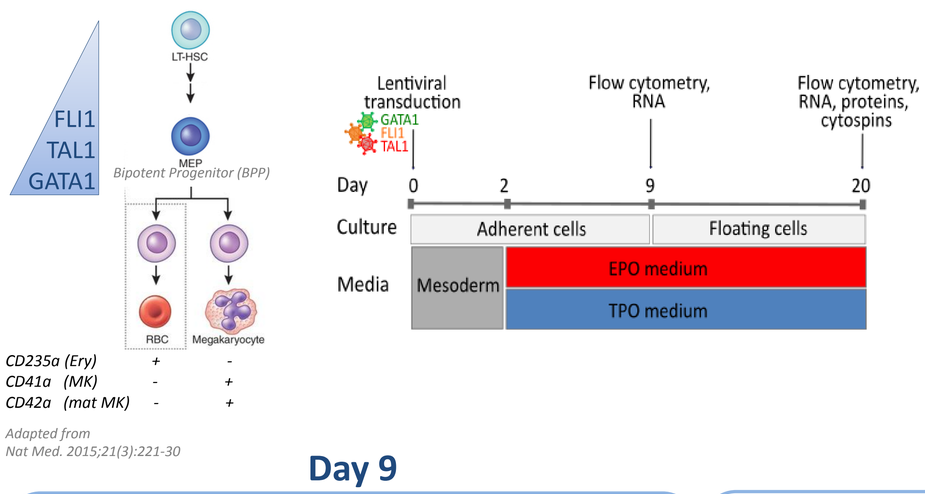
Forward Programming of human induced pluripotent stem cells to produce Red Blood Cells in vitro

José Ballester-Beltrán,[1] Amanda Dalby,[1] Chiara Lincetto,[1] Nicola Foad,[1] Ernest Turro,[1,2] Marloes R. Tijssen,[1] Thomas Moreau[1,3] and Cedric Ghevaert[1,3]

[1]Department of Haematology, University of Cambridge and NHS Blood and Transplant, Cambridge Biomedical Campus, Cambridge, United Kingdom. [2]Medical Research Council Biostatistics Unit, Cambridge Institute of Public Health, Cambridge, United Kingdom. [3]Wellcome Trust-Medical Research Council Cambridge Stem Cell Institute, Tennis Court Road, Cambridge CB2 1QR, UK.
Presenter: jb2023@medschl.cam.ac.uk

