





# Platelet production in vitro: Opportunities for front line medicine

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**THOR June 22** 

**Department of Haematology** 

### Disclosures

#### Consultant Haematologist for NHS Blood and Transplant

Consulting for

- BitBio (Cambridge, UK)
- Platelet Bio (Boston, USA)
- Xap Therapeutics (Cambridge, UK)
- RedC Biotech (Israel)

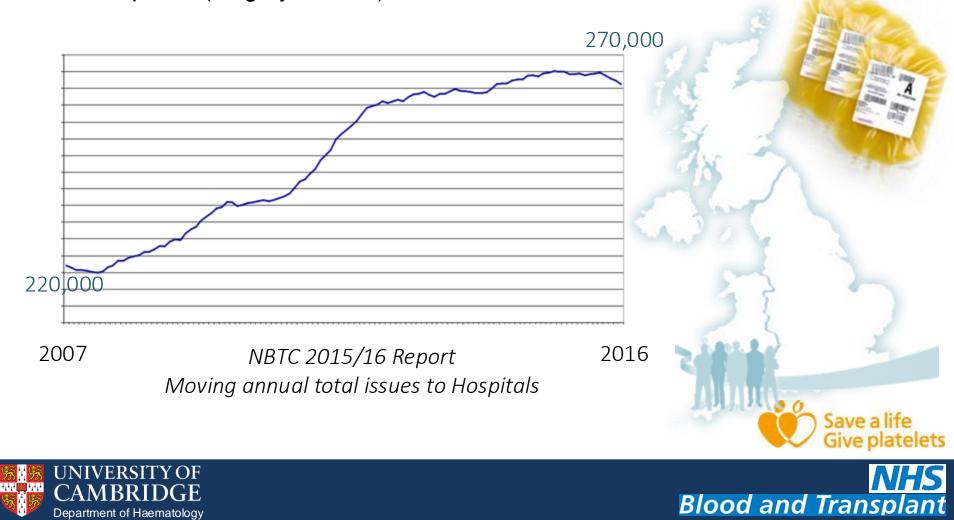




### **Platelets – transfusion needs**

### 280,000 platelet units per year in the UK (£58M)

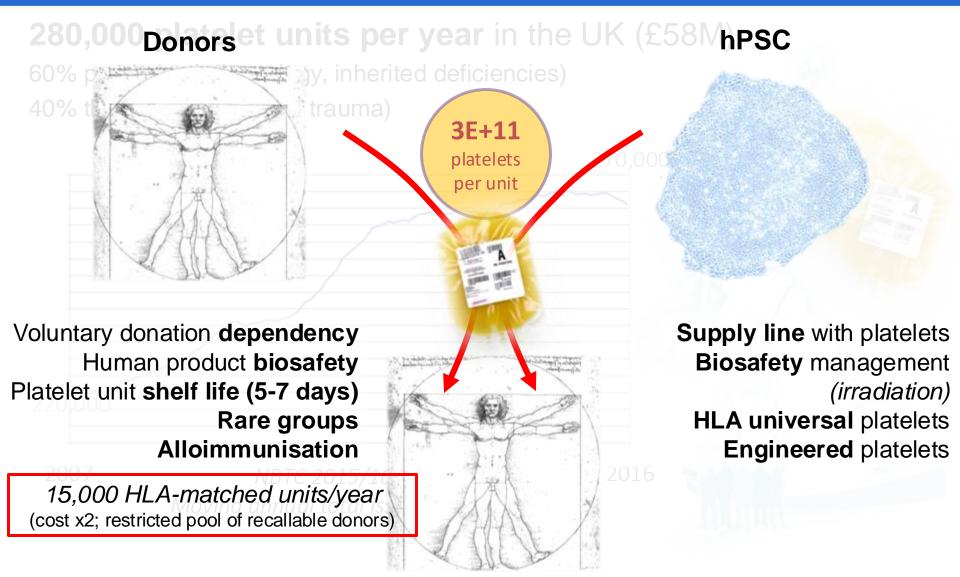
60% prophylactic (oncology, inherited deficiencies) 40% therapeutic (surgery, trauma)



### **Platelets – Sources**

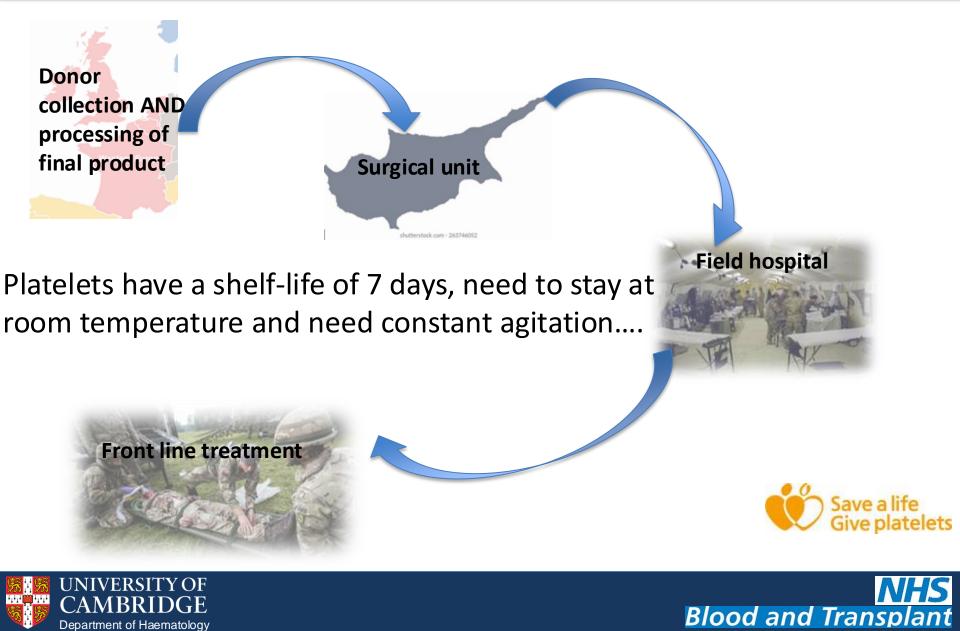
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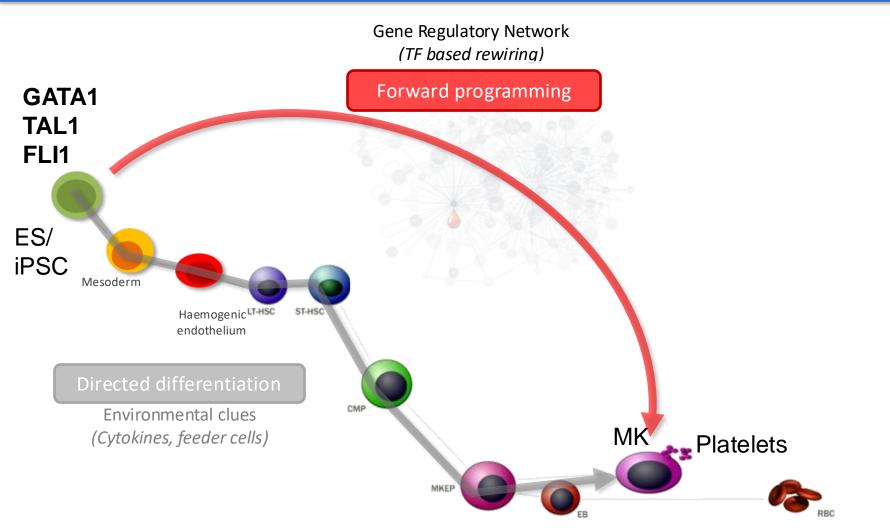




