Developing *ex-vivo* leukaemia models through multi-omic characterisation of the bone marrow microenvironment

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1. Background

B-Cell Acute Lymphoblastic Leukaemia (B-ALL)

ALL is the most common paediatric cancer, with B-ALL resulting from aberrant proliferation of B-cell progenitors. B-ALL has at least 26 recognised subtypes, each associated with differing age groups, treatment regimens, and clinical prognoses.

The Bone Marrow Microenvironment (BMM)

The BMM is made up of niches that support healthy haematopoiesis. However, these niches can also protect leukaemia cells. For example, mesenchymal stromal cells (MSCs) can transfer mitochondria or release extracellular cyst(e)ine to reduce reactive oxygen species-induced B-ALL cell death following chemotherapy [1-3].

2. Justification and Aims

Poor understanding and representation of patient BMM complexity in 2D culture or murine models often renders promising preliminary therapeutic results difficult to study or replicate. This, alongside ethical considerations of animal use in research, highlights the need for improved *ex-vivo* models.

We aim to characterise the leukaemic vs healthy BMM to determine possible metabolic dependences or signatures which can be used to curate representative 3D primary hydrogel models and exploited through novel or combination therapies.

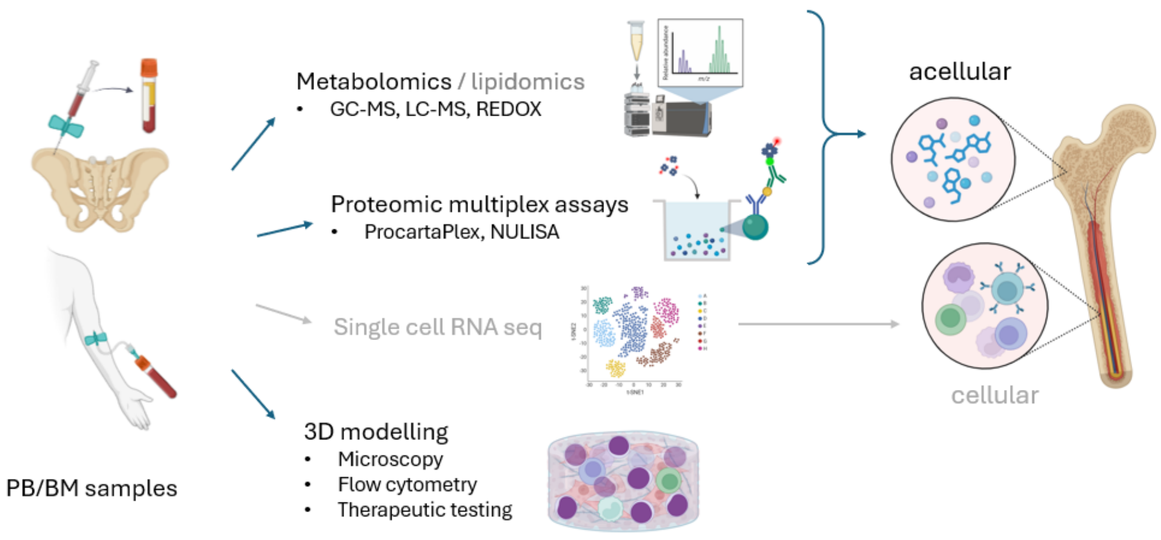
3. Methods

Profiling of healthy vs leukaemic bone marrow (BM) and peripheral blood (PB) plasma:

- NULISA multiplex immunoassay
- Gas chromatography-mass spectroscopy

3D culture of human B-ALL and stromal cell lines:

- PeptiGel (fully synthetic, self-assembling hydrogel)
- SEM (KMT2A-AFF1), REH (ETV6-RUNX1) and HS5 (MSC) cell lines
- Cells are grown in 50 or 100 ul of gel for up to 14 days