Forward Programming



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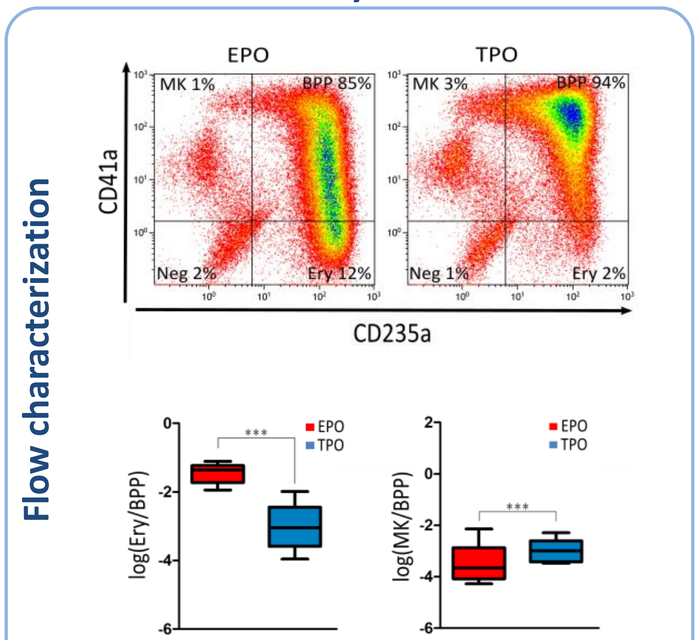
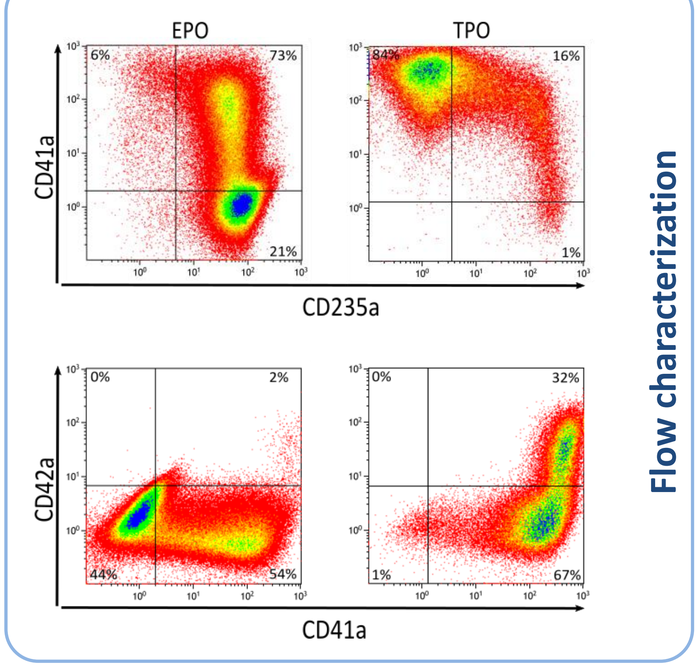
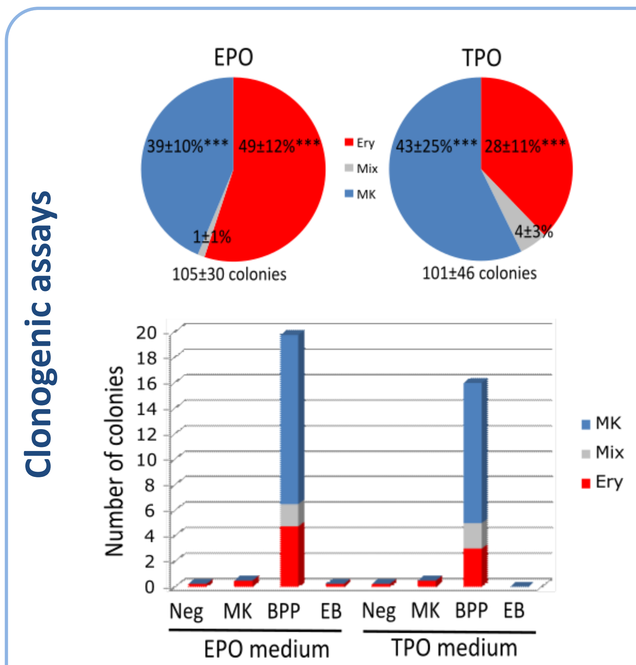
Forward Programming (FoP) relies on the fact that cell identity is controlled by limited sets of transcription factors (TFs). Exogenous expression of three key haematopoietic TFs (GATA1, TAL1 and FLI1) was previously shown to produce mature megakaryocytes (mat MKs) from induced pluripotent stem cells (*Nat Commun. 2016;7:11208*). Here we describe how FoP generates a bi-potent progenitor cell (BPP), capable of producing both MKs and erythrocytes (Ery) in permissive cytokine conditions.