#### **Platelets – the journey to the front line**



### From iPSCs to Megakaryocytes and Platelets

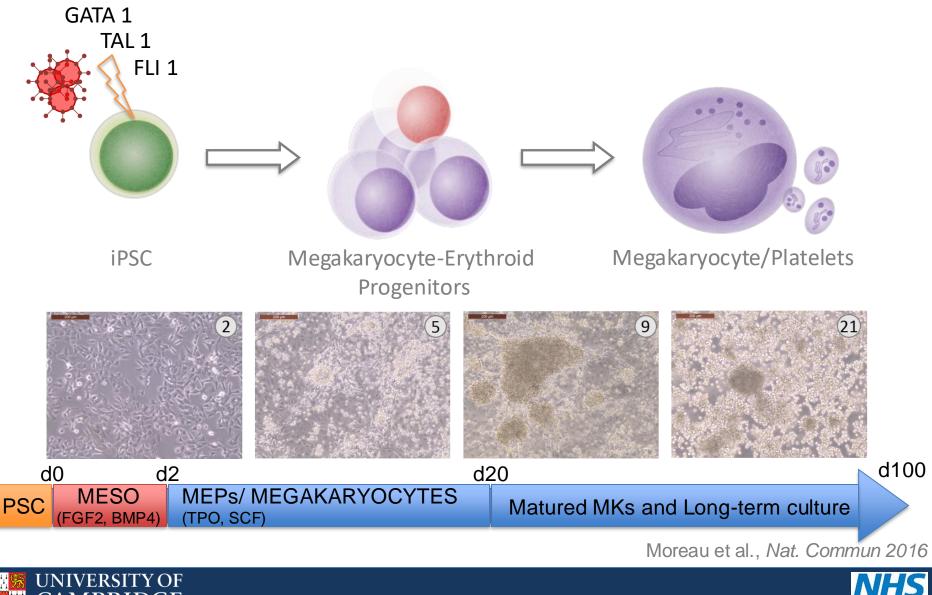


Moreau et al., Nat. Commun 2016



**NHS** Blood and Transplant

### **Forward Programming**



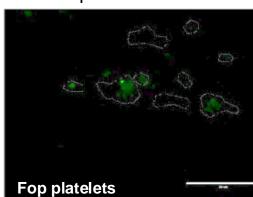
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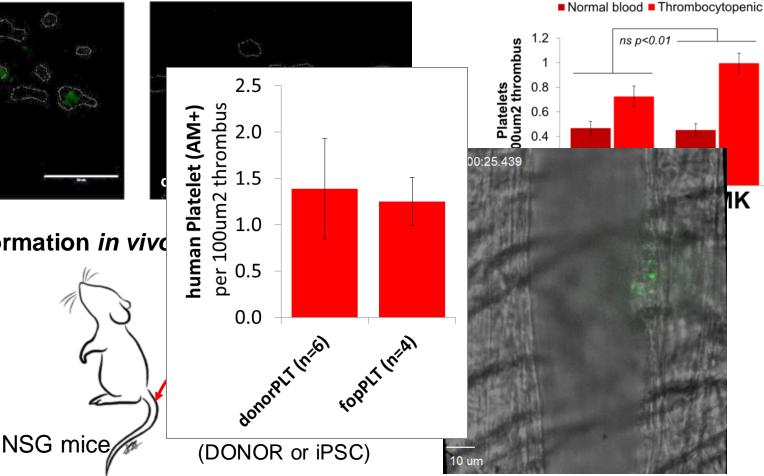
### **Platelet functionality**

Thrombus formation in vitro (Collagen, flow chamber)

Spiked calceinAM platelets (1E+7/ml)



Thrombus formation in vive





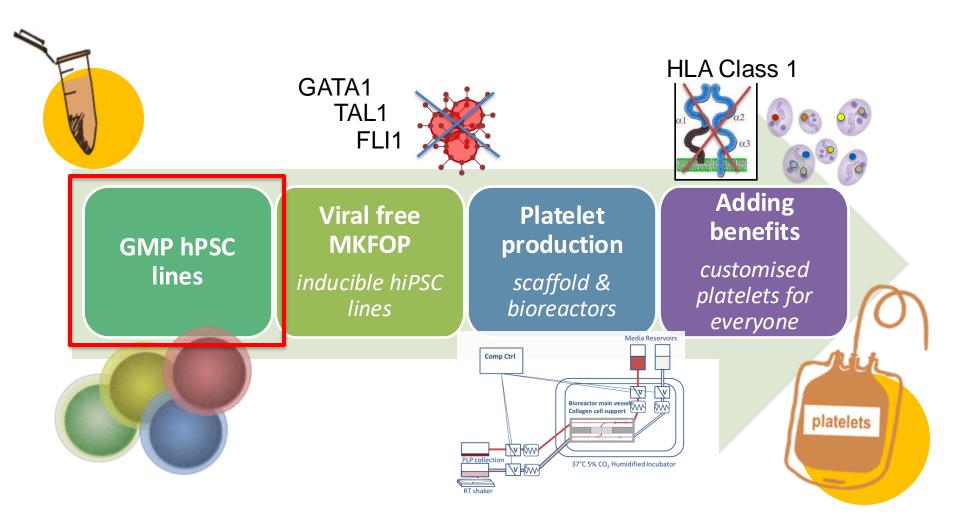


- Scaling up and manufacturing process
- Quality assurance and safety/ regulatory considerations
- Adoption by health providers and costs