4. Results: Profiling the BMM

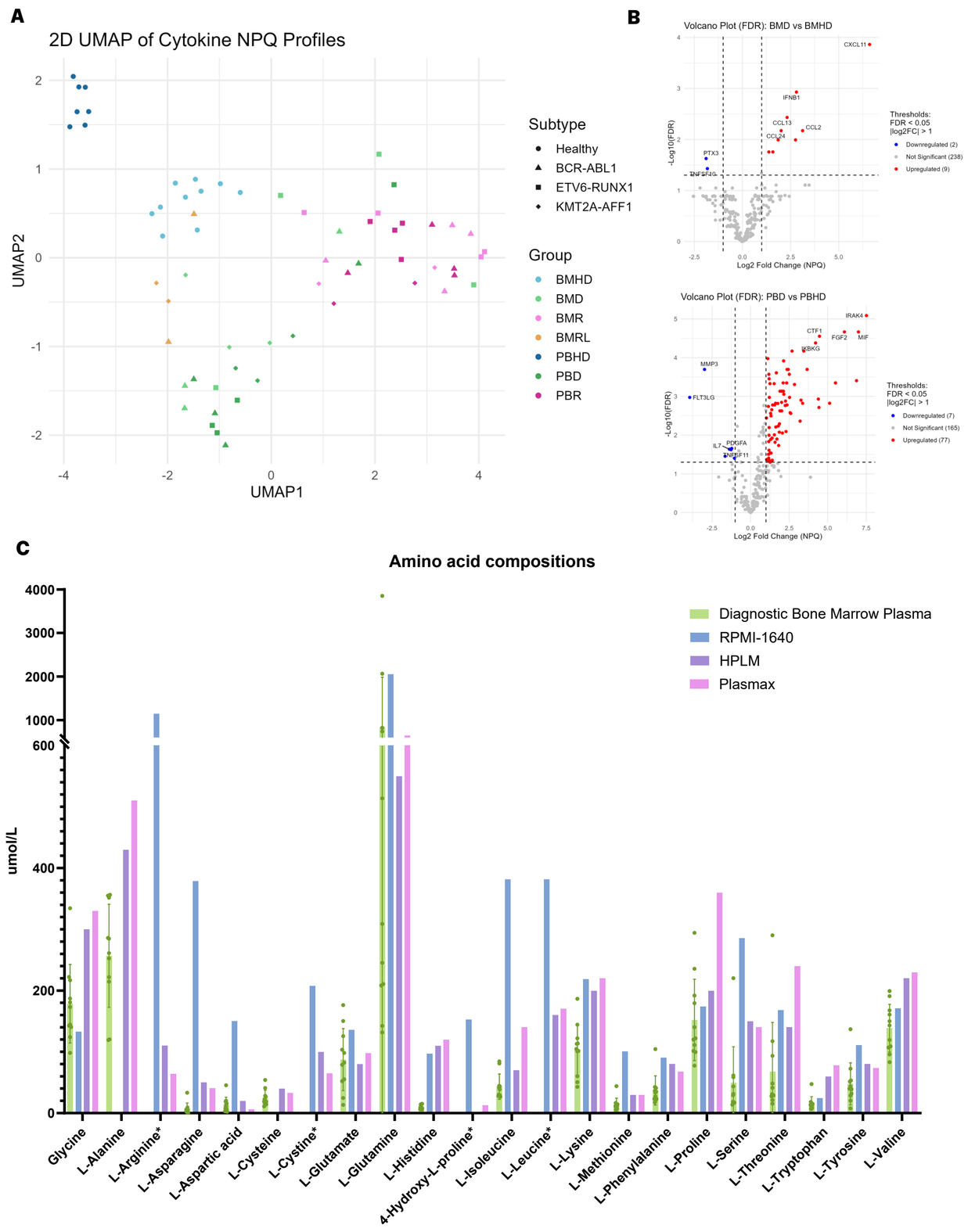


Figure 1: Proteomic and metabolomic profiling of B-ALL vs healthy samples. A) UMAP clustering of NULISAseq cytokine profiles. B) NULISAseq significant up and downregulated cytokines in diagnostic bone marrow (above) or peripheral blood (below) samples. C) Amino acid concentrations in diagnostic bone marrow plasma (N=11) by GC-MS vs RPMI-1640, HPLM, and Plasmax manufacturer concentrations.

