DEVELOPMENT OF A REPEATED CHALLENGE POTENCY ASSAY FOR EVALUATION OF IMMUNE CELL-MEDIATED CYTOTOXICITY IN VITRO

Denise Sullivan, Sayem H Bhuiyan*, George Huang, Danielle Califano, Stacie Chvatal, Daniel Millard. Axion Biosystems, Atlanta, GA, USA; Apexigen, San Carlos, CA, USA

Background: The development of immunotherapies relies on the use of in vitro potency assays—which are key for understanding complex interactions between immune (effector) cells and cancer (target) cells. Although a variety of in vitro assays are used to characterize the cell-mediated cytotoxicity of engineered immune cells, they often are unable to differentiate between various CAR T products that may be susceptible to exhaustion or reduced potency that can impede complete tumor eradication. Thus, in vitro potency assays that can recapitulate conditions of high tumor burden, induce T cell exhaustion, and allow for changes in T cell killing kinetics to be monitored in real-time are critical. Here, we describe an in vitro potency assay that quantifies immune cell-mediated cytotoxicity in response to repeated challenges with target cells.

Methods: The repeated challenge assay was performed with two separate protocols, each providing a unique assessment of the effector cell function. In the first method, SKOV3 target cells were seeded into a 96-well microplate with embedded electrodes in the substrate that detect the attachment and proliferation of target cells. HER-2 CAR T cells were added at 24 hours post SKOV3 cell seeding at E:T = 1:1. Cytolysis of the target cells was calculated by comparing treated wells to no treatment control wells. Every 24 hours, additional SKOV3 target cells were added to repeatedly challenge CAR T potency, with total tumor cell killing computed from the area under the impedance curve.

Results: The repeated challenge condition reached complete killing after the 6th tumor cell challenge (144 hours), whereas the single challenge condition reached complete killing within 96 hours of effector cell addition. Flow cytometry revealed that the effector cells proliferated significantly more for the repeated challenge condition (4.7x increase) than the single challenge condition (1.5x increase). In the second method, the CAR T cells were removed after each challenge, counted, and added to a new plate SKOV3 cells at E:T=1:1. Here, the E:T ratio was kept constant for each challenge, allowing a direct comparison of the time to kill 50% of the SKOV3 population for each subsequent challenge. The KT50 decreased from the first challenge (19.9 hours) to the fourth challenge (83.5 hours). Flow cytometry revealed a reduction in CD8+ cells for the repeated challenge condition, indicating a change in the make-up of the effector cell population.

Conclusions: The paradigms described above facilitate a label-free, in vitro workflow to assess potency of immune effector cells across repeated challenges.

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