### Moving towards transfusion medicine

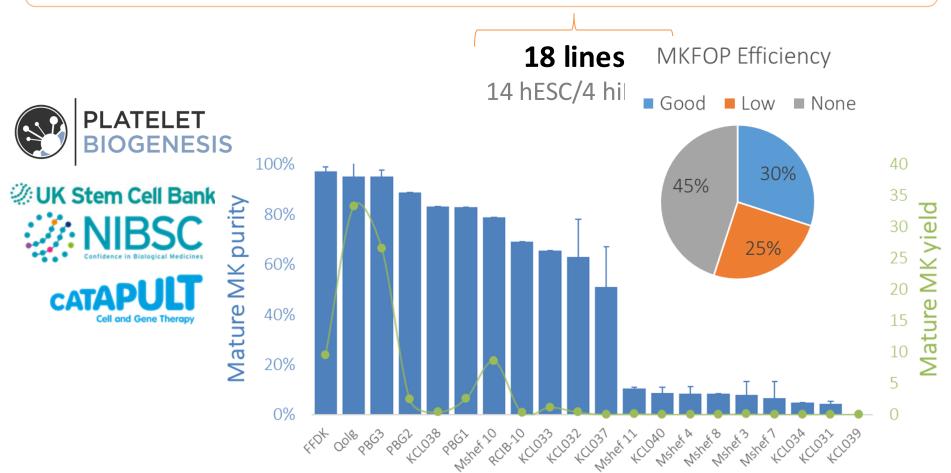






### Identifying the seed material

Identify GMP-grade hPSC lines with high MK programming potential

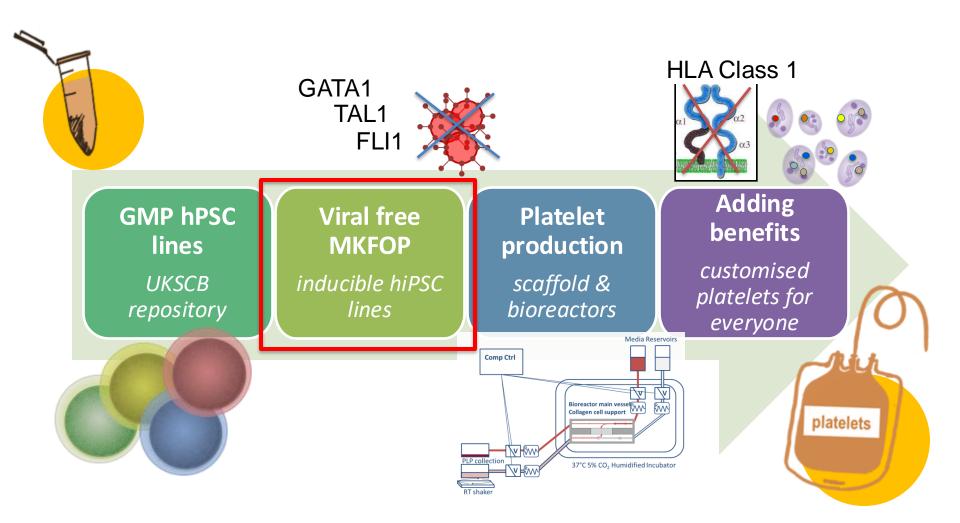


Evans et al, Blood Advances 2021





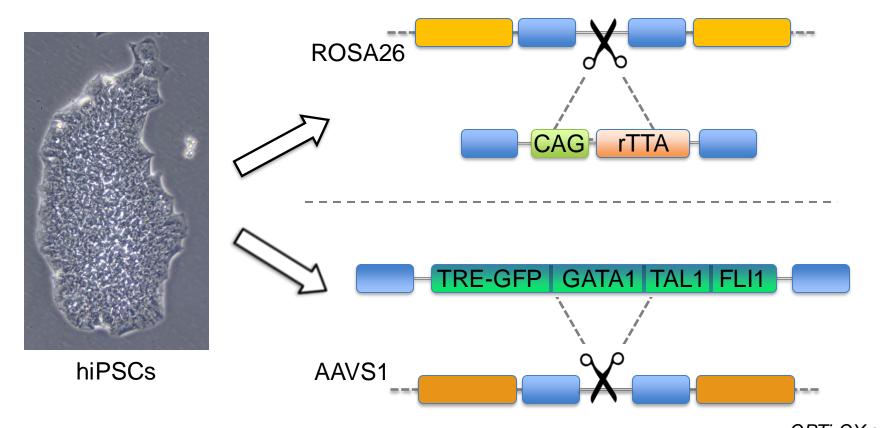
### Moving towards transfusion medicine







### Inducible FOP – optimized TET-ON system



rTTA=reverse tetracycline transactivator TRE= tetracycline responsive promotor element

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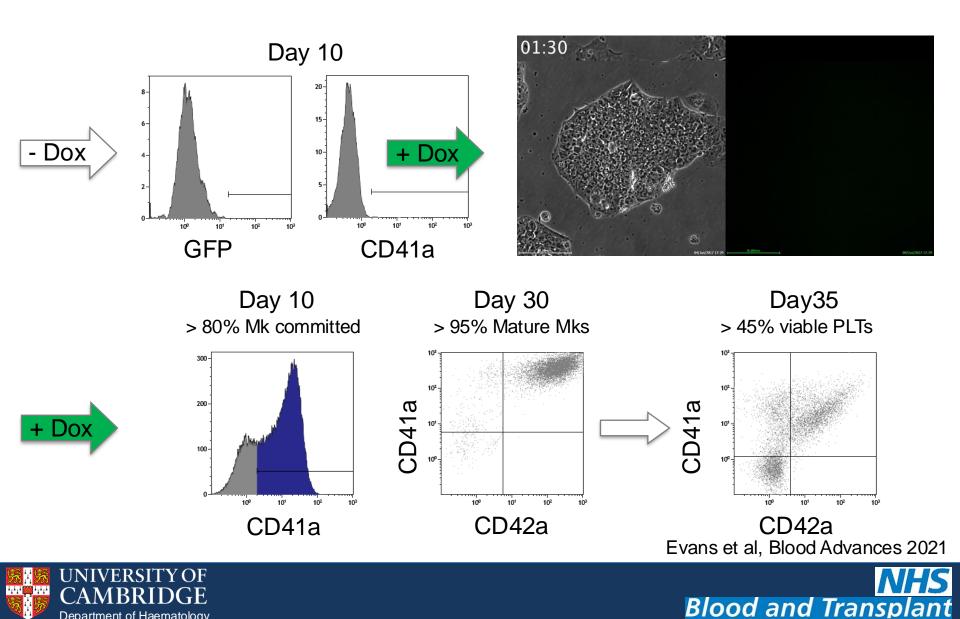
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OPTi-OX system Pawlowski *et al., Stem Cell Reports 2017* Evans et al, Blood Advances 2021

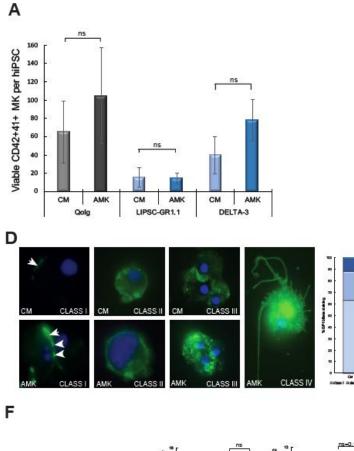


### **Mks and Platelets from inducible FOP**

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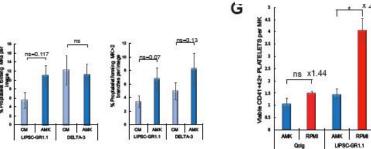


### **Optimising media for MK culture**

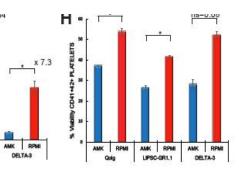


# AMK: bespoke culture medium with individual compounds available at GMP grade

Component	Company	Cat. No.	GMP Compliant	ml	Concentration
Basal Media IMDM w/o phenol red	ThermoFisher Scientific (Gibco <sup>™</sup> )	21056 (500ml)	V	500	1x
Chemically defined lipid concentrate	ThermoFisher Scientific (Gibco <sup>™</sup> )	11905-031 (100ml)		5	1x
Insulin-Transferrin- Selenium (ITS -G) (100X)	ThermoFisher Scientific (Gibco <sup>™</sup> )	41400-045 (10ml)	V	5	1x
2-Mercaptoethanol	ThermoFisher Scientific (Gibco <sup>™</sup> )	21985-023 (50ml) @55mM	$\checkmark$	0.5	55μΜ
Bovine Serum Albumin (BSA) 30%	Biosera	SA-296 (1000ml)	BSE Free	8.4	0.5%



