Development of CAR T therapy for ALL-B using the point-of-care approach

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Introduction

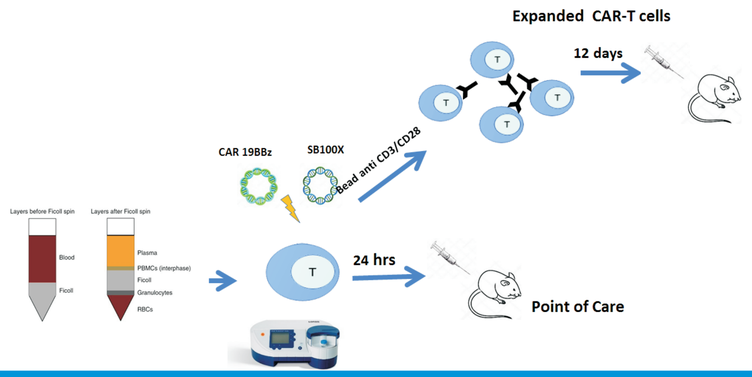
Recently, CAR-T cell immunotherapy was approved for the treatment of acute B cell leukemia (B -ALL) and some lymphomas with promising results. However, the major drawbacks of CAR-T treatments are the high costs, being prohibitive for many of the patients. One of the key aspects impacting the therapy price is the requirement to transport the leukocytes to and from specialized centers to cultivate and gene modify the cells, the use of viral vectors and the need to expand these cells to be re-infused into the patient. We developed an alternative low-cost approach to genetically modify T cells to express CARs using the transposon-based Sleeping Beauty (SB) system and electroporation. This combination allows the development of a so-called point-of-care (POC) approach, which consists in the production of the cellular product in the same place of the patient and reinfusion immediately without expansion of these cells. This approach may reduce the costs of these promising therapies, making them more accessible.

Objective

We herein describe an alternative protocol involving effectiveness to modify T lymphocytes with the SB Transposon and electroporation and functional activity of the CAR-T cell therapy generated using the point-of-care (POC) technique on in vivo models of immunodeficient mice engrafted with human B-ALL cells.

Methodology

- Peripheral blood mononuclear cells (PBMC) were isolated using Ficoll and electroporated using the Nucleofector IIb device combined with plasmids encoding 19BBz CAR in the pT3 SB transposon backbone and SB100x transposase.
- Tumor cells were inoculated intravenously into NSG mice (RS4;11 GFP+ or Nalm-6 GFP+) and were treated with human T cells expressing the 19BBz CAR generated in the POC approach (24 hours after transfection) or with T cells activated with TransAct beads and expanded cells 8 days (human).
- Tumor burden was monitored by analyzing GFP+ cells in blood samples. Animal welfare was monitored daily.
- All animal experimentation was performed after approval of the Institutional Research Ethics Committee.



