INNOVATIVE BONE MARROW CAR-T CELL MANUFACTURING FOR AML: ENHANCING VIABILITY AND TUMOR MIGRATION

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INTRODUCTION

Manufacture CAR T lymphocytes is challenging for patients suffering acute myeloid leukemia (AML) due to several limitations, such as high number of circulating blasts, lymphopenia and dysfunctional T cells. Accordingly, current methods fail to produce CAR-Ts for the majority these patients. When production is achieved, response rates range 30-49%, lower than those reported in B ALL or multiple myeloma. Thus, immunotherapies such as CARTs represent an unmet medical need for AML.

AIM

To develop a new CAR-T manufacturing method that solves this production challenge for AML, which we refer to Immunocoaching T Cells (ICTs).

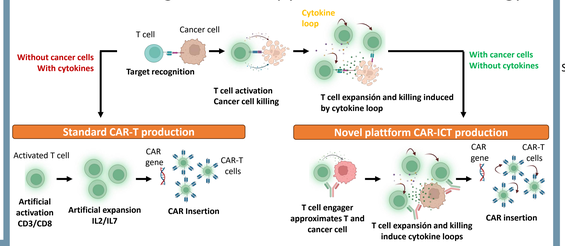
Key innovations: 1) TILs as source of T cells. 2) Ex vivo activation with T cell engagers and Immuno-oncology factors. 3) Expansion with live tumor cells CAR transfection. 4) Double selection to select T cells. 5) Simple 2-step automated manufacturing enables decentralized production.

METHOD

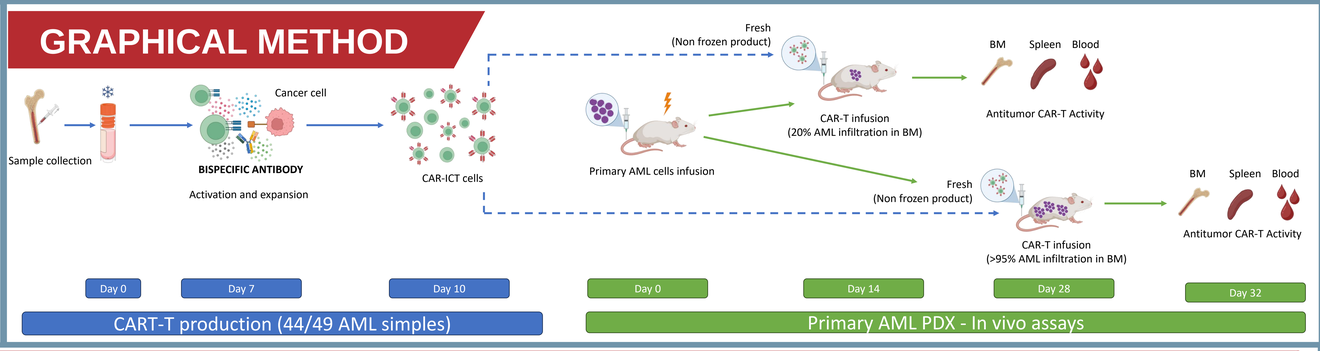
- 49 cryopreserved BM samples with > 60% of blast cells from AML patients were included in the study. For CAR-ICT cells manufacturing, AML whole BM samples were cultured with a bispecific antibody CD3-CD123 in combination with IL2, IL15, IL21, PD1, TIM-3, TIGIT, CTLA4 IDO-1, A2A and JQ1 for 7 days, in the presence of blasts. After that, ICTs or Healthy Donor (HD) T cells were transduced with a D28 & 4-1BB CD123 lentivirus (MOI=5) in combination with BX795. Before intravenously injection to the PDX mice at day 10, a double magnetic bead selection removes 1st remaining AML cells and 2nd positive selection of T cells. Minimal Residual Disease testing is used as a quality control to ensure no tumor cells are present in the final CAR-ICT therapy product. The CAR T HD were produced following standard methods: HD PBMNCs were separated by ficoll, isolating CD3+ T cells with magnetic beads, cultured in IL-2 + IL-15 before transduction. Flow cytometry was used to monitor blast cell death, T cell expansion and activation, and subsequent transduction and phenotype
- To test the activity and migration of these cells, a PDX model using primary AML cells in immunocompromised NSG mice was used to evaluated the activity of these cells. To generate the model, 5x10^6 blasts were intravenously infused via the tail vein of the animals. For activity studies, $2.5x10^6$ to $5x10^6$ CAR+ T cells were infused two weeks post-infusion, when > 20% BM infiltration was achieved. Two weeks later, the animals were sacrificed, and a flow cytometry analysis was conducted to assess CAR-T cell activity and various markers in the BM, peripheral blood, and spleen. For studies on CAR-T cell migration to tissues and viability, a similar process was followed, but $5x10^6$ CAR-T cells were infused at four weeks, with > 90% BM tumor infiltration, and 1 week later after the animals were sacrificed for studies. Additionally, cytokine production was studied in vitro.