ALSO DESCRIPTION



#### Evans et al, Blood Advances 2021

Simplified Process Flow Diagram (PFD)

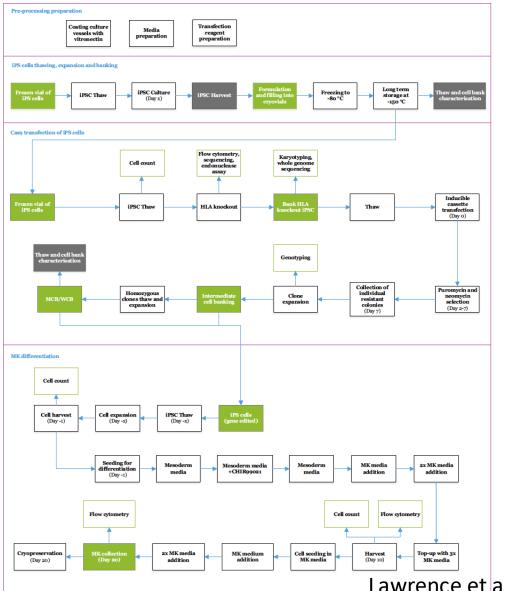
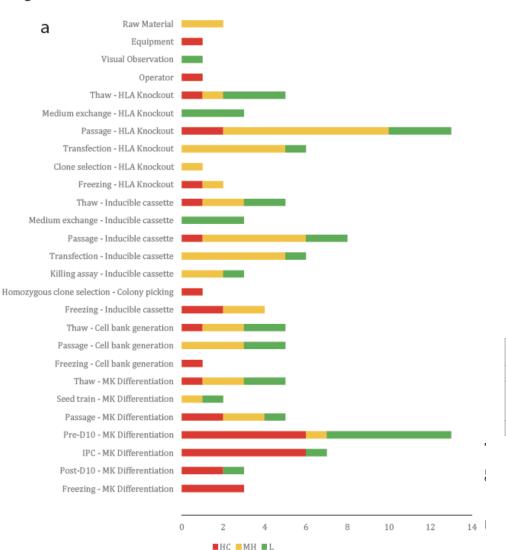
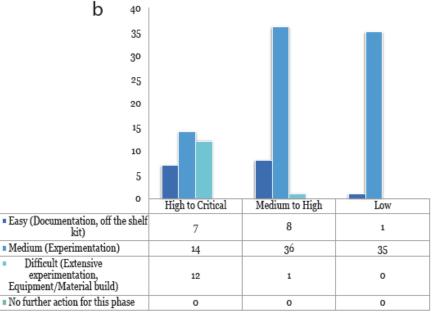
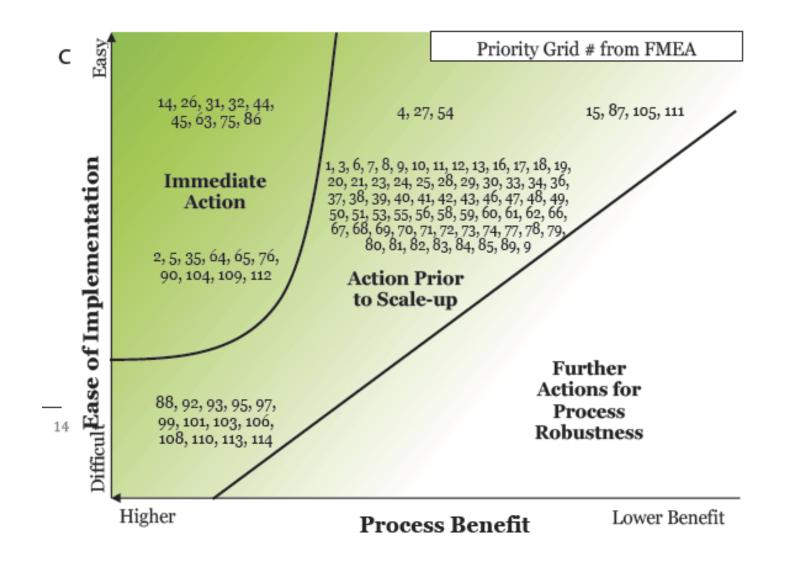
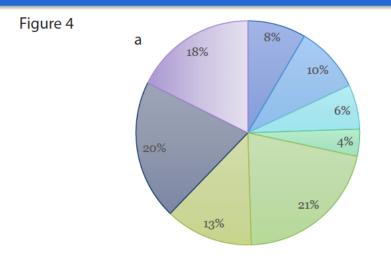


Figure 3

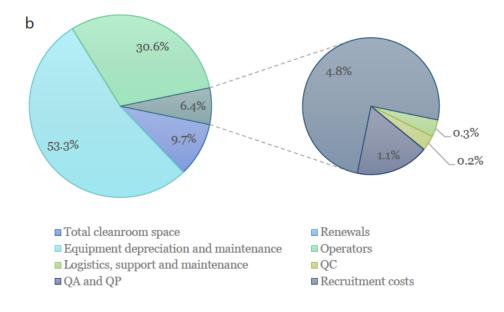








■ 6 well plates ■ BMP4 ■ E6 ■ E8 ■ SCF ■ TPO ■ Vitronectin VTN-N ■ Other

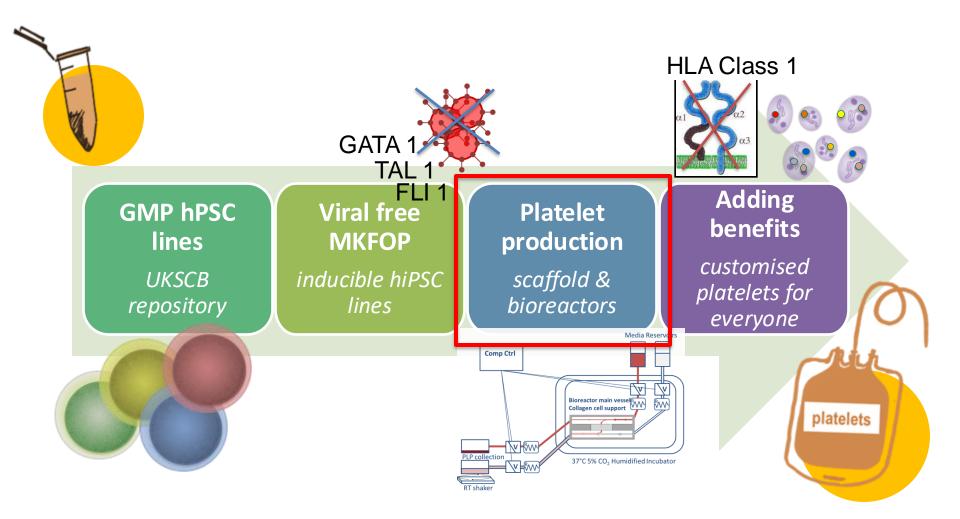


### Current estimated production costs of 1 unit of platelets

### £150,000 or 170,000 euros

....if we produce 100 functional platelets per MK....