Results

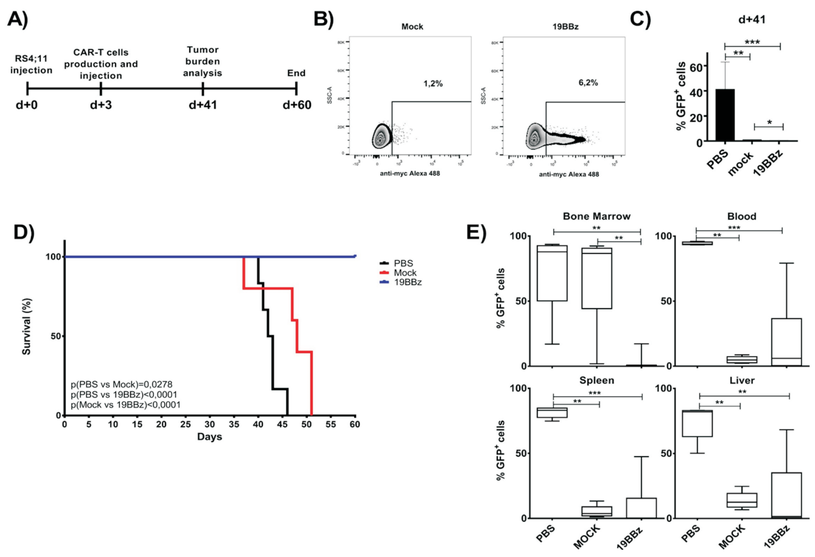


Figure 1: Evaluation of the potential antileukemic effect of the point-of-care approach. (A) Timeline of the experiment. NSG mice were inoculated with $5x10^6$ RS4;11 GFP tumor cells and, after 3 days, treated with CAR T cells electroporated 4 hours before treatment. (B) Expression of 19BBz CAR in T cells 24 hours after electroporation. Mock condition represents the electroporation of PBMC without 19BBz plasmid. A total of $6,2x10^5$ CAR-T cells were injected. (C) Animal blood was collected on day 41 for analysis of tumor burden of RS4;11 GFP by flow cytometry. (D) Kaplan-Meier plot of survival data (PBS n=6; Mock n=5; 19BBz n=9). (E) After euthanasia, tumor burden in blood, bone marrow, spleen and liver were analyzed by flow cytometry. The survival curve was analyzed by log-rank test and for organ analysis the Mann-Whitney test was used for paired comparisons. Consider * p <0.05, ** p <0.01, *** p <0.001.

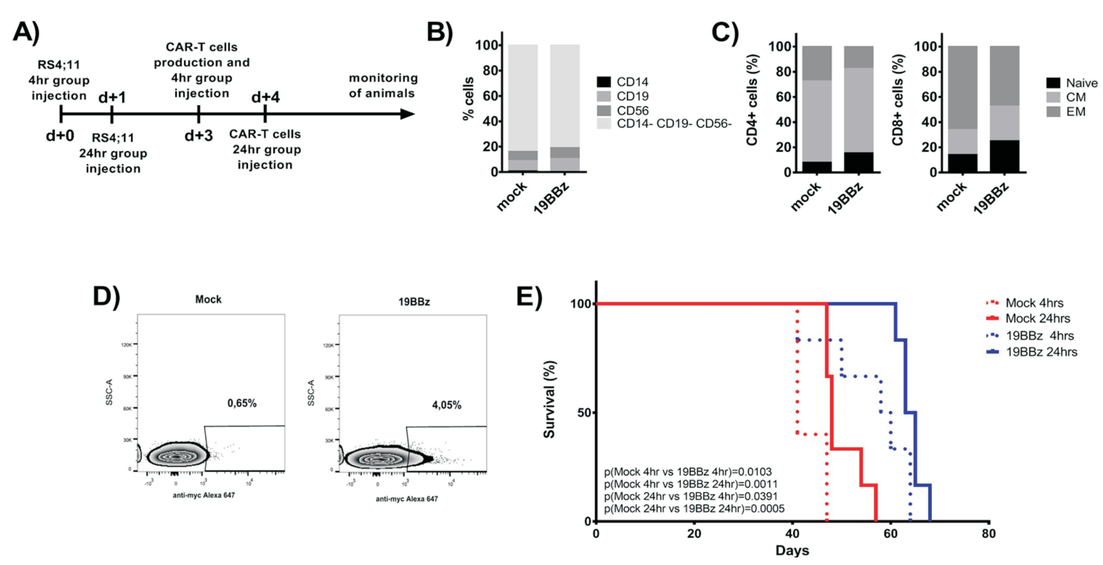


Figure 2: Comparison of antitumor activity in treated animals with cells electroporated 4 or 24 hours earlier. (A) Timeline of the experiment. Animals were treated with CAR T cells from the same donor, but at different times after its production: 4 or 24 hours after electroporation. (B) Immunophenotypic characterization of cells and (C) memory phenotype characterization evaluated in CD4+ and CD8+ T cells and represented by Naive (CD45RO-), Central Memory (CM, CD45RO+ and presence of either CD62L or CCR7 markers) and effector Memory (CD45RO+CD62L-CCR7-) 24 hours after electroporation. (D) Expression of 19BBz CAR in T cells 24 hours after electroporation. A total of $4x10^6$ CAR-T cells were injected.(E) Kaplan-Meier plot of overall survival data (Mock 4hrs n=5; Mock 24hrs n=6; 19BBz 4hrs n=6; 19BBz 24hrs n=6). The survival curve was analyzed by log-rank test.

