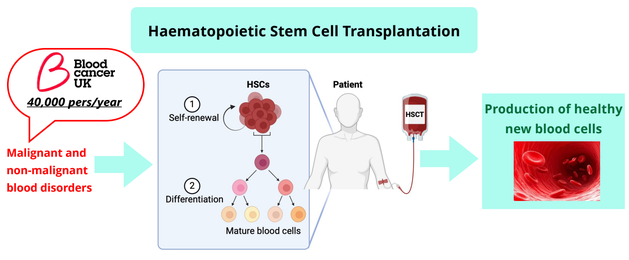
Bioengineering the human foetal liver microenvironment with 3D co-culture systems to support hepatic specification and haematopoiesis

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Background

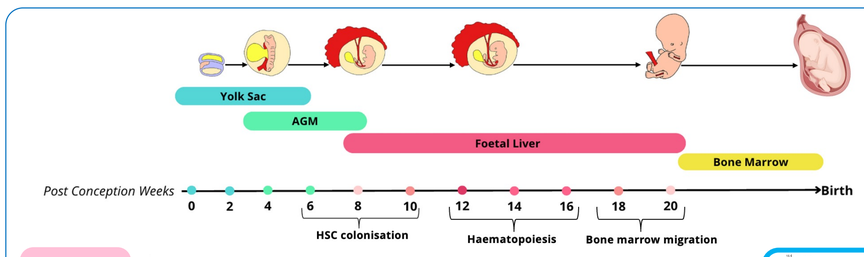
Blood cancers such as leukaemia, lymphoma and myeloma are the 5th most common cancer type in the UK, with patients relying on allogeneic Haematopoietic Stem Cell (HSC) transplants to replenish their HSC pool. However, HSC quiescence and difficulties expanding HSCs *ex vivo* remain key limitations. The foetal liver (FL) is the primary haematopoietic organ between 8-18 pcw, before HSC migration to the bone marrow. While the Bone Marrow (BM) niche has been extensively studied, the specific microenvironmental cues including stromal cells and ECM driving haematopoietic stem progenitor cell (HSPC) expansion in the FL remain poorly understood.



Aims

Explore the role of the Foetal Liver (FL) cellular and intracellular compartments in the haematopoiesis and to recapitulate *in vitro* the conditions supporting HSPC expansion in the FL. Overall, we aim to develop a 3D HSC *in vitro* culture environment and unlocking the FL haematopoietic niche through analysis of various components of the FL throughout development.

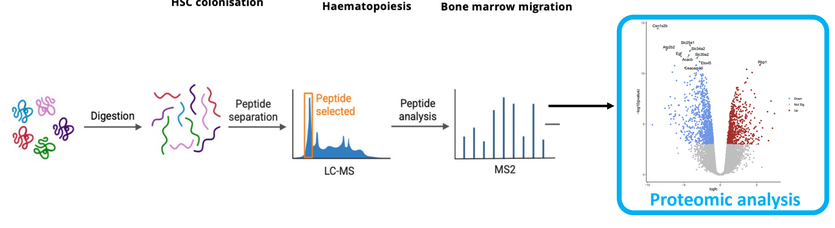
Materials & Methods



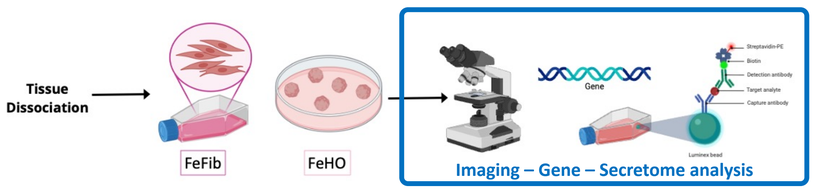
To characterise the human FL niche, we analysed its key cellular and molecular components including stromal cells, secreted proteins and cytokines across development.

Foetal Liver

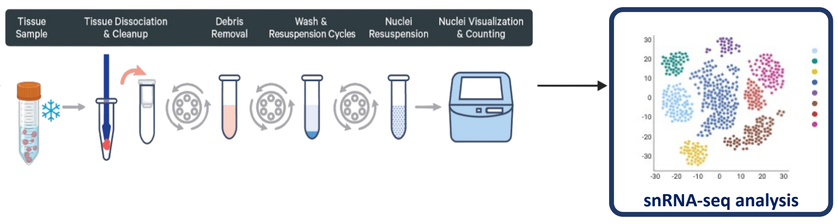
8-20pcw



Proteomic analysis compared HSC colonisation (8–10 pcw) versus Post-HSC colonisation (12–20 pcw) stages across 28 FL tissue samples by TMT-labelled proteomics.



