences in viability, cell diameter, or cumulative PDL in Platform 2, indicating a reduced sensitivity to cytokine modulation (n=3). Notably, the divergent responses observed across platforms suggest that cytokine effects act within a broader platform context, where additional platform-specific factors likely contribute to the overall process behavior.

Cytokine Conditions Differentially Impact Gene Modification Across CAR T Platforms

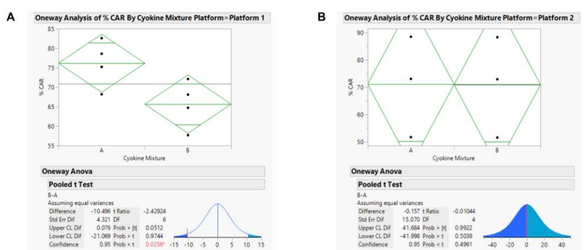

Figure 3. Cytokine effects on gene modification efficiency.
(A) In Platform 1, cytokine mixture A is associated with higher CAR transduction efficiency compared with cytokine mixture B (n=4).
(B) In contrast, transduction efficiency is comparable between cytokine conditions in Platform 2, indicating no detectable effect of cytokine milieu under these conditions (n=3).

Cytokine-Driven Modulation of CAR T-Cell Memory Differentiation

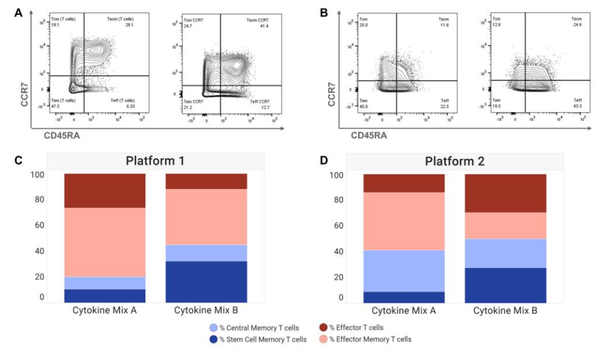

Figure 4. Effects of cytokine conditions on memory phenotype.
(C) In Platform 1, cytokine mixture B is associated with an increased proportion of less differentiated memory subsets, including a higher frequency of Tscm cells, compared with cytokine mixture A, as assessed by CCR7/CD45RA flow cytometry.
(D) In Platform 2, cytokine mixture B similarly favors a shift towards less differentiated memory phenotypes relative to cytokine mixture A, although the magnitude of the effect is reduced compared with Platform 1. Still, an increase Tscm fraction is observed in the final drug product.

Platform- Dependent Cytokine Effects on Short-Term Functional Potency

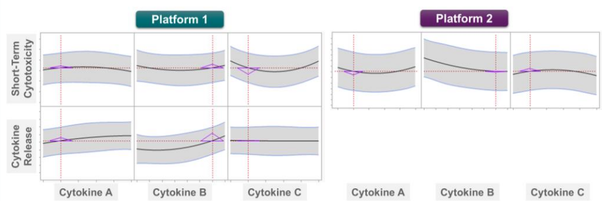

Figure 5. Effects of cytokine conditions on short-term functional responses.
Using a DOE approach, individual effects of each cytokine on short-term functionality were evaluated across platforms. In Platform 1, Cytokine A demonstrates a nonlinear, predominantly negative association with short-term cytotoxicity, whereas in Platform 2 it exhibits a positive effect. Cytokine B shows a stronger contribution to functional response in Platform 2 compared with Platform 1. In contrast, Cytokine C displays opposing effects between the two platforms, further highlighting platform-specific functional sensitivities to cytokine conditions.

Platform- Dependent Cytokine Effects on Long-Term Functional Potency

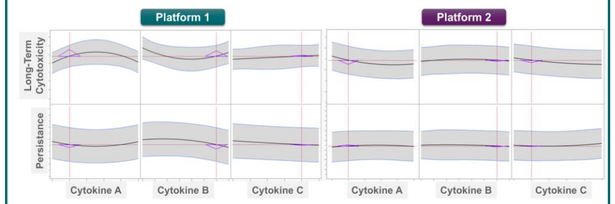

Figure 6. Effects of cytokine conditions on long-term functional responses.
The same DOE framework was applied to evaluate cytokine effects on long-term cytotoxicity and persistence across platforms. Cytokine A exhibits opposing effects on long-term cytotoxicity between Platform 1 and Platform 2. Cytokine B contributes more strongly to long-term cytotoxicity and persistence in Platform 1 relative to Platform 2, while Cytokine C shows minimal contribution to long-term functional responses in both platforms. Consistent with these trends, persistence is modulated primarily by Cytokines A and B in Platform 1, whereas all cytokines exhibit limited impact on persistence in Platform 2.

RESULTS

- Modifying the signaling environment during allogeneic CAR-T manufacturing produced platform-dependent biological outcomes.
- While such interventions reinforce stem-like memory states and potency in Platform 1, Platform 2 shows reduced responsiveness and diminished long-term performance.
- These data underscore the importance of product-specific signaling environment engineering strategies and demonstrate that mechanisms observed in one CAR-T design cannot be generalized across platforms.
- Using a DOE approach, individual cytokine effects and their interactions can be assessed, enabling evaluation of how combined cytokine inputs influence the desired response, providing a data-driven approach to tailor process conditions for each platform.

ABBREVIATIONS

CAR, chimeric antigen receptor; QbD, quality be design; SDM, scale-down model; PDL, population doubling level; DOE, design of experiment; CCR7, C-C chemokine receptor types 7; CD45RA, protein tyrosine phosphatase, receptor type C; Tsm, stem memory T cell.

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DISCLOSURES

All authors are current or former employees of Allogene Therapeutics, Inc

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