Day 9

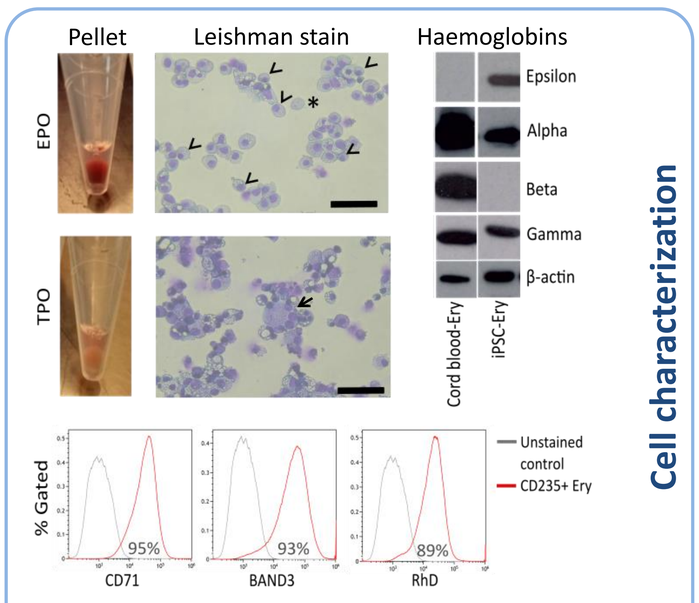


Day 20

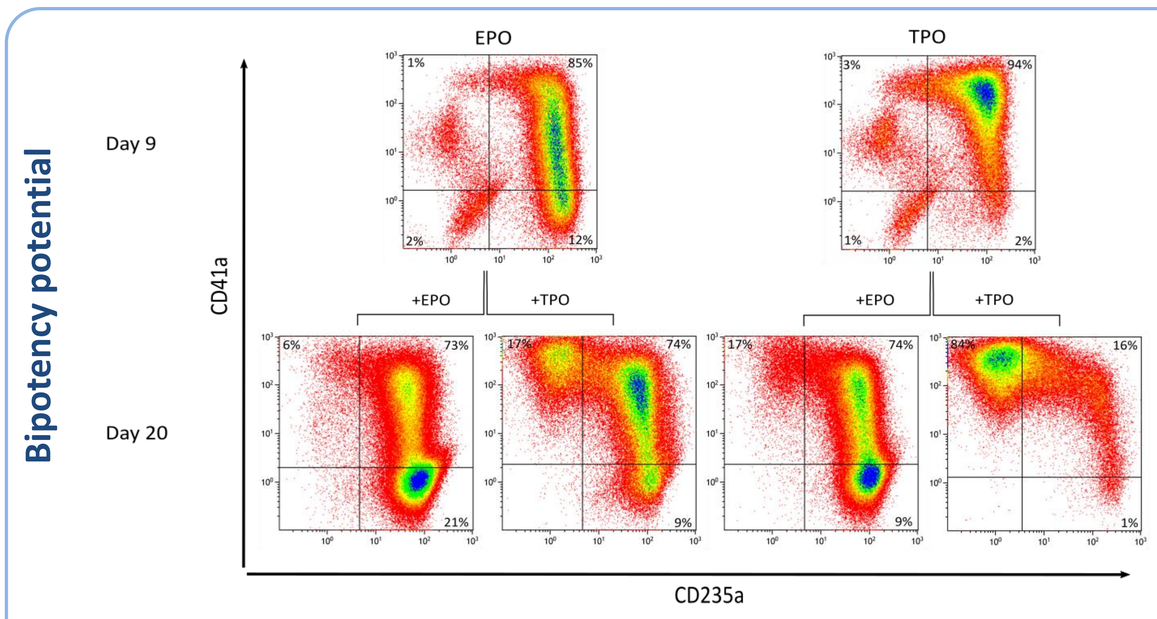




CD235a+/CD41a+ cells are bi-potent progenitors (BPPs) able to give rise to erythroblasts and megakaryocytes



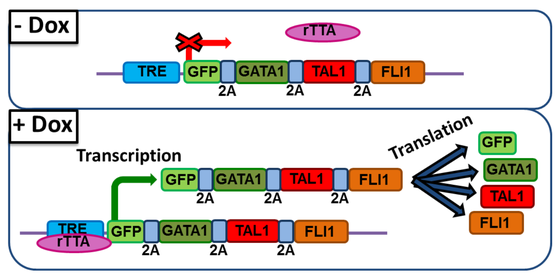
EPO media triggers the generation of basophilic normoblasts (<), with low percentage of poly- and orto-chromatic normoblasts and enucleated reticulocytes (*). CD235a+ cells also display markers of red cell maturation such as CD71, Band3 and RhD, as well as expressing haemoglobins at the protein level.