FL fibroblasts and organoids were derived from 8–20 pcw samples, expanded up to 15 passages, and characterised alone or in co-culture by Luminex cytokine analysis and gene expression profiling.



snRNA-seq was performed on 16 FL samples (8–18 pcw) using the 10x Genomics GEM-X Flex v2 kit, multiplexed into 4 pools and demultiplexed with Cell Ranger multi v10.0.0. After QC filtering, 264,578 high-quality nuclei were normalised, batch-corrected (Harmony) and clustered (resolution 0.3) following cell cycle regression, yielding 25 transcriptionally distinct populations annotated using canonical FL markers.

Results

Differentially expressed extracellular matrix protein between 8-10pcw and 12-20pcw within the foetal liver

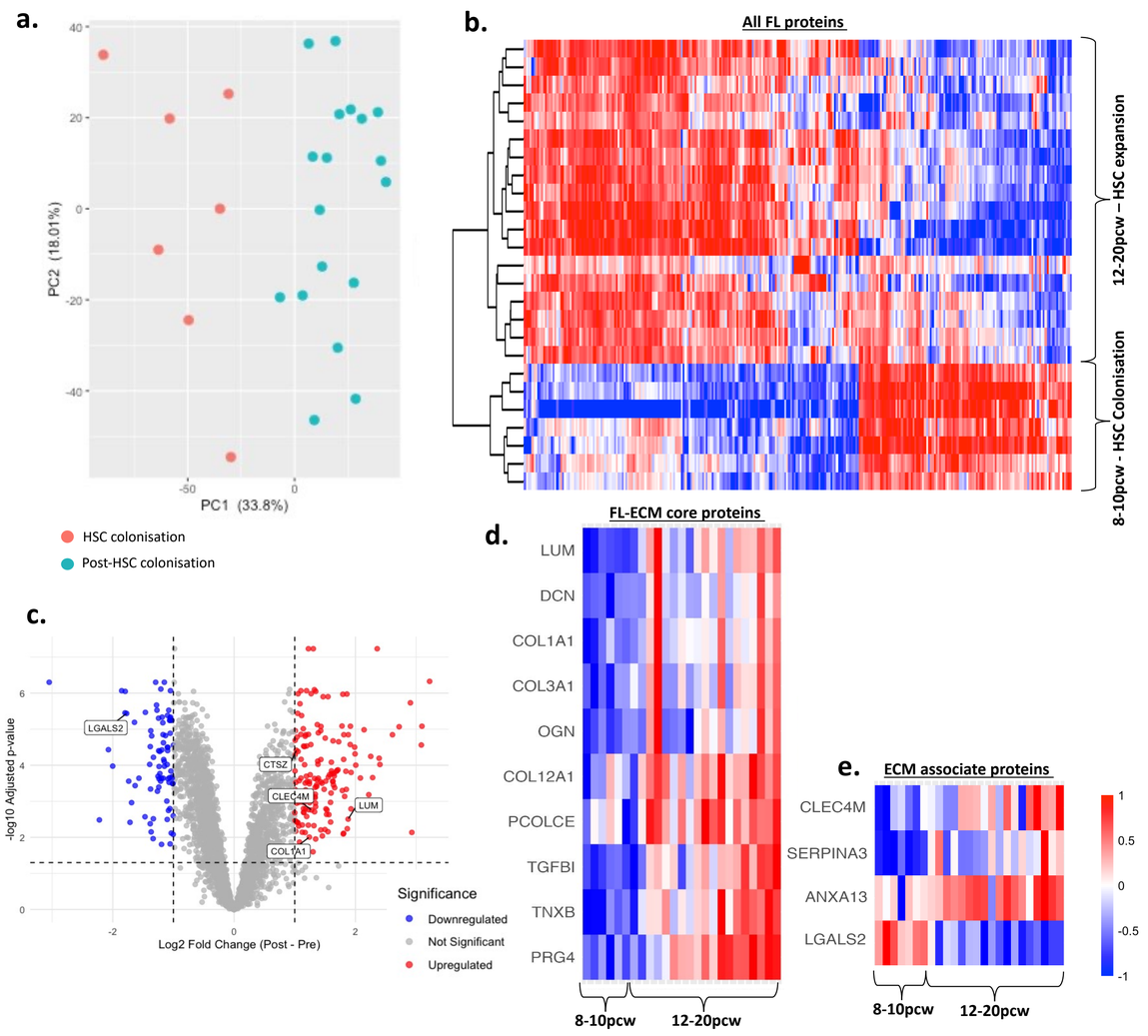


Figure 1 a. PCA plot of foetal liver samples analysed via proteomics. b. Heatmap with dendogram of upregulated and downregulated proteins between 8-10pcw vs 12-20pcw of the FL. Significance was calculated with a log2 fold change of >1.5, and a p-adjusted value of <0.05. **c.** Volcano plot of all proteins detected via TMT-labelled proteomics, comparing 8-10pcw to 12-20pcw samples. **d.** Heatmap of significant ECM core proteins found when comparing 8-10pcw to 12-20pcw stages. **e.** Heatmap of significant associated ECM proteins detected between 8-10pcw to 12-20pcw stages.

