EXPERIMENTAL PROTOCOL: In vivo CAR-T cell models

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Introduction

For in vivo CAR-T cells assay it is important to consider the latency of engraftment of the target cells we would like to test. So it is highly recommended to do a pilot experiment with 2-3 mice and inject the same amount of target cells we would like to use in our experiments. Engraftment will be followed by periodical blood or BM analysis. This protocol is specifically designed for analysis of B-ALL models.

In vivo protocol

1. Use ten-week-old non-obese diabetic (NOD) Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice (The Jackson Laboratory) or corresponding immunodeficient mouse strain.
   For B-ALL cell lines (NALM6 or SEM), NSG mice could be intravenous (i.v.) or intratibial (i.t.)-injected (no irradiation needed) with 1×10^5 cells. It is preferred to inject them intravenous since it is easier, it works similarly and we can perform one BM aspirate more (in total we can do 3 i.t. injections separated by 6 weeks in alternative legs).
   For PDXs cells, inject $0.5–1.0\times10^6$ B-ALL cells i.v. (or i.t.) injected in sublethally irradiated (2 Gy) NSG mice.

2. If target cells are Luc+, mice are followed-up weekly by bioluminescence imaging (BLI) using an in vivo imaging system (IVIS, Lumina III; Perkin-Elmer).

3. Peripheral blood (PB) analysis are performed weekly (in the case of cell lines) or beweekly (in the case of PDXs, starting at week 6. This depends on the latency of the PDX cells).

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PB cells are stained with the following antibodies:

		1 sample
PBS		40uL
HLA-ABC	PE	2uL
CD45	BV510	2uL
CD22	APC	1uL
CD19	BV421	1uL
CD10	PECy7	1uL
CD3	PerCP	1uL

- Cells are incubated 20 min RT.
- Add 2mL BD Buffer Lysis. Incubate 10 min RT.
- Add 2 mL of PBS.
- Spin 5 min 1500 rpm. Remove supernatant.
- Wash with 2 mL PBS.
- Spin 5 min 1500 rpm. Remove supernatant.
- Add 200uL PBS.
- Read in cytometer.

4. Ideally, inject the CAR-T cells when the target cells are detected in blood (1%) or in BM (10%). This percentages are illustrative and depend on the aggressiveness of the disease. Perform a PB/BM/BLI analysis the day before the injection. Inject i.v. 4-5×10^6 CAR T cells (or desired amount) in 100uL of volume in PBS.

5. Followed-up periodically by PB/BM/BLI analysis the evolution of the target cells.

6. At the desire endpoint, sacrifice the mice and collect PB, bones, spleen and liver to assess leukemic burden and CAR T cell persistence by flow cytometry. We do the above staining for the cells of all organs: Human cells are identified with HLA-ABC and CD45; T cells are identified with CD3 and inside these CAR T cells are the GFP+ ones (if the CAR vector contains the GFP reporter); CD19, CD22 and CD10 are used to identified the target cells in case of B-ALL leukemia. It is important to include the marker the CAR is targeting to identified a problem of antigen downregulation or if persistent target cells have lost the specific targeted antigen. Include one ore two additional markers that identified the target cells. A representative gating strategy is shown below (from Zanetti *et al.*, Mol Ther, 2021).

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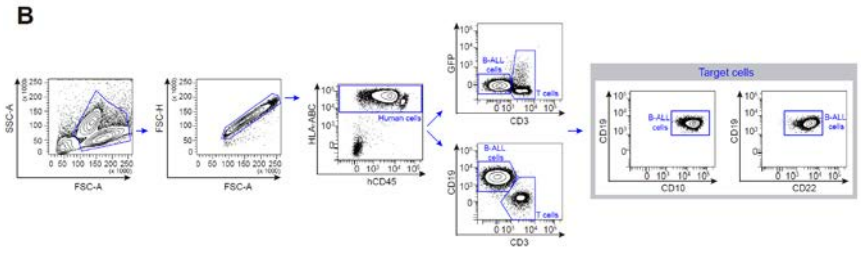
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SSC-A
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hCD45
Human cells
GFP
CD3
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CD19
CD3
Target cells
CD19
CD10
CD22