BIO_13 - A low-cost process of lentiviral vectors production for cell therapy

Laura Libânio de Souza[2]; Renata Schmieder Pivetta[1]; Najla Santos Pacheco de Campos[1]; Eloah Rabello Suarez[2].
[1]Unifesp
[2]Universidade Federal do ABC - UFABC

Introduction: Lentivirus production is a critical stage for the development of several cellular therapies, including chimeric antigen receptor (CAR) T cell generation, since the lentiviral vector is used to introduce the chimeric receptor gene into T cells. The quality of the lentiviral vectors produced can directly affect the effectiveness of the therapy, which may result in low CAR expression or high T cell cytotoxicity, with a consequent reduction in antitumor capacity. Moreover, the production of these vectors usually uses very expensive ultracentrifuges or high cost sedimentation reagents.

Objectives: In this context, this project aims to optimize a low-cost production of a lentiviral vector (G36/ZsGreen) for CAR T therapy, improving the efficiency of CAR transduction and expression, without compromising T cell viability.

Methodology: Lentiviral vectors will be produced by transient transfection of five plasmids into 293T cells using polyethyleneimine (PEI). Different variables were evaluated for optimizing the concentration of viral particles using a low-cost self-produced reagent that requires lower velocity for sedimentation in the centrifuge. Besides, we tested different values of a multiplicity of infection (MOI); cell densities for virus titration and filtration conditions. The evaluation of CAR expression in transduced cells was done by flow cytometry.

Results: Using different MOI values (1-20), we initially found that the T cell viability ranged from 10-40% 48 hours after transduction. Lower MOI values (1-0.0625) were evaluated using a pre-centrifugation condition, verifying that transduction levels varied between 15-25% of positive cells. Under these conditions, the viability levels reached 50-85% after 120 hours.

Conclusion: With this project, we hope to optimize and cheapen the lentiviral vector production for CAR T therapy, improving its effectiveness and contributing to the development of lower-cost and high-efficiency treatments for cancer patients. The project was approved by the institutional review board and CIBio (Technical opinion number 6839/2020).

Keywords: CAR T; Low cost method; Cell therapy vector