ELSEVIER

Blood 142 (2023) 3450–3451

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Manufacturing, Characterization and Anti-Tumor Activity of Allogeneic CD19-CAR Cytokine-Induced Killer (CIK) Cells Engineered with Non-Viral Sleeping Beauty Transposon System and Armored with IL-18

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Rationale & Objective:
Adoptive cell therapy by chimeric antigen receptor- engineered cytokine-induced killer (CAR-CIK) cells has demonstrated high therapeutic potential with minimal alloreactivity and little to no toxicity in B-cell acute lymphoblastic leukemia patients, but further development is required to improve anti-tumor activity and persistence *in vivo*. The expression of the proinflammatory cytokine interleukin-18 (IL-18) has been shown to greatly enhance CAR-T cells potency and persistence. The purpose of the work described here was to develop a workflow for the manufacture of functional CAR-CIK cells, using the non-viral *Sleeping Beauty* transposon system, that co-express a second generation CD19-targeted CAR and the proinflammatory cytokine IL-18, hereafter referred to as CARCIK-1918 cells. The CAR molecule expressed by these cells includes an attenuated CD28 intracellular domain for enhancement of anti-tumor activity and *in vivo* persistence. Characterization of the manufactured armored CARCIK-1918 cells and evaluation of their anti-tumor activity *in vitro* and *in vivo* were performed.
Methods:
PBMCs isolated from healthy donors were cultured with IFN-γ, stimulated with anti-CD3 and IL-2, electroporated for non-viral genetic modification with *Sleepy Beauty* transposase RNA (SB100X) and pT4-1928'z-IL18 bicistronic plasmid DNA, and cultured for 15 days (17-day process). In process and final product characterization included monitoring of cell culture composition, cell expansion and viability, and evaluation of the expression of CAR, CIK proteins CD56 and NKG2D, and memory phenotype markers using flow cytometry. The final product CARCIK-1918 cells were further characterized by *in vitro* stimulation with CD19 $^{+}$ REH tumor cells to assess tumor killing activity and IL-18 secretion.
CARCIK-1918 *in vivo* efficacy and persistence were also investigated in an NSG/Daudi mouse model. Mice were grafted with CD19 $^{+}$ Daudi tumor cells and, after 2 days, received an intravenous injection of 5x10 ^{6} or 10x10 ^{6} CARCIK-1918 cells. Daudi-grafted untreated mice were used as controls. Survival was monitored, and tumor burden and CAR-CIK cell persistence evaluated by using flow cytometry to assess human CD19 $^{+}$ and human CD3 $^{+}$ cells respectively, in peripheral blood, bone marrow, spleen, and kidney.
Results:
We manufactured >15 lots of CARCIK-1918 cells. After electroporation on day 2, CAR expression was already detected at day 7 and increased during the expansion culture. After the 17-day process, the range of harvested cells was 4.4E+09 to 1.6E+10 viable cells, with a cell viability >94%, and a fold expansion that varied among lots (range 20.1 - 161.4). The final CARCIK-1918 cells were predominantly CD3 $^{+}$, expressed both the CAR (range 13 - 81%) and CIK-specific markers CD56 (range 27 - 88%) and NKG2D (range 59 - 86%) at variable levels among batches, and showed mostly a central memory/effector memory phenotype. CARCIK-1918 cells demonstrated potent and specific *in vitro* cytotoxicity towards the CD19 $^{+}$ REH target cell line. Cells secreted IL-18 only in the presence of CD19 $^{+}$ target cells (range 62.17 - 560.58 pg/mL).

In addition, CARCIK-1918 cells had a substantial anti-leukemic activity *in vivo*. CARCIK-1918 injection resulted in a significant dose-dependent reduction of tumor growth and improved survival, as compared with untreated NSG/Daudi mice. CARCIK-1918 cells persisted *in vivo* in mouse peripheral blood up to 90 days. Post-mortem analysis demonstrated that CARCIK-1918 cells from both dose levels persisted over time and limited the level of leukemic cell dissemination in the animals, with $10x10^{6}$ regimen allowing an almost total control of the disease progression in all tissue examined.

Conclusion:

In conclusion, this study demonstrates that manufacturing of armored CAR-CIK cells using *Sleepy Beauty* non-viral transposon system and 2 nd generation CD19 CAR with an attenuated CD28 costimulatory domain and armored with IL-18 is feasible and allows rapid and efficient expansion of highly potent CARCIK-1918 cells. These cells demonstrated potent and specific anti-tumor activity *in vitro* and a robust anti-leukemic activity *in vivo*. These findings provide the first reported successful production of IL-18 armored CAR-CIK cells offering a promising strategy for the treatment of B-cell malignancies.

Disclosures Contreras-Ruiz: *CoImmune Inc:* Current Employment, Current holder of *stock options* in a privately-held company. **Pace:** *CoImmune Inc:* Current Employment, Current holder of *stock options* in a privately-held company. **Kohnke:** *CoImmune Inc:* Current Employment, Current holder of *stock options* in a privately-held company. **Viruet:** *CoImmune Inc:* Current Employment, Current holder of *stock options* in a privately-held company. **Adams:** *CoImmune Inc:* Current Employment, Current holder of *stock options* in a privately-held company. **Krisko:** *CoImmune Inc:* Current Employment, Current holder of *stock options* in a privately-held company. **Leesnitzer:** *CoImmune Inc:* Current Employment, Current holder of *stock options* in a privately-held company. **Velie:** *CoImmune Inc:* Current Employment, Current holder of *stock options* in a privately-held company. **Tettamanti:** *CoImmune Inc:* Research Funding. **Pisani:** *CoImmune Inc:* Research Funding. **Melita:** *CoImmune Inc:* Research Funding. **Biondi:** *Novartis:* Speakers Bureau; *Agmen:* Speakers Bureau; *Galapagos:* Membership on an entity's Board of Directors or advisory committees; *BMS:* Membership on an entity's Board of Directors or advisory committees; *CoImmune:* Membership on an entity's Board of Directors or advisory committees, Research Funding. **Rambaldi:** *Abbvie:* Honoraria. **Tcherepanova:** *CoImmune Inc:* Current Employment, Current holder of *stock options* in a privately-held company, Patents & Royalties. **Brentjens:** *R.J.B. has licensed intellectual property to and collect royalties from BMS, Caribou and Sanofi. R.J.B. received research funding from BMS. R.J.B. is a consultant to BMS, Atara Biotherapeutics Inc, Coimmune, Triumvira and was a consultant for Gracell Bi:* Consultancy, Current equity holder in publicly-traded company, Current holder of *stock options* in a privately-held company, Membership on an entity's Board of Directors or advisory committees, Patents & Royalties: BMS, Caribou and Sanofi, Research Funding. **Nicolette:** *CoImmune Inc:* Current Employment, Current holder of *stock options* in a privately-held company, Patents & Royalties.

https://doi.org/10.1182/blood-2023-181005