Experimental Plan

Callum Malcolm

2023-12-07

Contents

Project 1: A20 Modelling the BL Tumour Microenviroment

Overview

- Using A20 to model BL immune TME
  - Look specifically at T-cell infiltration
  - Immune evasion mechanisms deployed by BL
- Compare results to patient data
- Apply findings to humanised mouse models of BL PDX

Background

- The impact of immune TME in BL is unclear
- BL

Experimental Plan

E1: NSG vs BALB/C A20 injection

E1 Overview

- Compare between immunocompetent and immunocomprimised mice
- Basic actors to compare
  - Tumour growth rate
  - Tumour size

Injection Plan

Group	Strain	Location	Cell Injection
1	BALB/c	Sub-cut	A20
2	BALB/c	IP	A20
3	NSG	IP	A20
4	NSG	IP	A20

IHC Panel

- Compare markers between tumour types
- Burkitt IHC indentification:
  - CD10+ (B-Cell Germinal Centre)
  - Bcl-2-
  - Ki-67%hi (proliferation index)

  Potential IHC Panel Markers

Cell Type	Marker
Proliferation marker	Ki67
B cell	CD20
T cells (all)	CD3
T Cells (cytotoxic)	CD8
T cells (helper)	CD 4
Dendritic Cells	CD11c
Macrophage	F4/80

Flow Panel

- Options are:
  - Standard T Cell
  - TRegs
  - B-cells
  - DC Mono CD11c
  - Th17
  - TfH

Standard T Cell

Cell Type	Marker
T Memory	CXCR3
Naive Immune Cells	CCR7
Naive T Cells	CD45RA
Th17	CCR6
GC B-Cells	CD38
TRegs (Helper)	CD4
Macrophage	HLA-DR
T Cells (all)	CD3
T Cells (Cytoxic)	CD8

E2: Trial BL Therapies

- CAR drugs
- Bi-Specific antibodies (check that they can be applied to mice)
- Rituximab comparison
  - Clonal dynamics of Rituximab treatment
    - WILDseq

E3: Immune focused CRISPR Screen

- Immune compromised vs Immune competent
  - Think very carefully about specific mouse models (some still have macrophages, NK cells, ect.)
  - JAX Lab Article

Project 2: Spatial Transciptomics of BL

11 Samples of BL acquired from VIVO BioBank

Looking at characterizing the Human BL TME - Publically available BL Data - VISIUM internal samples - Mibiscope Internal Samples

Publically available datasets analysis - Talk to Jamie again

- 10x Visium transcriptomics
  - T Cell Dynamics
    - Infiltration
    - Exhaustion
  - NK cell dynamics
  - Places to get it done:
    - CI (no)
    - Source Bioscience
    - Wellcome Sanger Institute

        - Teichmann Lab used 10x Visium
- MIBIScope Comparison
  - Does Protein expression map onto spatial gene expression?
  - Collaborate with Nina in Germany