MODIFICATION OF T LYMPHOCYTES WITH LENTIVIRAL VECTORS FOR EXPRESSION OF ANTI-CD19 CHIMERIC ANTIGEN RECEPTORS (CAR)

Pablo Moço, Fundação Hemocentro de Ribeirão Preto
pablomoco@usp.br
Daianne MC Fantacini, Fundação Hemocentro de Ribeirão Preto
Marcela CC Freitas, Fundação Hemocentro de Ribeirão Preto
Kamilla Swiech, Faculdade de Ciências Farmacêuticas de Ribeirão Preto
Dimas T Covas, Fundação Hemocentro de Ribeirão Preto
Virgínia Picanço-Castro, Fundação Hemocentro de Ribeirão Preto

Key Words: Immunotherapy, CAR-T, CD19, Jurkat, Lentivirus, Copy number

The use of immunotherapy with modified T lymphocytes with chimeric antigen receptor (CAR) has been proven effective in the treatment of leukemias and lymphomas resistant to chemotherapy. CAR possess an extracellular domain derived from variable regions of antibodies and costimulation intracellular domains of T lymphocytes. CD19 protein has been shown to be an ideal target because it is expressed on most B-cell tumors as well as normal B cells, but not in other types of cells. Recent clinical studies involving anti-CD19 CAR T-cells have shown excellent responses in a variety of B-cell tumors, even in patients with relapse after high-dose chemotherapy. This study aimed to produce CD4+ lymphocyte lineage Jurkat (ATCC® TIB-152 ™) modified with a second generation anti-CD19 CAR with 4-1BB as intracellular costimulation domain. Lentiviral vectors were produced in HEK293T (ATCC® CRL-3216 ™) transiently transfected with plasmids containing the coding sequence of the CAR, viral envelope VSV-G, and viral capsid. The viral titer was calculated by real time PCR after transduction of HEK293T cells, resulting in 1.65×10^5 IU/mL. The literature indicates an MOI (multiplicity of infection) from 5 to 10 IU/cell for transduction of lymphocytes. A new batch of virus was produced, and the supernatant was ultracentrifuged at 19200 rpm (Beckman Coulter, SW28 rotor) in order to concentrate the viral particles. The viral titer of the concentrated batch was 1.26×10^8 IU/mL. This new titer is compatible with the necessary to infect 10^7 cells, amount of pre-expansion cells necessary to obtain the number of cells suitable for infusion into patients (2.5×10^9 to 5×10^9 cells). Then, the infection of Jurkat was performed in a 6-well plate with RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 2 µg/mL Polybrene®, and centrifugation at 1000 rpm for 20 minutes at room temperature. After 16 hours of incubation (37°C, 5% CO_2 and 85% humidity), the medium was exchanged for fresh RPMI 1640 10% FBS. After additional 48 hours of incubation under the same conditions, the cells were collected and was their DNA was extracted. We obtained by real-time PCR that the number of integrated viral copies per genome was 35.3 ± 4.5 (mean ± standard deviation) for transduction with MOI of 5 IU/cell. While for MOI of 10 IU/cell, it was obtained 42.6 ± 0.1 copies per genome. It was observed that there was not a significant increase in viral copies when the MOI increased from 5 to 10. This may occur because cell's surface receptors have been saturated by the large number of viruses. The lentiviral vector used by us has been shown to transduce T lymphocyte satisfactorily. The next steps of the study are the transduction of T lymphocytes from healthy donors and verification of the CAR receptor effectiveness to bind to CD19 of cell B lymphocyte lineages. Grant #2016/08374-5, São Paulo Research Foundation (FAPESP).