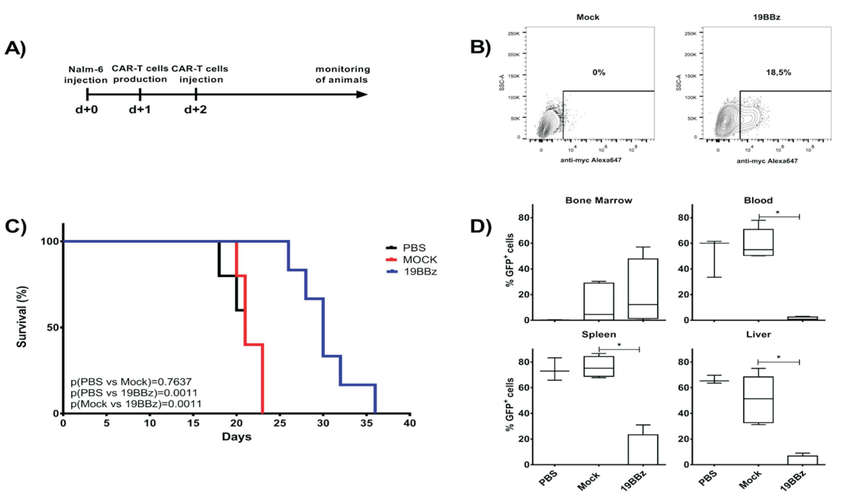


Figure 3: Effectiveness of point-of-care (POC) approach in animals engrafted with Nalm-6. (A) Timeline of the experiment. NSG mice were inoculated with 10^5 Nalm-6 GFP cells and were treated after 2 days with CAR T cells produced 24 hours earlier. (B) Expression of 19BBz CAR in T cells 24 hours after electroporation. A total of $7x10^5$ CAR-T cells were injected into each animal (C) Kaplan-Meier plot of survival data (PBS n=5; Mock n=5; 19BBz n=6). (D) After euthanasia, tumor burden of blood, bone marrow, spleen and liver were analyzed by flow cytometry. The survival curve was analyzed by log-rank test and for organ analysis the Mann-Whitney test was used for paired comparisons. Consider * p <0.05, ** p <0.01, *** p <0.001.

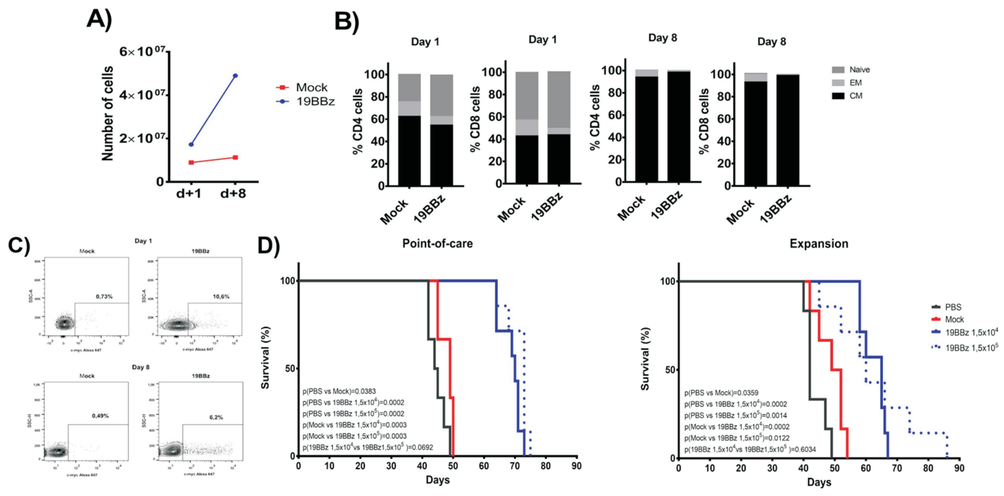


Figure 4: Comparison of the efficiency of antitumor activity between the Point-of-care approach and cells expanded with anti-CD3/CD28 coated beads. (A) Comparison of the expansion of CAR+ cells. Cells from this donor were used to treat NSG mice engrafted with RS4;11 in the POC or expanded cells approach. (B) Memory phenotype was evaluated at day 1 and day 8 after electroporation in CD4+ and CD8+ T cells and were represented by Naive (CD45RO-), Central Memory (CM, CD45RO+ and presence of either CD62L or CCR7 markers) and Effector Memory (CD45RO+CD62L-CCR7-). (C) Expression of 19BBz CAR in T cells 24 hours after electroporation and at day 8 of the expansion protocol. (D) Kaplan-Meier plot of survival data (POC group: PBS n=6; Mock n=6; 19BBz $1,5x10^4$ n=7; 19BBz $1,5x10^5$ n=7. Expansion group: PBS n=6; Mock n=6; 19BBz $1,5x10^4$ n=7; 19BBz $1,5x10^5$ n=7). The survival curve was analyzed by log-rank test.

Conclusion

- The POC approach has been shown to be effective in increasing survival in NSG animals grafted with RS4;11 or Nalm-6
- There is antitumor activity in treated animals 4 or 24 hrs after CAR-T electroporation, however, 24 hrs later allows pre-characterization of the cells used.
- Comparison of cells generated by the POC approach with expanded cells with anti-CD3 / CD28 beads showed no difference in survival curves.
- The POC-based approach for generating CAR T cells has shown promising, indicating that refinements can be made to achieve maximum efficiency.

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