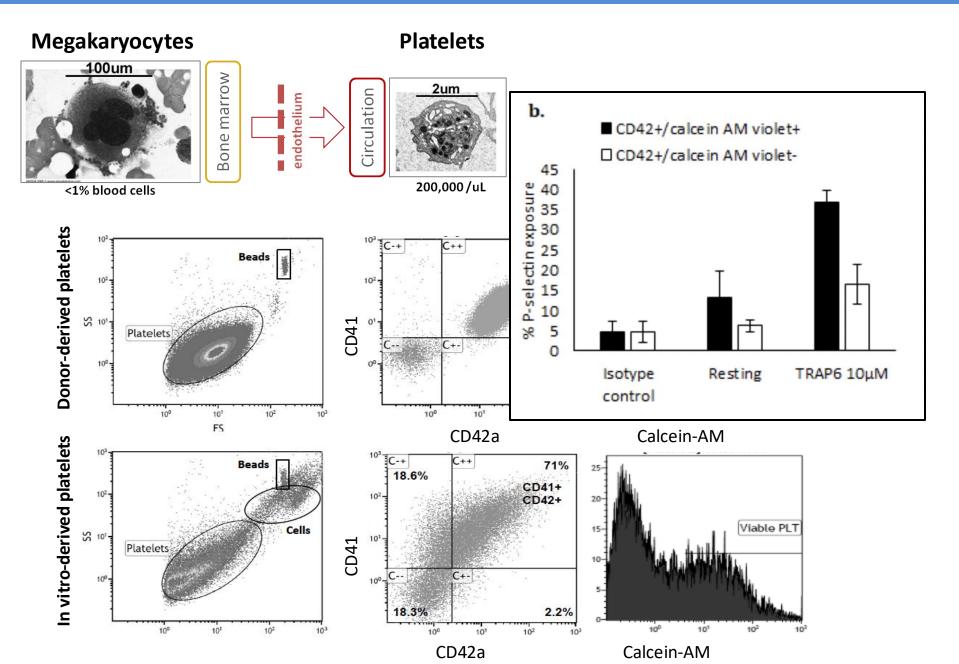
### Moving towards transfusion medicine







## **Platelets: defining a cell**



## Platelets: defining a cell

# Defining the cell we are going to give to patients in a central pillar of product safety and efficacy.

World Health

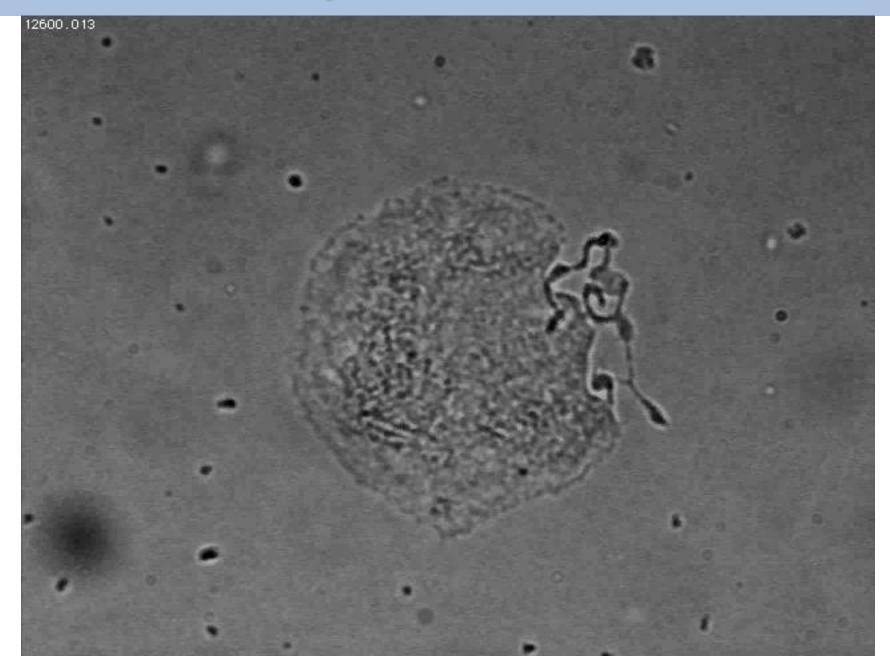
**Organization** has agreed that a "standard" should be made against which academic and commercial venture that are making platelets for clinical use should test their product.

This will be a lyophilized platelet prep, stained with calcein AM to test viability and expressing by CD41 and CD42b for flow cytometry.

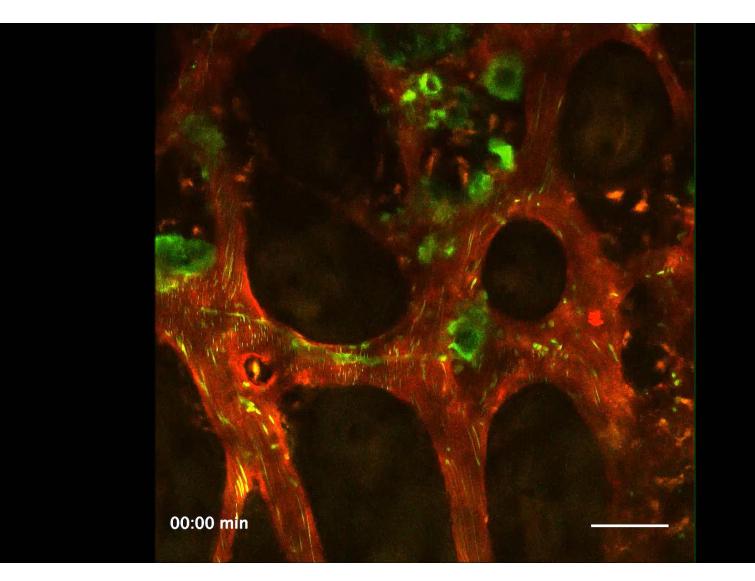
A further standard expressing P-selectin and activated  $\alpha$ IIb $\beta$ 3 may come subsequently

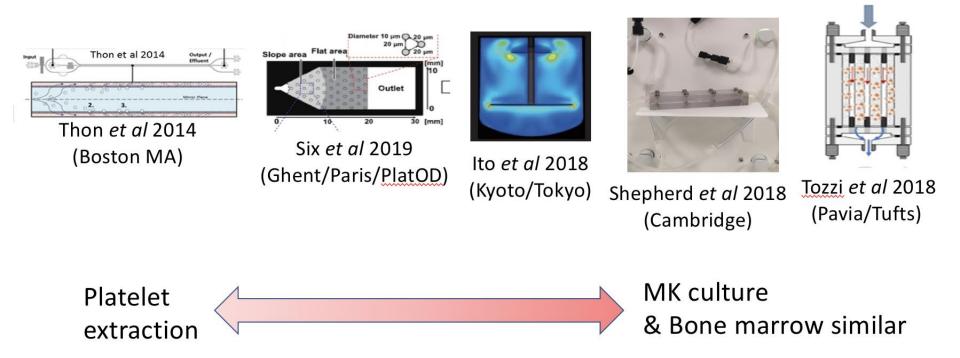
Bouet et al., Bull Acad Natl Med. 2020 Mookerjee et al., Platelets 2020

