# Unlocking the Haematopoietic Potential of Amniotic Fluid Stem Cells


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## Introduction

- The amniotic fluid (AF) is a unique source of accessible foetal stem cells with therapeutic potential.
- CD117 is a marker of amniotic fluid stem cells (AFSCs) able differentiate into all haematopoietic lineages in vitro[1] and repopulate the haematopoietic system in murine xenografts. [2]
- To obtain adequate cells for therapy, CD117+ AFSCs need to be expanded.
- This work investigates the characterisation, isolation, and expansion of AFSCs to clinically relevant numbers whilst maintaining their haematopoietic potential.

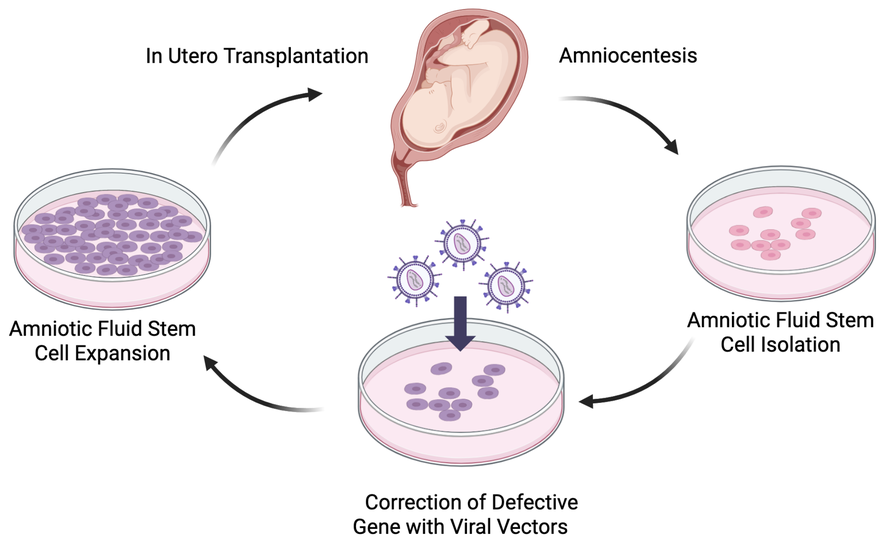
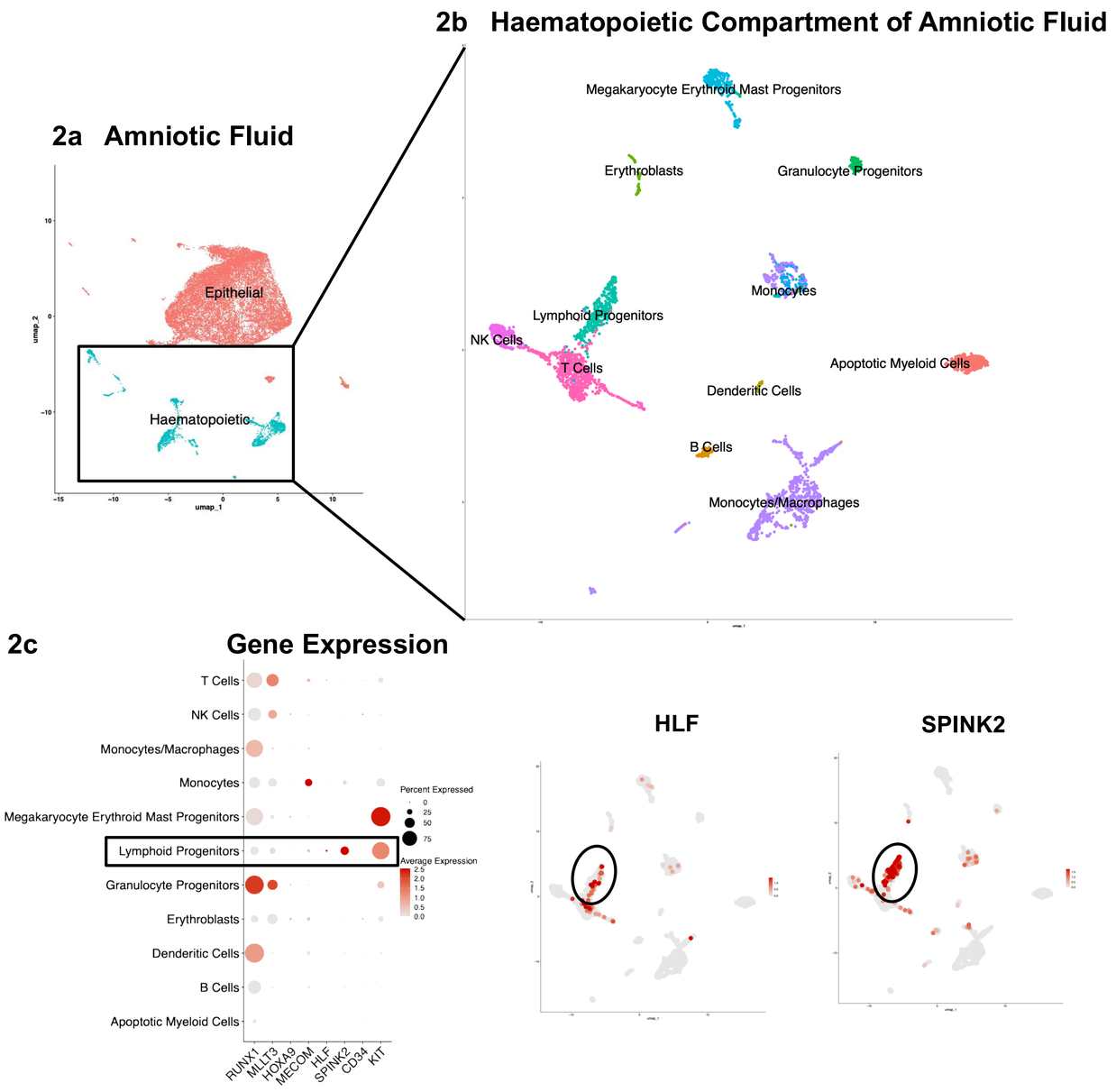



