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Abstracts
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Anti-CD19 CAR-T cells are efficient against CD19-positive 3D bioprinted solid tumor models
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Application of CAR-T cell demonstrated remarkable success in treatment of hematologic malignancies (HM), however this novel cell therapy is yet to prove its efficiency against solid tumors (ST). Poor clinical performance of CAR-T therapy in ST is primarily accounted for biological differences between ST and HM. Therefore it is important to develop models simulating in vivo conditions for testing effectiveness of CAR-T therapy against ST. In current study we evaluated anti-CD19 CAR-T cells against several 3D bioprinted solid tumor models. We constructed plasmid with 2nd-generation anti-CD19 CAR and also recombinant vector containing CD19 gene under control of internal ubiquitin C promoter and puromycin resistance gene. T-cells obtained from healthy donor were activated and transduced with lentivirus. CD19-positive cells were generated by transduction of MDA231, MDA468, A431, H522 solid tumor cell lines with CD19_p2a_PuroR recombinant lentiviral vector and further incubation with puromycin for selection. After that anti-CD19 CAR-T cells were applied onto CD19-positive tumor cell 3D constructs bioprinted using hydrogel composition. Efficacy of anti-CD19 CAR-T cells was assessed using viability assay and confocal microscopy. We propose that reported approach might be useful for screening and evaluating CAR-T against 3D solid tumor models.

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Consequences of maternal microchimerism upon CAR-T cell treatment
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With the use of efficient gene transfer technologies, T cells can be genetically modified to stably express antibody receptor (chimeric antigen receptors [CAR]) on their surface, conferring new antigen specificity. The consequences of maternal microchimerism (MM) in newborns of CART-treated women and the risk for newborns to suffer from B cell depletion are unknown. MM is acquired by an infant during pregnancy. Currently, two CART19 constructs are used in clinics. To stay close to the clinical setting, we cloned two 2-cistronic-lentiviral constructs containing CAR19-CD28 or CAR19-4-1BB and mCherry connected with T2A site using a lentiviral construct and tested them in vitro and in vivo. To achieve adequate transduction efficiency (TE), lentiviral constructs were concentrated and TE efficacy was confirmed using several methods. As immune-competent female bl/6 mice were used, preconditioning with cyclophosphamide was necessary to ensure engraftment of transferred CAR-T cells. Two cyclophosphamide concentrations were tested to determine a safe (effect on reproduction and on offspring rate) but effective cyclophosphamide concentration. Our observation showed decrease in lymphocyte population, but neither mal-formations nor effects on offspring rates were seen. Subsequently, mice were pre-treated with cyclophosphamide and dosed with 1x106 CAR-T cells (CAR19-CD28-mCherry, CAR19-4-1BB-mCherry or CAR19-mCherry ctrl). Localization and effects of CAR19 T cells were analyzed in both treated female mice and offspring. Furthermore, to ensure all facets on MM, we further improved the CAR-19 construct by cloning 3-cistronic-retroviral constructs consisting of Pmscv-P2A-mCherry-T2A-CAR19CD28 or 4-1BB-IL-12. Functionality is assessed by IncuCyte and Amnis technologies and ongoing in vivo mouse studies.

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Automated end-to-end manufacturing solutions for CAR-T immunotherapies
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Autologous cell therapies, such as CAR-T cells for cancer therapy, pose major cost and manufacturing challenges. The ideal solution to many of these challenges is to have the entire manufacturing process performed via a closed and automated system specifically designed to meet the needs. Our work using the novel CocoonTM system highlights the successful translation of a typical manual CAR-T process into the closed and automated CocoonTM system to reduce cost and maximize process efficiency and quality. The CAR-T process was performed using critical parameters such as starting inoculation of 100 million PBMCs, CD3/CD28 activation, IL-2 and IL-7 supplemented into T-cell growth media for culture expansion with an optimized and defined feeding strategy. The system was programed to run the entire process after inoculation automatically, without manual intervention. The in-process samples were drawn for cell counts and viability. At the end of the harvesting process, FACS analysis and killing assay were performed, the CAR-T cells reached approximately 2 Billion cells. Automated runs and associated manual controls were able to maintain both CD4+ and CD8+ T cell subsets. There was a higher detection of NGFR in the CD4 fraction than in the CD8 fraction in all samples. In summary, automated CAR-T process in the Cocoon system yields a healthy populations of T cell subsets. This system is a viable solution to translate labor-intensive CAR-T process into a fully automated system, thus allowing scalability, high yield, reduction of manufacturing cost, and better process control to yield high quality CAR-T cells.

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Analysis of lentivirus integration site distributions in CTL019 immunotherapy
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Tisagenlecleucel (CTL019) is an cancer immunocellular therapy reprogramming autologous T cells with a transgene encoding a chimeric antigen receptor (CAR), to target and destroy CD19 positive malignant cells. This report investigates