# **Proplatelet formation**

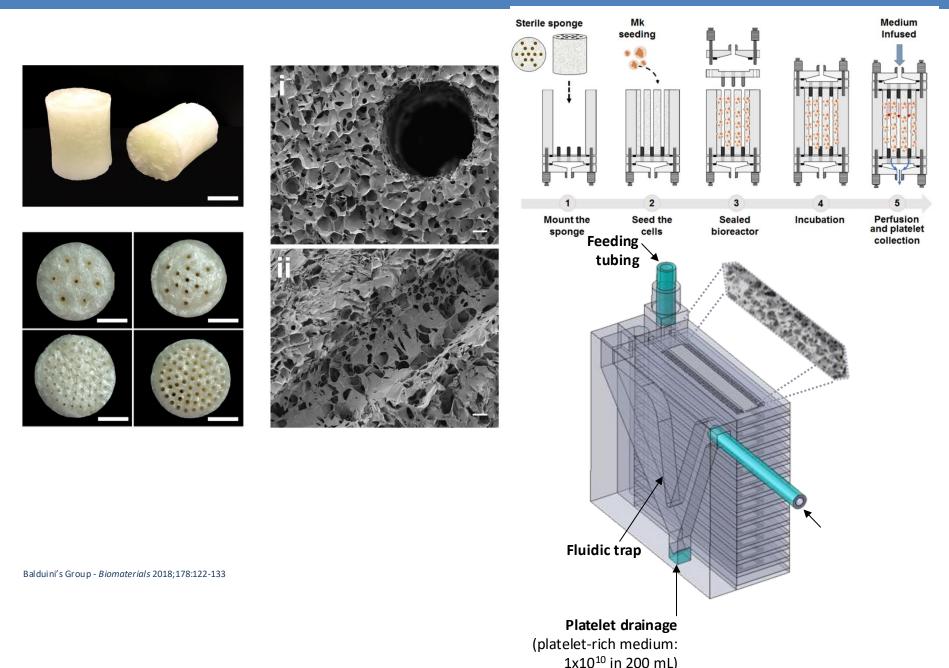


# **Proplatelet formation**





### From 2D to 3D bioreactors



### **Bioreactors for platelet production....and final product prep**

#### (1) Platelets release



Bioreactor that triggers platelet release by cultured MKs 6 5 Platelets per MK 1

Pass2

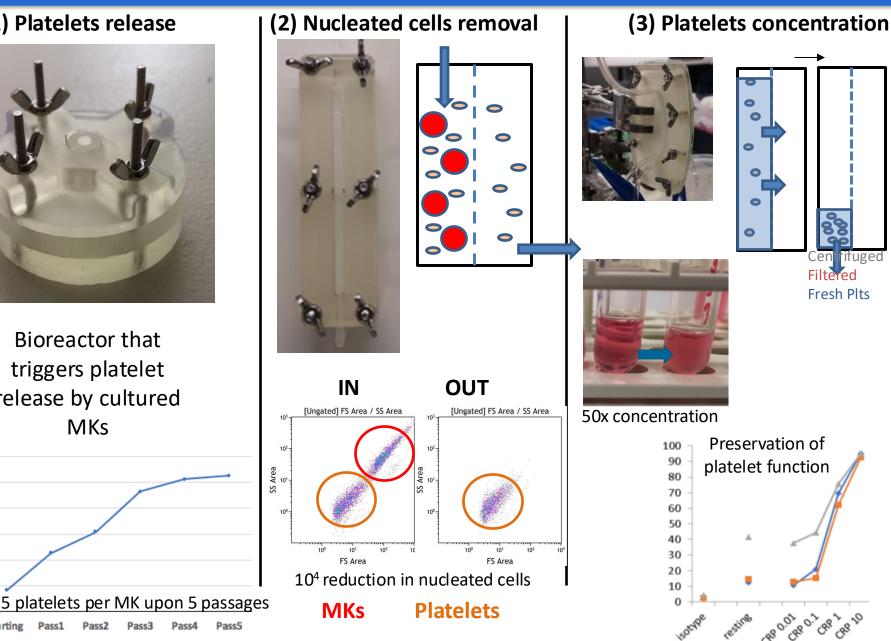
Passa

Pass4

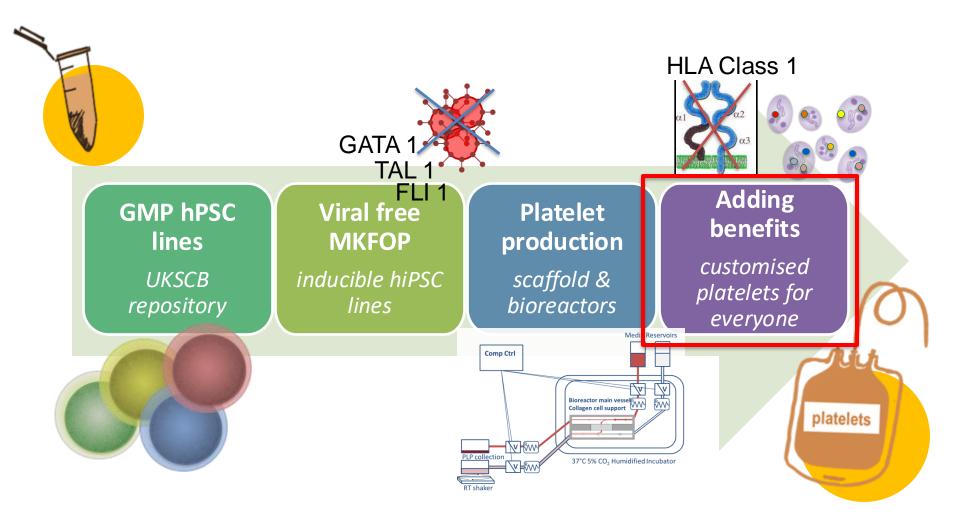
Pass5

Starting

Pass1



### Moving towards transfusion medicine

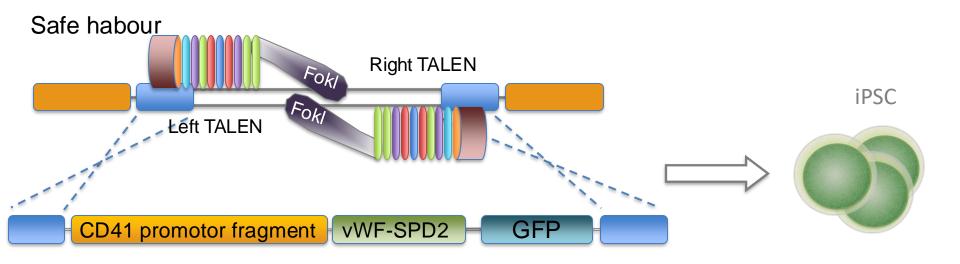


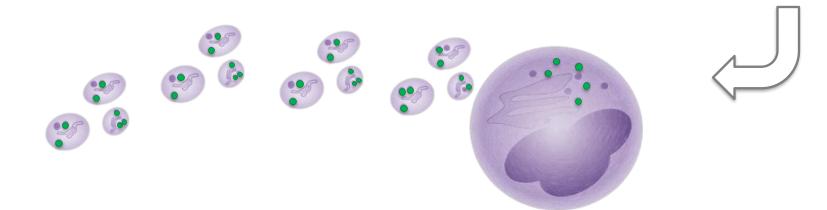


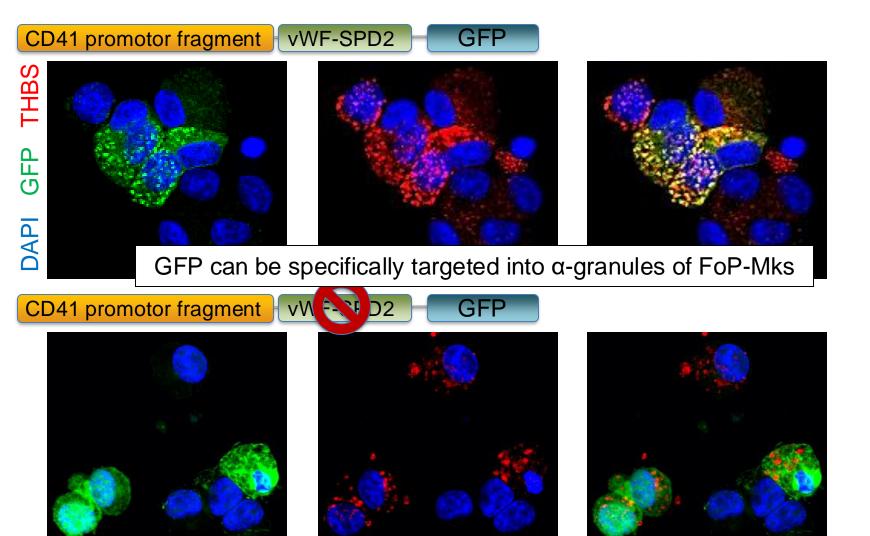


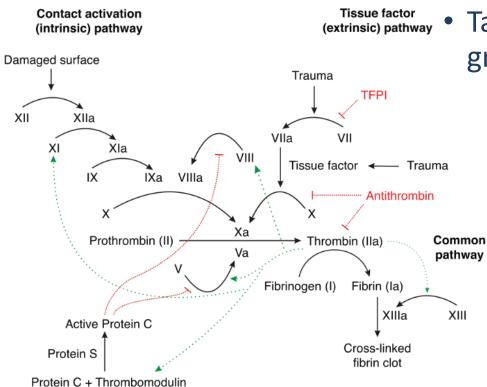
Control

Name	Accession	Known platelet protein	Known to be released / exceptosed	easate identified using MudPIT	mRNA rank in platelets	Name		Known platelet protein	Known to be released/exceytosed	Funotion	mRNA rank in platelets
EINS IN THE PLATELET	RELEASATE KNO	WN TO	BE RELEASED FROM PLAT	ELETS		Adenylyl cyclase-associated protein 1 (CAF 1)	CAP1_MOUSE	No	Phagosomes from macrophages	Contains a WH2 actin-binding domain (as β-thymosin 4). Known to regulate actin dynamics. May mediate endocytosis.	174
rombospondin 1	TSP1_HUMAN	Yes	From platelet a-granules	On secretion, can bind $\alpha_{IB}\beta_{0},\alpha_{u}\beta_{0}$ and GPTV. Can potentiate apgregation	ation 213		TTA1_HUMAN	Yes	Exosomes from dendritis cells and phagosomes from macrophages	Cytoskeletaj protein involved in microtubule formation.	33
AG		-	1000	0	Al	and the second s	A1_HUMAN	Yes	From liver to plasma, from monocytes and exosomes of dendritic cells	Role in high-density lipoprotein binding to platelets.	-
0	1	1	101	AG	S. C.	9000	3_HUMAN	No	From liver cells and monocytes	Activator of the compliment system. Cleaved to α, β, and γ chains normally prior to secretion and is a mediator of the local inflammatory response.	y
Chick	110	88	etter dia	100	12.20	120025			From choroid plexus into cerebrospinal fluid (CSF)	Thyroid hormone-binding protein secreted from the choroid piexus and the liver into CSF and plasma, respectively.	1 –
A CONTRACT OF A	11	1	and the second second	D810501 . 7.547	19.00	R COST	F1_MOUSE D1_M	Yes	Exosome from dendritic cells	Actin demolymerization/regulation in cytoplasm.	31
6	60	1	Walter and	1	200		3_MO K1_M				<b>;</b> D4
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DITATION MINING MARTI				10000000	~ ~	Fleckstrin	FLEK_HL				
isoform 1S α-Actinin 2	AAC2 MOUSE	Yes	From platelet a-granules	Actin-binding and actinin cross-linking protein found in platelet α-granu	ules. —	Nidogen	NIDO_HL				
Latent TGF-8-binding protein 1L			From platelet a-granules	Interacts with thrombospondin on the platelet surface. Subunit of the TGF-B1 complex secreted from platelets.	017	Fibrinogen-type protein	OBVCM7			and the second	
Progotivator polypeptide	SAP_HUMAN		From lysosomes	Activator proteins for sphingolipid hydrolases (saposins) that stimulate hydrolysis of sphingolipids by lysosomal enzymes.	the 147	The GDF-dissociation inhibitor					
Fighelet glycoprotein 1b $\alpha$ chain	GFBA_HUMAN	Yes	Cleaved from platelet surface (glycocalicin)	Surface membrane protein of platelets that participates in formation of platelet plug by binding A1 domain of von Willebrand factor.	20	2 The GTF ase activating protein	Q92512				
Vitamin K-dependent protein S			From platelet <i>a</i> -granules	Collactor to protein C in the degradation of coagulation factors Va and V		Transgelin	TAG2_H			•	
FF4 variant	FF4V_HUMAN	Yes	From platelet $\alpha$ -granules From platelet $\alpha$ -granules	Platelet-specific chemokines with neutrophil-activating properties. Acute phase protein, similar to compliment, inhibits proteinases.	340						
x <sub>2</sub> -macroglobulin	A2MG_HUMAN	Yes									
