ORT_02 - Improvement Car-T Cell therapy with ultra-fast protocoland il-15 membrane bound addition

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Introduction: Despite the advancement of new technologies for immunotherapy, gene therapy is far from democratized. Among them is CAR-T cell therapy, which has a great response in B-cell tumor patients but is very expensive. Basically, this therapy occurs with leukapheresis to remove the cells, taken to specialized laboratories, activated to proliferate, genetically modified with viral vectors to insert CAR, expanded for about 15 days, and returned to the hospital to treat the patient. This logistics can last about 1 month. In this project, we propose an ultra-fast protocol to decrease the time, cost, and complexities of CAR-T cell therapy. We use a transposon-based non-viral vector called Sleeping Beauty (SB) or PiggyBac (PB) that allows us to not activate the cell before gene insertion and consequently, the non-obligation to expand these cells. We insert CAR into T cells and in less than 24h we use these cells to treat grafted mice with leukemia. This protocol is called Point-of-care (POC) approach.

Objectives: Develop and refine an ultra-rapid protocol for CAR-T cell therapy.

Methodology: Mononuclear cells were isolated by Ficoll and electroporated using the Nucleofector IIb with SB plasmids encoding 19BBz CAR and SB100x transposase. For PB, we electroporated 10ug 19BBz PB with 20ug PBase. For *in vivo*, NSG mice were injected iv. $5x10^6$ RS4;11 or 10^5 Nalm-6 and after 3 days were treated with recently electroporated CAR-T cells.

Results: CAR expression on day 1 following electroporation ranged between 5-15% in all experiment with SB. Both mice models (RS4;11 and Nalm-6) were treated with our protocol to produce CAR-T cells (doses of $1x10^5$ and $7x10^5$ per mice, respectively) showed improved survival when compared to mice treated with mock electroporated cells and decreased tumor burden in blood and spleen was observed. Head-to-head comparison of 19BBz cells used in POC approach or expanded for 8-12 days *in vitro* showed similar antitumor activity *in vivo* against RS4;11 cells, leading to equivalent improvements in mice survival. After that, we added an IL-15 membrane bound receptor (mbIL-15) to CAR to improve cell persistence and animal survival and we used PB vector to insert the transgene. We noticed that the tumor burden evaluated by bioluminescence and the survival of animals that had 19BBz-mbIL15 was better when compared to only 19BBz (dose of $3x10^5$).

Conclusion: We can conclude that our proposed Point-of-care approach to CAR cell therapy can be explored as an alternative with less cost, time, and complexities. Furthermore, mIL15 added to CAR appears to bring benefits in fighting tumor in animal model.

Keywords: CAR-T cells, Immunotherapy, Point-of-care