Purification of CAR+ T Cells Reveals Impact of Untransduced Cells in CAR-T Drug Product

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Introduction

- Untransduced cells play an unknown role in the potency of CAR-T cell drug product.
- Purification of CAR+ cells may reduce risk of cytokine release syndrome, nonspecific killing, and graft versus host disease.
- Inserted the QBEND/10 epitope into the hinge domain of a CD33 CAR-T construct to enable immunomagnetic selection with CD34 microbeads.
- Achieved 95% CAR+ purity with greater than 75% recovery of CAR+ input cells.

Fig. 5: CAR-T Cell Dilution Cytotoxicity Assay

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- Target cell killing measured after 24-hour coculture with 50,000 effector cells.
- Untransduced (UTD) cells added to effector cells to model a range of CAR+ frequencies from 100% CAR+ to 25% CAR+.
- Effector samples used in 1:1 E:T ratio were diluted 1:5 to model lower E:T ratios.

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- Observed no substantial increase in potency or cytokine release when adding untransduced cells to effector cells.
- Full activation is not observed in UTD cells co-cultured with CAR+ and WT target cells



CAR Design and Purification Method. (A) Schematic of CAR design includes QBEND/10 CD34 epitope in hinge domain. (B) Two-round CAR purification method using commercially-available CD34 microbeads





Untransduced Cells Demonstrate Marginal Contribution to Cytotoxicity of CAR-T cells. (A) Total number of CAR+ and UTD cells used for each condition of the cytotoxicity assay in Fig. 5C. (B) Total number of CAR+ and UTD cells used for each condition of the cytotoxicity assay in Fig. 5D. (C) Target cell killing of WT (purple) or CD33KO (blue) Molm13 target cells at 1:1 E:T Ratio after 24-hour co-culture. Untransduced cells were added to each condition as shown in Fig. 5A. (D) Target cell killing of WT (purple) or CD33KO (blue) Molm13 target cells at 1:5 E:T Ratio after 24-hour co-culture. Untransduced cells were added to each condition of Th1 cytokine concentration in media collected following cytotoxicity assay shown in Fig 5C. Fig. 5C, D, and E are representative data of two donors.



Enrichment of CAR+ Cells Yields High Purity of CAR+ Cells and Selects Cells with Highest CAR Expression. (A) Flow cytometry analysis of CAR+ frequency of input and elution samples for first and second round of purification. Representative data from two donors. (B) Analysis of CAR expression in flow through and elution samples following each round of purification in two separate donors.



CAR Purification Does Not Increase CAR-T Cell Activation. (A) Quantification of CD69 expression in elution samples after 18 hours of satellite culture following purification using recombinant CD33 or anti-CD34 antibody. (B) Quantification of CD25 expression in input as well as flow through and elution samples from round 1 and round 2 after 24 hours of satellite culture following purification with CD34 microbeads. Representative data from two donors.



Enrichment of CAR+ Cells by CD34 Microbead Yields High Purity Sample with Excellent Recovery. (A) Quantification of CAR+ frequency in input and elution samples by flow cytometry. (B) Quantification of recovery of input CAR+ cells using purification method described in Fig. 1B. (C) Alluvial plots show recovery of CAR+ cells as a percentage of CAR+ input in two separate donors.

Untransduced Demonstrate Partial Activation When Incubated with CAR+ Cells and WT Molm13. (A) Total number of CAR+ and UTD cells used for each condition of the cytotox assay in Fig. 6C and Fig 6E. (B) Total number of CAR+ and UTD cells used for each condition of the cytotox assay in Fig. 6D. (C) Target cell killing of WT (blue) or CD33KO (green) Molm13 target cells at 1:2 E:T Ratio after 24-hour co-culture. Untransduced cells were added to each condition as shown in Fig. 6A. (D) Target cell killing of WT (blue) or CD33KO (green) Molm13 target cells at 1:5 E:T Ratio after 24-hour co-culture. Untransduced cells were added to each condition as shown in Fig. 6B. (E) Flow cytometry analysis of activation markers CD69 and CD25 following cytotoxicity assay shown in Fig. 6C. Representative data from two donors.

Conclusions

- CAR+ purification yields a product of greater than 95% CAR+ cells, with a recovery greater than 75% of CAR+ input.
- Purification by CD34 microbeads does not induce CAR-T activation.
- Untransduced cells do not substantially increase to CAR-T cell potency or cytokine release.
- Partial activation of untransduced cells is observed when co-cultured with CAR+ and WT targets.