Figure 1. Proposed therapeutic application with AFSCs as the source of autologous gene corrected cell therapy to treat haematological disorders in utero

## Characterisation of AFSCs at Single Cell Resolution

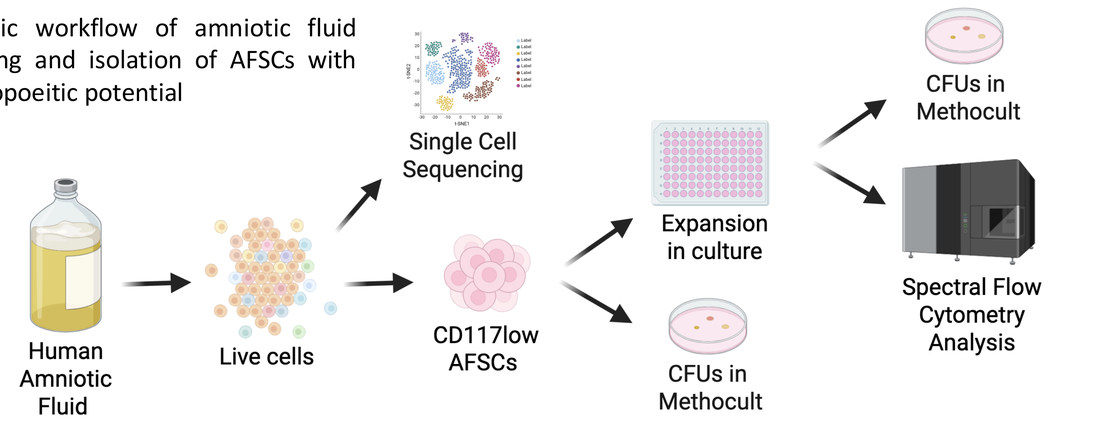
- 10X Genomics Single Cell RNA sequencing (scRNAseq) and surface proteomics (scCITEseq) performed on live cells isolated by FACS from three second trimester AF samples
- Two populations express CD117; megakaryocyte-erythroid-mast cell progenitors and lymphoid progenitors
- Lymphoid progenitors expresses fetal HSC signature genes HLF and SPINK2, with a CD45+ CD34- CD117low phenotype




**Legend - 2a** Single cell dimension reduction (UMAP) of live amniotic fluid cells isolated by FACS. **b** Annotated UMAP of the haematopoietic compartment of the amniotic fluid. **c** Gene expression of AF haematopoietic cells (scRNAseq). **d** Cell surface protein expression of AF haematopoietic cells (scCITEseq)