CONCEPT

Immuno Coaching T Cell Therapy Platform: Restores Biology

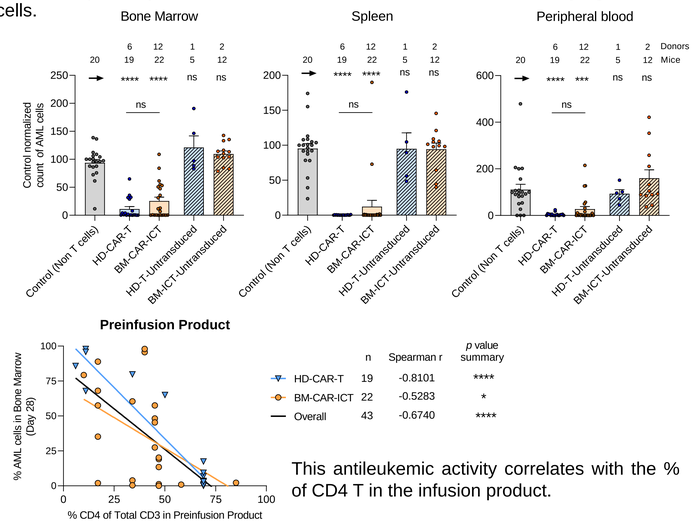


GRAPHICAL METHOD



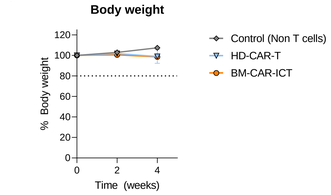
RESULTS

1. BM-CAR-ICT exhibit high killing activity vs control group in all three organs (BM, spleen and blood), at least comparable to HD-CAR-T produced by the standard method. Importantly, untransduced T cell don't show alloreactive activity against tumor cells.

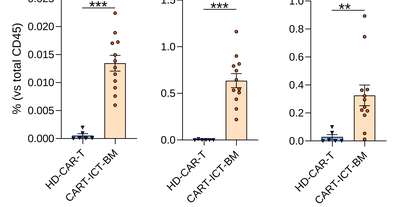


This antileukemic activity correlates with the % of CD4 T in the infusion product.

2. Notably, animals did not experience a decline in well-being during the study period.

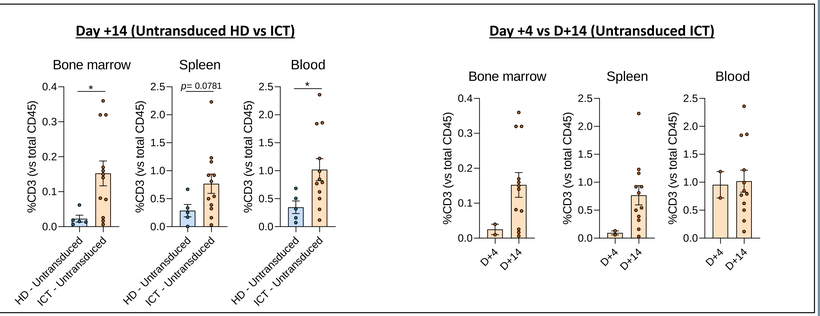


3. BM-CAR-ICT produced from four different AML samples showed substantially higher viability at 4 days in the PDX mice compared to CAR-T-HD produced by standard methods.



4. ICT cells show longer persistence in vivo, in the absence of any cytokine treatment to the mice.

After 14 days post-infusion, untransduced ICT cells from BM show longer persistence in BM, spleen and blood.



CONCLUSIONS

- The ICT method for CAR-T-CD123 production addresses challenges in CAR T production for AML.
- Demonstrates high antitumor activity in vivo without toxicity.
- Ensures persistence and viability within tumor tissues.

REFERENCES

1. Tambaro, F. P., Singh, H., Jones, E., Rytting, M., Mahadeo, K. M., Thompson, P., Daver, N., DiNardo, C., Kadia, T., Garcia-Manero, G., Chan, T., Shah, R. R., & Wierda, W. G. (2021). Autologous CD33-CAR-T cells for treatment of relapsed/refractory acute myelogenous leukemia. Leukemia, 35(11), 3282–3286. https://doi.org/10.1038/s41375-021-01232-

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