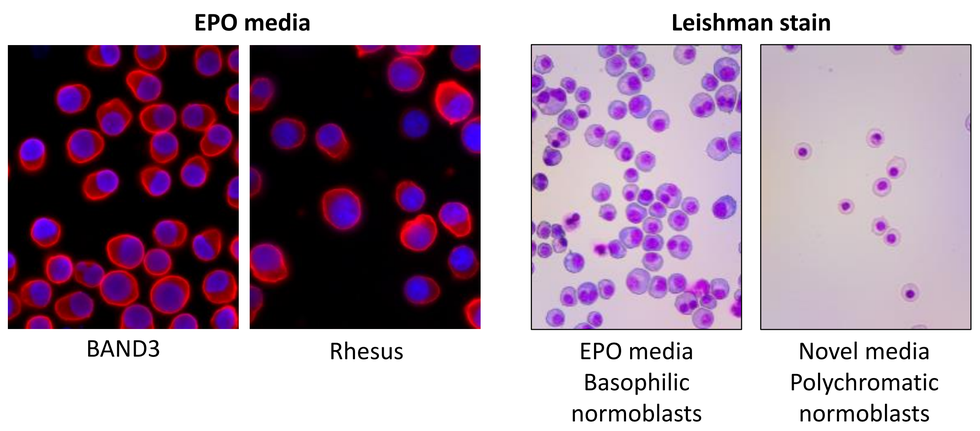
Cells were cultured for the first 9 days in EPO or TPO, then subsequently swapped over or maintained in the same media from day 9 onwards. These cross-over experiments show that BPP cells still have the potential to mature towards either lineage regardless of the cytokine used in the first phase of the culture.

Inducible cell line

Lentiviral transduction of iPSCs represents an issue for reproducibility and clinical application of the method. Therefore, an inducible line was engineered using the iOX system developed by the groups of Dr Kotter and Prof Vallier in Cambridge (A Bertero et al., Development 2016, patent 1619876.4).



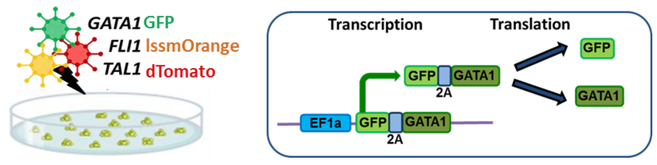
The two main components of the inducible TET-ON system were targeted to two genomic safe harbors. rTTA was inserted in the Rosa26 locus, and TRE+3 FoP TFs to the AAVS1 locus. A polycistronic cassette containing the coding sequence of the 3 FoP TFs, separated by 2A sequences, allows for a single mRNA transcript to be translated.



The inducible line also generates CD235+/CD41a- cells with BAND3 and Rhesus localized in the plasmatic membrane, which is a marker of maturity.

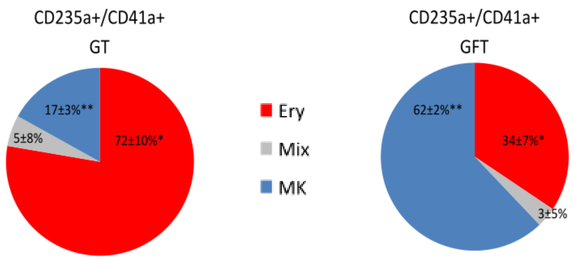
This cell line was used to screen several media and a new combinations of cytokines was found to trigger differentiation up to the polychromatic stage.

Rainbow vectors



In a similar way to the polycistronic cassette, the 2A system was utilised to generate 'Rainbow' vectors. Each rainbow vector encodes for one FoP TF and a fluorescent report gene. Allowing reporter and transgene expression to be tracked by flow cytometry. FoP with the rainbow vectors showed that day 9 BPPs are characterized by 2 main populations:

- cells expressing GATA1/FLI1/TAL1 (GFT)
- cells expressing GATA1/TAL1 (GT)



In clonogenic assays, these 2 different populations resulted in different colony potentials.

In liquid culture, TPO media results in CD235a-/CD41a+ MK cells which primarily express GFT, while EPO results in CD235a+/CD41a- Ery cells which primarily express GT.