5. Results: Modelling the BMM

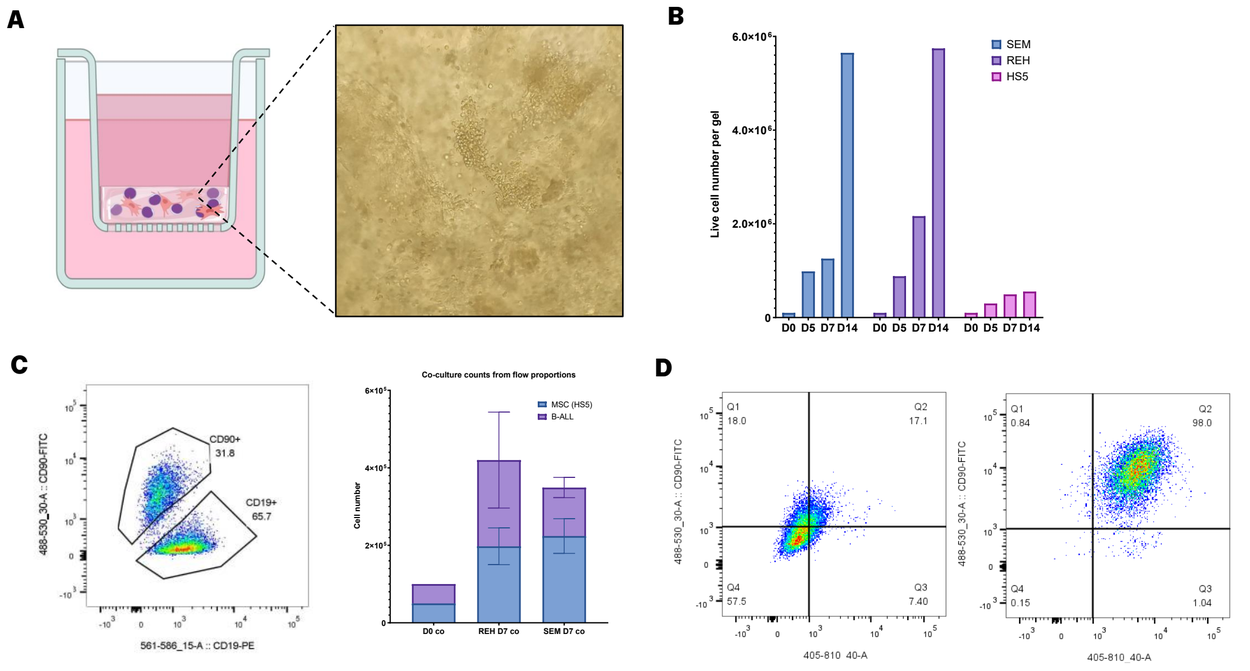
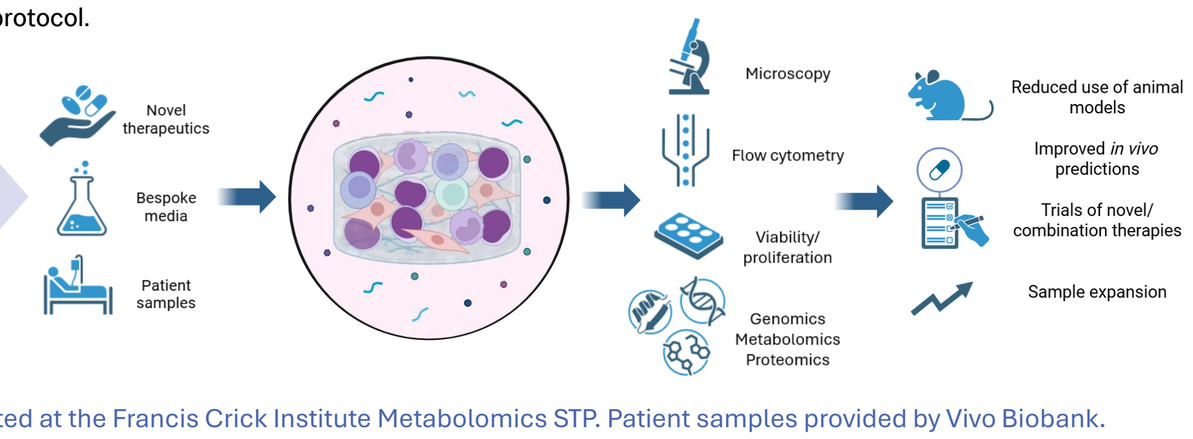


Figure 2: PeptiGel cell line optimisation. A) Cells are grown in 50ul (up to D7) or 100ul (up to D14) of hydrogel within transwell inserts. B) Cell line proliferation in gel monoculture. Cells were always ≥98% viable following extraction. C) Initial flow cytometry analysis following B-ALL co-culture with MSCs. D) Extraction and flow cytometry optimisation. Left: HS5 original extraction protocol. Right: new extraction protocol.

6. Conclusions and Future Work

- Initial profiling reveals clear environmental differences between standard cell culture conditions, healthy BM, and leukaemic BM.
- B-ALL and MSC lines grow with high viability in easy-to-use synthetic hydrogel models that facilitate downstream analyses.

Using our profiling results, we aim to further develop 3D culture conditions to better replicate the *in-vivo* microenvironment. This model can then be used to trial current and novel therapies (such as the BiTE Blinatumomab, or the cyst(e)ine degrader Cyst(e)inase) on primary patient samples.



[1]Burt *et al. Blood,* 2019 [2] Burt *et al*. *BloodAdv,* 2024 [3] Burt *et al*. HemaSphere 2024;8:(S1):p13. GC-MS conducted at the Francis Crick Institute Metabolomics STP. Patient samples provided by Vivo Biobank.