FL fibroblasts and hepatoblast organoids produce haematopoietic cytokines and exhibit gene expression differences according to developmental stage of derivation *in vitro*

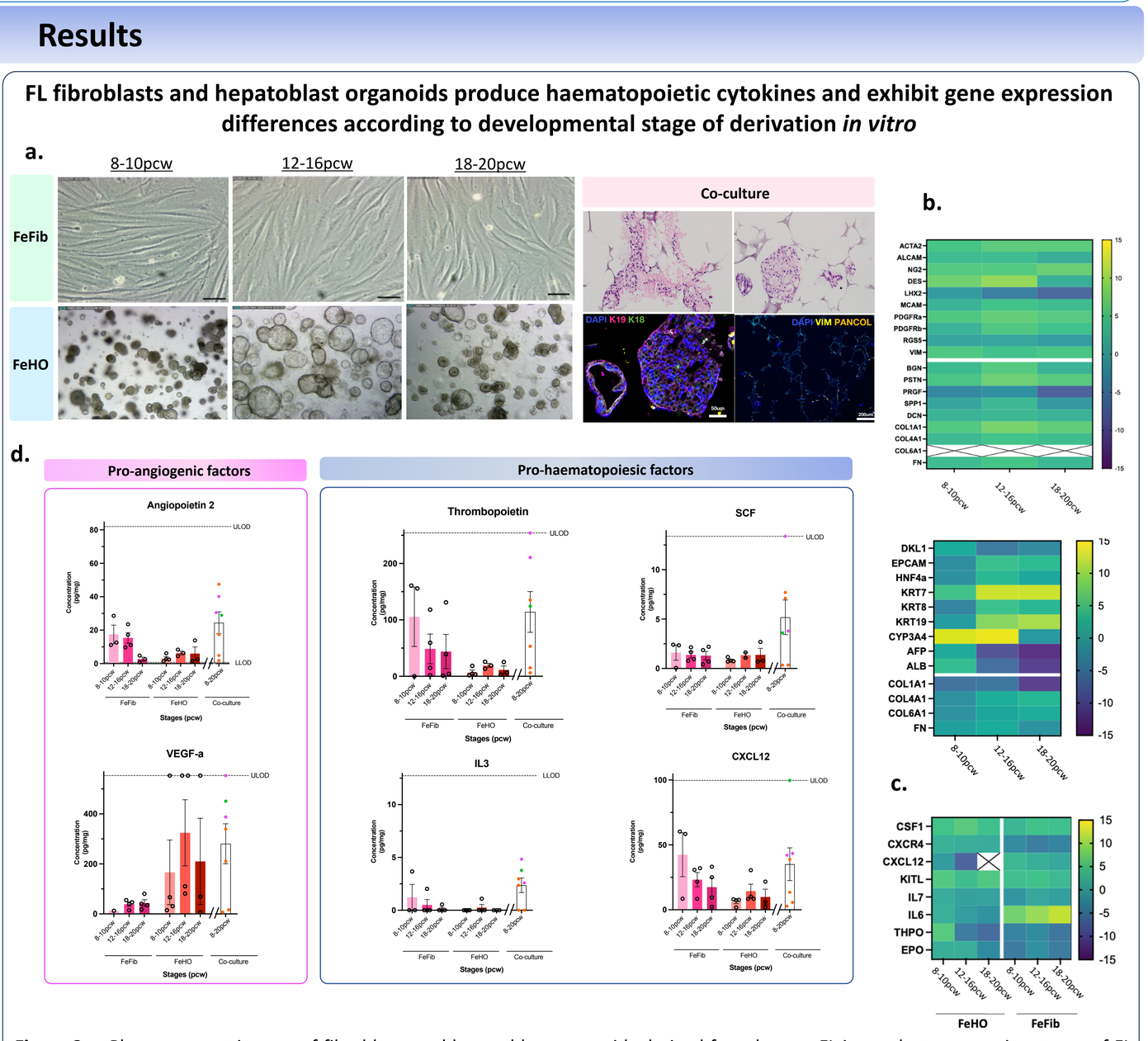


Figure 2 a. Phase contrast images of fibroblasts and hepatoblast organoids derived from human FL imaged at consecutive stages of FL haematopoiesis (scale bar = 100um) **b.** Characterisation of fibroblasts and organoid with phenotypic markers of fibroblast, hepatoblasts, cholangiocytes, stellate and parenchymal cells and ECM markers **c.** Expression of pro-haematopoietic markers found in foetal fibroblasts and organoids.**d**. Haematopoietic and angiogenic cytokine secretion from 8 to 20pcw in foetal fibroblasts and organoids (n=4). Upper/Lower Limit Of Detection (U/LLOD)

snRNA-seq of the human FL across gestational development (8-18pcw)

