

Experimental Plan

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2023-12-07

Contents

Project 1: A20 Modelling the BL Tumour Microenviroment	2
Overview	2
Background	2
Experimental Plan	2
E1: NSG vs BALB/C A20 injection	2
E2: Trial BL Therapies	3
Loncastuximab tesirine	3
E3: Immune focused CRISPR Screen	3
Project 2: Spatial Transcriptomics of BL	3
Paper Plan	4
Thesis ID	4
Publically Available RNAseq	5
Ideas	5
Ferritin as a drug delivery system	5

Project 1: A20 Modelling the BL Tumour Microenvironment

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 immunocompromised 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	Sub-cut	A20

IHC Panel

- Compare markers between tumour types
- Burkitt IHC identification:
 - 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
- Check what Swetha ordered
- Box 7 antibody sheet dropbox

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 (Cytotoxic)	CD8

E2: Trial BL Therapies

- Balb/c drug vs no drug vs WS-A20 drug vs WS-A20 no drug
 - Want to compare effect of GFP on immune cell infiltration
 - Look at clonal dynamics of rituximab treatment
- Glo-BNHL Trial
 - Odronextamab
 - * CD20xCD3 Bispecific antibody
 -

Loncastuximab tesirine

- Rituximab
- CAR treatments?
- 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 Transcriptomics of BL

11 Samples of BL acquired from VIVO BioBank

- Want to do stuff similar to these studies:

- * [PDAC Spatial Transcriptomics](#)
- * [Genomic/Transcriptomic analysis of Clinical BL Samples](#)

Paper Plan

Based on PDAC ST paper

- Panel 1:
 - Patient Data
 - a. Correlation survival studies between immune cell infiltration and survival
- Panel 1:
 - Patient sample characterisation
 - a. Work Flow (sample backgrounds, collection from sources)
 - b. Overview of all cell types profiled in the scRNA-seq cohort.
 - c. Overview of spatial transcriptomics cohort
- Panel 2
 - Characterize the tumour (Tumor vs stroma vs immune cells)
 - a. Differential pathway enrichment case-level tumor subpopulations
 - b. Tumor cluster pathway enrichment for specific case
 - c. Tumor cluster pathway enrichment for another specific case
 - d. UMAP of tumor subclusters for specific case
 - e. UMAP of tumor subclusters for specific case
- Panel 3
 - Genomic Landscape and oncogenic driver heterogeneity (??)
- Panel 4
 - Tumour cell heterogeneity in ST data(?)
- Panel 5
 - Immune Populations in TME
- Panel 6
 - Mibiscope

Thesis ID

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

Publically Available RNAseq

1. The iDEP or TACITuS pipeline for transcriptomic profiling and GSEA/pathway analysis.
2. The CIBERSORT immune-cell deconvolution algorithm to obtain cell-fractions and gene expression/enrichment scores of tumor-infiltrating lymphocytes.
3. The GEPIA2 database for survival analysis correlating to immune infiltration and/or differential gene expression.

Ideas

Ferritin as a drug delivery system

- Does BL have increased Tfr1 expression relative to normal cells/B cells?