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Abstracts

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Anti-CD19 CAR-T cells are efficient against CD19-positive 3D bioprinted solid tumor models

A Valiullina[1] R Sayarova[1] A Petukhov[1 2] M Zhuravleva[1] E Bulatov[1] A A Rizvanov[1]

1: Kazan Federal University, Kazan, Russia 2: Institute of Hematology, Almazov National Medical Research Centre, Saint Petersburg, Russia

Application of CAR-T cells demonstrated remarkable success in treatment of hematologic malignancies (HM), however this novel cell therapy is yetto 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

A Tastanova[1] B Christen[1] A Del Rio Espinola[1] D Ibrahimaibo[1] P Ulrich[1] T Rubic-Schneider[1]

1: Novartis Pharma AG

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 immuno-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 malformations nor effects on offspring rates were seen. Subsequently, mice were pre-treated with cyclophosphamide and dosed with 1×106 CAR-T cells (CAR19-CD28-mCherry, CAR19-4-1BB-mCherry or CAR19-mCherryctrl). 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

Y Shi[1] J O'Conner[1] D Dutta[1] E Abraham[1]

1: Lonza

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

C Nobles[2] J Everett[2] F Male[2] J Gregg[2] S Reddy[2] F D Bushman[2] A Del Rio-Espinola[1] P Ulrich[1] S Kassim[1] J Hamilton[1] J Moggs[1]

1: Novartis Pharma AG 2: University of Pennsylvania School of Medicine

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