CHIEF SCIENTIST OFFICE

CODE: ETM/432

RESEARCH PROJECT BRIEFING

TITLE – this does not need to be the original grant title

AIMS

Haematopoietic stem cell (HSC) transplantation is a standard treatment for many malignant and non-malignant haematological disorders. The shortage of suitable HSC donors for some patient groups is a major obstacle for clinical transplantations. Here, we aimed to develop novel criteria to identify the best umbilical cord blood (UCB) samples containing potent HSCs and lymphomyeloid progenitors, which would be of great benefit for patient treatment and would improve clinical outcomes.

KEY FINDINGS

1) Our transplantation model (grafting samples of UCB into immunocompromised NSG recipient mice) revealed 4 main types of haematopoietic repopulation kinetics.

2) We identified UCB samples, which gave considerable repopulation with cell types relevant to clinical outcomes: T- cells; Neutrophils; NK cells; Platelets; Erythrocytes and correlated their production with expression of some genes in CD34+ cells of fresh UCB samples.

3) We found correlations between the presence of committed progenitors (CFU-C) in fresh UCB samples and quality of in vivo repopulation.

WHAT DID THE STUDY INVOLVE?

1) 24 UCB samples were each transplanted into 6 sub-lethally irradiated NSG recipients (50,000 CD34+ cells/ recipient) and repopulation monitored over the 6 months period using 14-parameters flow cytometry to detect levels of human haematopoietic (CD45+) repopulation and subsets of lymphoid and myeloid cells. Specifically, we used CD3, CD4, CD8, CD14, CD33, CD66b, CD56, CD57, CD235a, CD41, CD42 antibodies amongst others.
2) Remaining CD34+ cells from each UCB sample were analysed i. by RNAseq to determine gene expression and ii. in the methylcellulose (CFU-C) assay.
3) Graphs showing kinetics of repopulation by human CD45+ as well as lymphoid and myeloid cells during 6 months post transplantation were generated (tissue analysis was also performed in the end of the study).
4) Large gene expression datasets were generated using Illumina sequencing. Bioinformatics analysis was employed to correlate gene expression with kinetics of repopulation by human CD45+ cells and individual cell subsets (only selected specific markers are indicated): i. T-cells: CD4+CD8-; CD4-CD8+; ii. NK-cells: CD56+; ii. B-cells: CD19, IgM; iii. Monocytes: CD14; iv. Granulocytes: CD66b; v. Platelets CD41a/CD42b; vi. Erythroid cells: CD235a.

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

We developed a methodology, which can potentially allow clinicians to select UCB samples, tailored for transplantation in patients with specific needs. To this end, levels of expression of specific genes (correlating with production of desired cell types) can be detected by qRT-PCR in UCB CD34+ cells in clinical labs before selecting a UCB sample for transplantation. We have determined multiple differentially expressed genes with a significant p-value correlating with the size of various lymphomyeloid populations at different time point post transplantation. For example, if a stronger graft-versus-leukaemia effect is required, it may be appropriate to detect low expression of LRRK2 as it correlates with T-cell production; if high and fast neutrophil generation is required it may be appropriate to detect low levels of MYBPC1. We are now defining a cut-off value of linear regression slopes for numerous genes, which will be of practical use in the clinical scoring of samples to predict transplantation outcomes (this will be an important conclusion for the publication).

To the best of our knowledge, this is the first successful attempt to select parameters of UCB samples useful for clinical transplantations. Furthermore, this study opens up the perspective of further analysis to more accurately predict the transplantation outcome using UCB samples. We are currently looking for funding, which would allow us to perform an accurate single-cell transcriptome analysis and get access to results of clinical transplantations.

WHAT IMPACT COULD THE FINDINGS HAVE?

- Patients may receive a better treatment (safer and more predictable).
- Policy, potentially, can be changed to perform selection of UCB for individual patients and diseases.
- Practice, potentially, can incorporate novel steps in defining optimal UCB samples for transplantations.
- Economic implications - potentially significant savings as a result of more efficient treatment.

HOW WILL THE OUTCOMES BE DISSEMINATED?

We are currently considering:

- Publishing the data in a professional journal in haematology.
- Presentation at an UK and international conference(s).

CONCLUSION

We have made the first important step in defining characteristics of UCB samples potentially predictive for clinical transplantations outcome. Our data warrants further investigation in this direction (funding to be sought in near future).

RESEARCH TEAM & CONTACT

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Additional Information
E.g.: Date the project was completed and the amount of funding received