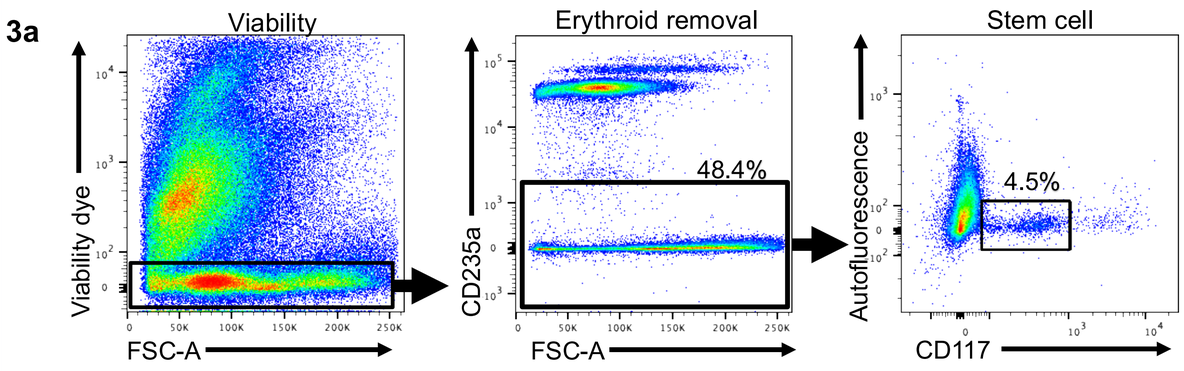
## Methods

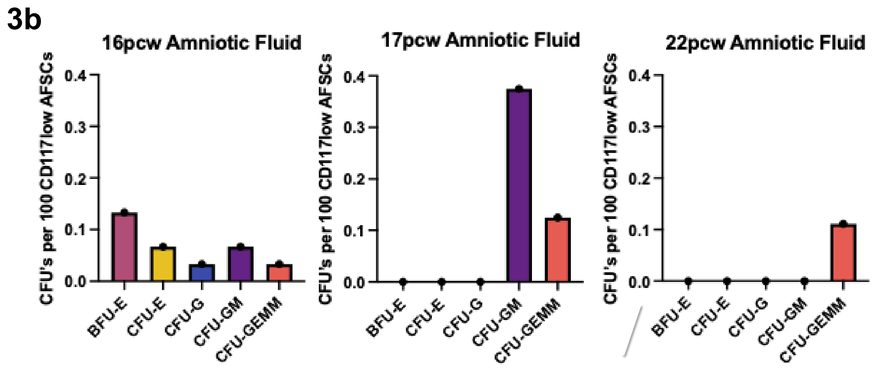
- Schematic workflow of amniotic fluid processing and isolation of AFSCs with haematopoeitic potential

