Spin-based transduction of human T cells

A Production of retrovirus

Day 1 (**Monday**):Seeding of packaging cells

- Plate FLYRD18 cells in RPMI media (10% FCS and Pen/Strep) into MW6 @ 3E5 cells/well in 2 mL

Day 2 (**Tuesday**): Transfection

- Virus-packaging cells should be approx. 70% confluent
- Allow serum-free medium (SFM; Optimem) and XTREMEGENE to warm up to RT (pulse vortex to mix)
- Leave (or remove until) around 2 mL of medium in well (for MW6; see table for other formats)
- For each condition to transfect prepare SFM, add XTREMEGENE directly to medium & mix (flick tube; see table for amounts)
- Incubate 5 minutes at RT
- Add retroviral plasmid DNA and flick to mix
- Incubate for 15-20 minutes at RT
- Spread transfection reaction over the culture dish and mix
- After ± 2 hrs add back half of regular culture medium amount (2 mL for MW6) and incubate

Day 4 (**Thursday**): Harvest of virus supernatant

- Remove media supernatant from culture dish
- Centrifuge at 2000 RPM for 10 minutes to avoid carry-over virus-producing cells
- Virus can be snap-frozen and stored at -80C and/or used directly for retroviral transduction

B Activation of human PBL

Day 2 (**Tuesday**): Bead-activation of PBLs

- Thaw buffy-coat cells and dissolve in Buffer 1 (PBS with 5% human serum)
- Count cells and determine amount of CD3+ cells (assume approx. 50% or determine by FACS)
- Two CD3/CD28 beads (Human T cell expander; Invitrogen/Dynal) per CD3+ cell are required (cell count = # of beads to use). Wash an appropriate amount of beads twice with Buffer 1 using a magnet. Dissolve beads afterwards in volume of Buffer 1 appropriate to adjust PBL density to 10 x 10^6 **CD3+** cells/mL
- Incubate cells for 30 minutes at RT on a tumbler
- Place cells on a magnet to remove supernatant with non-bead bound cells
- Resuspend bead-bound cells at a density of 0.5-0.75 x 10^6 **CD3+** cells/mL (assume 100% recovery of initial CD3+ cells) in RPMI media (Gibco; supplemented with 10% human serum and P/S) containing IL-15 (Peprotech 10 ug/mL stock, dilute 2000x; 5 ng/mL final) and rh-IL-2 (Novartis 3E6 IU/mL, dilute 30.000x; 100 IU/mL final).
- Plate 2 mL/well into a tissue-culture treated 24-well plate

C Retroviral transduction

Day 3 (**Wednesday**): Retronectin-coating of 24-well plates for spin-transduction

- Prepare a 10 ug/mL retronectin (Takara) solution in PBS
  Storage of retronectin: we dilute the stock to 50 ug/mL in PBS and store it in the fridge for several weeks. Add 0.5 mL per well into a non-tissue-culture-treated 24-well plate
- Store in the fridge O/N

Day 4 (**Thursday**): Retroviral transduction

- Remove retronectin from coated plate, add 0.5 mL of blocking solution (2% BSA in PBS) and incubate for 30 minutes at RT. Remove blocking solution and add 1 mL of PBS. Leave PBS in plate.
- Harvest activated PBLs and count
- Remove PBS from retronectin-coated plate
- Resuspend PBLs at ~0.5E6 cells/mL in RPMI medium (with 10% HS and P/S) containing 10 ng/mL IL-15 and 200 IU/mL rh-IL-2 and seed 1 mL/well. Add 1 mL virus for 2x dilut.
- Spin plate at 2000 RPM for 90 minutes at RT with slow acceleration (‘3’) and no brake (‘0’).

Day 5 (**Friday**):

- Split cells (1 mL to new plate) and add 1 mL fresh RPMI media (supplemented with 10% human serum and Pen/Strep) containing 10 ng/mL IL-15 (dilute 1000x for 5 ng/mL final) and 200 IU/mL rh-IL-2 (dilute stock 15.000x for 100 IU/mL final)

Transduction efficiency is determined at Day 8 (Monday after) by antibody stain (CD3, CD4, CD8, MM/murTCRcβ).

Transfection parameters:

	for MW24	**for MW6**	
Cell seeding	5E4	3E5	**Cells/well**
SFM (uL)	50	100	**SFM (uL)**
XTRGene (uL)	1.5	9	**XTRGene (uL)**
DNA (1 ug/uL)	0.5	3	**DNA (1 ug/uL)**
Add to well (uL)	50	100	