Evaluation of CAR T cell expansion

Note: In this set of experiments, perform lentiviral transduction on T cells as conducted traditionally – and assess transgene expression prior to initiating experiments.

Experimental Condition 001

Day 0

1. Media administration – Using a G-Rex 100M, add **980 mLs** of complete T cell media.
2. Cell administration – Resuspend 100E+06 transduced T cells in **10 mLs** of media and transfer to the G-Rex 100M.
3. Growth factor administration – In **10 mLs** of media, calculate a final IL2 concentration of 100 U/mL for a total G-Rex volume of 1000 mLs. This will correspond to a total concentration of 100,000 U of IL2 per G-Rex 100M. [This is a general guideline but the same principle will apply to any other growth factor used in culture, such as IL7 or IL15. Simply calculate the final cytokine concentration in the G-Rex 100M by multiplying the cytokine concentration/mL by a 1000.]

Day 1 to 10

- The culture can be monitored by sampling the media surface and measuring the glucose and lactate concentrations. [This information will be important to collect to better understand the kinetics of cell growth as well as determine the ideal day of cell collection. In general, glucose concentration should not drop below 50 mg/dL and lactate concentration should not be higher than 12 mg/dL.]
- Cytokine administration should follow the traditional schema. [For example, 100,000 U of IL2 can simply be added to the culture using a micropipette every 2-3 days without mixing or stirring the device.]

Day 10

Gently place the G-Rex in the hood for 5-10 minutes and remove 900 mLs of media using an aspiration pipette. Use the residual 100 mLs to resuspend the cells in the culture by stirring the device. Using a pipette gun, mix thoroughly and harvest the final product in a collection tube. [The final cell number should be between 1.5-2.5E+09 cells/G-Rex 100M.]

In general, it is important not to mix or stir the G-Rex throughout the culture as T cell growth can be affected by this disturbance.

Experimental Condition 002

Perform as Experimental Condition 001 but simply decrease the seeding density outline in Step 2 to 50E+06 transduced T cells per G-Rex 100M.

2. Cell administration – Resuspend 50E+06 transduced T cells in **10 mLs** of media and transfer to the G-Rex 100M.

Experimental Condition 003

Perform as Experimental Condition 001 but simply decrease the seeding density outline in Step 2 to 25E+06 transduced T cells per G-Rex 100M.

2. Cell administration – Resuspend 25E+06 transduced T cells in **10 mLs** of media and transfer to the G-Rex 100M.

This series of experimental conditions will determine the minimum seeding density required for the culture to perform. In general the higher the seeding density, the more consistent and robust performance but this can also lead to the least fold-expansion as the maximum number of cells derived from the G-Rex 100M seems to be a constant related to the cell type. Therefore, the purpose of these experiments is to determine the minimum number of cells that can be tolerated for your culture conditions, thereby evaluating a high seeding density (Experimental Condition 001) of 1E+06 cells/cm^2, an intermediate seeding density (Experimental Condition 002) of 5E+05 cells/cm^2 and a low seeding density (Experimental Condition 003) of 2.5E+05 cells/cm^2.