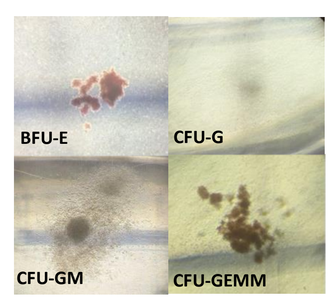


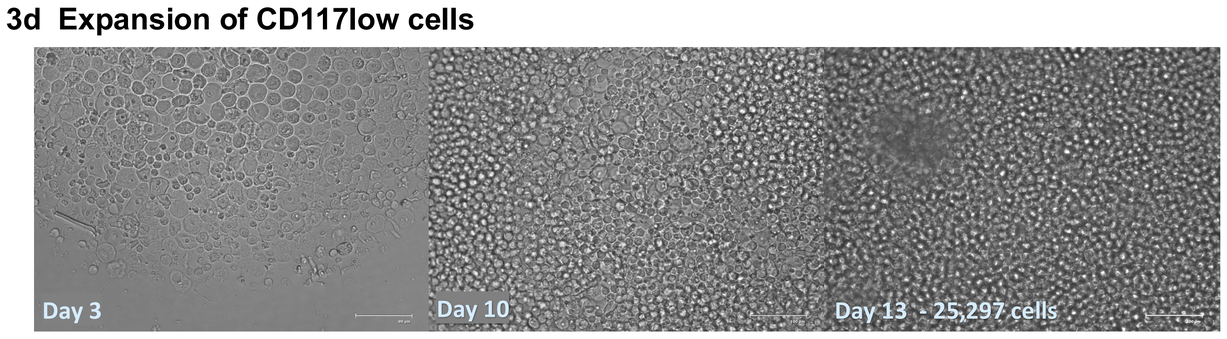

## Isolation and Expansion of AFSCs

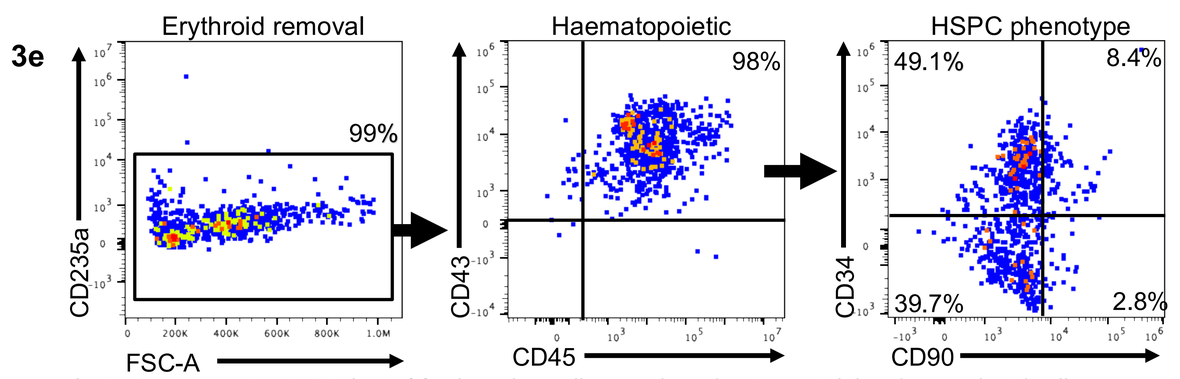
- A FACS strategy has been developed to isolate rare CD117low cells from second trimester AF
- 1:300-900 AF CD117low cells can differentiate into myeloid lineages in vitro
- Isolated AF CD117low cells expand in media, optimized for expansion of cord-blood derived HSCs[3], whilst retaining a HSPC like phenotype (CD235a- CD45/43+ >50% CD34+)




**Legend - 3a** Representative FACS plots of fresh AF, live cells are selected using a viability dye, erythroid cells are removed by negative selection of CD235a, CD117low cells are isolated. **b/c** Colony-Forming Units (CFU) in MethoCult™ derived from AF CD117low cells **d** Expansion of 2,177 (FACS count) AF CD117low cells in StemSpan™II supplemented with cytokines and small molecules[3], counted on day 13 by FACS with sorting cell counting beads . **E** Spectral flow cytometry analysis of cell surface markers of expanded AF CD117low cells

## Key Findings and Future Directions

- The amniotic fluid contains a small number of AFSCs with a haematopoeitic phenotype (CD45+) and expression of fetal HSC signature genes (HLF and SPINK2)
- These cells have a CD117low phenotype and can be isolated by FACS.
- Isolated CD117low cells can differentiate into myeloid lineages in vitro and can be expanded whilst retaining a HSPC like phenotype (CD45/43+ CD34+ CD90+/-)
- Next, we must assess functionality of expanded cells; by their ability to differentiate into haematopoeitic lineages in vitro and reconstitute the haematopoeitic system in murine xenograft models

## Acknowledgements


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1. Ditadi, A., de Coppi, P., Picone, O., Gautreau, L., Smati, R., Six, E., Bonhomme, D., Ezine, S., Frydman, R., Cavazzana-Calvo, M. and André-Schmutz, I., 2009. Human and murine amniotic fluid c-Kit+ Lin− cells display hematopoietic activity. Blood, The Journal of the American Society of Hematology, 113(17), pp.3953-3960.
2. Ramachandra, D.L., Loukogeorgakis, S., Antoniadou, E., Subramaniam, S., Shangaris, P., Tedeschi, A., Manson, A., Carmo, M., Blundell, M., Howe, S. and Flake, A.W., 2016. Human Amniotic Fluid Stem Cells Have Hematopoietic Potential In Vivo. Blood, 128(22), p.5719.
3. Fares, I., Chagraoui, J., Gareau, Y., Gingras, S., Ruel, R., Mayotte, N., Csaszar, E., Knapp, D.J., Miller, P., Ngom, M. and Imren, S., 2014. Pyrimidoindole derivatives are agonists of human hematopoietic stem cell self-renewal. Science, 345(6203), pp.1509-1512.