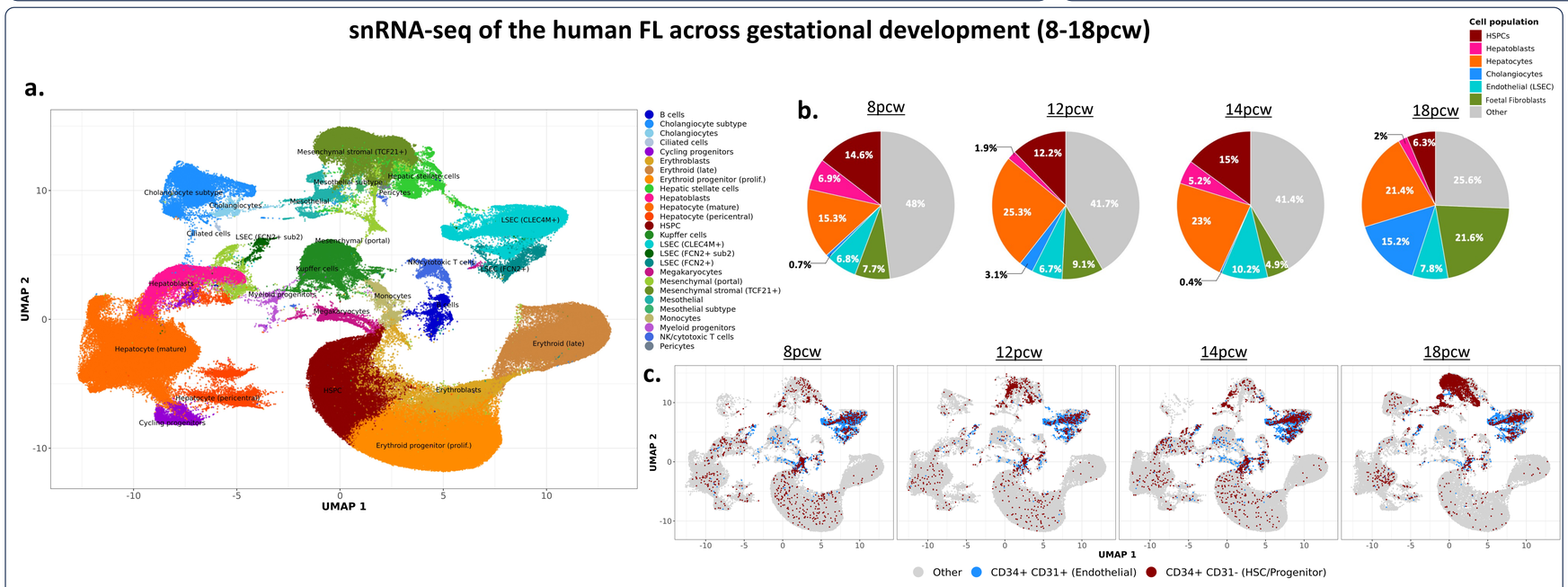


Figure 3. snRNA-seq of the human FL across gestational development. **a**. UMAP visualisation of 264,578 nuclei from 16 foetal liver samples spanning 8–18 (pcw), annotated into 25 distinct cell populations following Harmony batch correction and differential cell cycle regression. **b.** Pie charts showing the proportion of relevant cell populations at 8, 12, 14 and 18 pcw, highlighting dynamic changes in the cellular composition of the foetal liver niche across development. Key haematopoietic, stromal and parenchymal populations are identified, including haematopoietic progenitors, mesenchymal/fibroblast cells, hepatoblasts, hepatocytes, cholangiocytes and endothelial cells (LSECs). **c**. UMAPS of CD34+CD31+/- cell populations across gestational stages from 8 to 18pcw.

Conclusion & future prospectives

Proteomics of FL samples identified significant ECM core and associated proteins across HSC colonisation (8–10 pcw) and expansion (12–20 pcw) stages.

Fibroblast and hepatoblast organoid characterisation revealed increased haematopoietic cytokine secretion during peak haematopoiesis (8-10pcw), supported by upregulation of hepatoblast and haematopoietic markers at 8–10 pcw.

SnRNA-seq of 16 FL samples (8–18 pcw) identified 25 transcriptionally distinct cell populations, including a CD34/RUNX1/MYB/GATA1 co-expressing progenitor population and a progressive decrease in cycling cells with gestational age.

Identified ECM proteins will be added into co-culture systems with HSCs, fibroblasts, organoids and endothelial cells to model the FL niche and improve HSPC expansion *in vitro*.

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