α <sub>2</sub> -maproglobulin α-aptinin 4	AAC4_HUMAN	Yes	From platelet $\alpha$ -granules	Actin-binding and actinin cross-linking protein found in platelet α-granu interacts with thrombospondin on the platelet surface.	ules. 407	Vinculin WD-repeat protein	VINC_HU WDR1_H		1000		
	AAC4_HUMAN PLATELET RELEA VTDB-	Yes	From platelet $\alpha$ -granules	Actin-binding and actinin cross-linking protein found in plateiet α-grapu interacts with thrombospondin on the plateiet surface. IN PLATELET8 Carries vitamin D sterols. Prevents actin polymerization. Has T lympho		WD-repeat protein			1.0		
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α-gptinin 4 ECRETORY PROTEINS IN THE Vitamin D-binding protein	AAC4_HUMAN PLATELET RELEA VTDB- HUMAN	Yes ABATE N No	From platelet a-granules OT PREVIOUSLY IDENTIFIED From liver to plasma Exosomes from dendritic cells, B cells, entercoyles, tumor	Active-binding and aptimin erose-linking protein bound in platelet e-grapul interacts with thrombospondin on the platelet surface. IN PLATELET alerois. Prevents actin polymerization. Has T lympho surface association.	ocyte —	WD-repeạt protein Superoxide dismutase (SOD) 78-kDą glucose-reląted protein	WDR1_H SODC_H GR78_M		÷		•
α-gptinin 4 ECRETORY PROTEINS IN THE Vitamin D-binding protein	AAG4_HUMAN PLATELET RELEA VTDB- HUMAN B2MG_HUMAN HBA_HUMAN	Yes No No	From platelet a-granules OT PREVIOUSLY IDENTIFIED From liver to plasma, Exosomes from dendritic cells, B cells, enterocytes, tumor cells, and r cells Exosomes from dendritic cells and phagosomes in maprophages	Actin-binding and actinin cross-linking protein bund in plateist orgranu interacts with thrombogondin on the plateist surface. IN PLATELETS Carries vitamin D sterols. Prevents actin polymerization. Has T lympho surface association. Is the ß chain of the major histocompatibility complex (MHC) class I	ooyte — 3	WD-receil protein Superoxide dismutase (SOD) 78-kDa glucose-related protein Bromodomain and PHD finger-containing protein 3 (fragment)	WDR1_H SODC_H GR78_M BRF3_H	ł			•
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$\alpha$ -actinin 4 ECRETORY PROTEINS IN THE Vitamin D-binding protein $\beta_{g}$ -microglobulin Hemoglobin $\alpha$ chain	AAG4_HUMAN PLATELET RELEA VTDB- HUMAN B2MG_HUMAN HBA_HUMAN	Yes ASATE N No No No Yes	From platelet a-granules OT PREVIOUSLY IDENTIFIED From liver to plasma, Exosomes from dendritic cells, B cells, enterocytes, tumor cells, and r cells Exosomes from dendritic cells and phagosomes in maprophages	Activ-binding and actinin cross-linking protein hund in plagetel u-granu linteracts with incomboosond on the plajetel surface. IN PLATELETS Garlies vitamin D steroits. Prevents actin polymeritation. Has T lympho surface association. Is the jo chain of the major Histocompatibility complex (MPC) datas 1 molecule. Oxygen Hangsont: Polentiajes plajetel aggregation through thromboxa recession. Discolves fortin hisbool doits, porteolytic factor in fissue remodeling. tu hingdion, and inflammation.	ocyte — 3 ane 21 umor —	WD-receil protein Superoxide dismutase (SOD) 78-kDa glucose-related protein Bromodomain and PHD finger-containing protein 3 (fragment)	WDR1_H SODC_H GR78_M BRF3_H	-			•
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Lingdrin 4 CoRETORY PROTEINS IN THE CoRETORY PROTEINS IN THE Vision De-Ninkowski By-mioroglobulin Preprince Beatrapaterin Provide kinace. M2 baayme Addin, aprils amooth muscle Addin 14-3-3 protein (J6	ААСЦЛИМАН РГАТЕLET RELEA УТВВ НШМАЙ НВА_ТИМАН НВА_ТИМАН НВА_ТИМАН ПРЕ_ТИМАН КРУ2_ДИОИSE АСТА_ТИМАН АСТА_ТИМАН 432_МОUSE НЕМО_ТИМАН	Yes No No No Yes Yes Yes Yes Yes	Prom bigbleter organises Of PREVIOUSLY UBENTIFED From Netro bysing Beochertenoorde, summ cells, and Terlis Borle, retroorder, summ cells, and Terlis Borle, retroorder, summ cells, and Terlis Borle, retroorder, summ arronbages Prom Netro bysing Border extremosition Border, summary Border, summary	Activitizing and aptimic ross-linking protein hund in plageter argumulinerate. White homotopoond not the plageter surface. IN PLATELETS Games Variantia Statistical Statistics (MHC) dataset in a surface subscription. Is the joing in the major instrument again polymetrization. Plas 1 lympho surface subscription. Is the joing in the major instrument again polymetrization. Plas 1 lympho dataset is a subscription. Discrimes from in blood costs, porteolytic batter in tissue remodeling. In Imagina, and antigramation. Presures the major issues of plagooytosis (MAPF), while enhance leaking the Playettic and antigrameters. Invasion, and retrainmation. Invasion and retrainmation. Major cytockeletaj protein. External function unknown. External function unknown. Involved Intragellularly in signal franculated however, may have a role in regularing exceptors.	ogyte — 3 ane 21 umor — 61 — 11	WD-reset protein Superoxide dtimutate (SOD) 74-K0a glucose-related protein frege-containing protein 3 fragment) Tim Similar to hesptocetular continuers Similar to hesptocetular antigen 99 PK/SG30 PNA-binding protein Hypothetical protein Hypothetical protein Hypothetical protein Filamin fragment (hypothetica) 54-K02 protein)	WDR1_H   SODC_H   GR78_M   BRF3_HL   D6W242   O99JW3   O98YX7   O98YX7   O98YX5   O98X55   Y586_HU   O99K02   FLNA_HL			Substrate for caspage-3. Actin-binding protein that binds to integrin-β3 domain. Associates with the gain cytochetion near adhesion plaques. Binds α gatinh and VASP.	
s-gatin 4 CRETORY PROTEINS IN THE CRETORY PROTEINS IN THE Exhibit of the state of	AACLJUMAN PLATELET RELET HUMAN EEMAJUMAN HEAJUMAN HEAJUMAN TITREJUMAN NYYQJMOUSE ACTAJUMAN ACTEJUMAN HEAJUMAN	Yes ABATE N No No No Yes Yes Yes Yes Yes Yes	From platelet organizes Of PRE-VOUSLY UBENT-BEO From Netro bytama Brosomes from denditic cells, Brock, and Toats Brock, and Toats Brock and Toats From Netr Into plasma From Netr Into plasma Brock excores Brosomes from Brock, and mathongke Brosomes from Brock, and mathongke Broomes in mathongke Broomes in mathongke Broomes in mathongke Arrow Aret by plasma From Netro by plasma From Netro by plasma	Activitizing and aptimic roces-linking protein hund in plageter ungraup Interactive Min homobogondh on the plageter ungrau. NP IA-TELET8 Carries vitamin D stendor, Prevente gain polymetragion. High T lympho surfage apposition. Is the jul ohan of the major instoomragibility complex (MFC) dags 1 moticule. Oxygen harpstonic Polentiages plageter aggregation through thromboxa receptor. Dissolves think in blood doits, porteolytic faptor in tissue remotelling to imagion, and information. The state remotelling to imagion, and information and approximate of happortoide. Major opticule tage of gloodysis: Presented gla an autoantiper by dentific cells. Major opticuletaj protein. External function unknown. Edetmal function unknown. Involved Intrapelikary in slopal happoladus. However, may happe a, role in regulating escolydola. Hapen-binding protein with metajloporteringae domains.	ooyte — 3 ane 21 ano — 61 — 11 on 63 — 9	WD-receip protein Superoxide dismutate (SOD) 78-K03, glucose-velated protein freque-containing protein 3 (tragment) Tim Similar to hepatocelular carcinoma-sascotated antigen 53 FKS330 FN4-chandra protein Hippothetical protein Flagmin fragment (hypothetica) S4-t03 protein) Flagmin fragment (hypothetica) S4-t03 protein) Flagmin fragment (hypothetica) S4-t03 protein)	WORIGH SOCC,H SOCC,H BRF3,H BRF3,H OBWZ42 O99,W3 O98/V37 O99/V3 O98/V37 O99/V3 O90/V3	Yes	No evidence	Actin-binding protein that binds to integrin- $\beta$ 3 domain. Associates with the actin cytoskeleton near adhesion plaques. Binds $\alpha$	: 145 gram and a



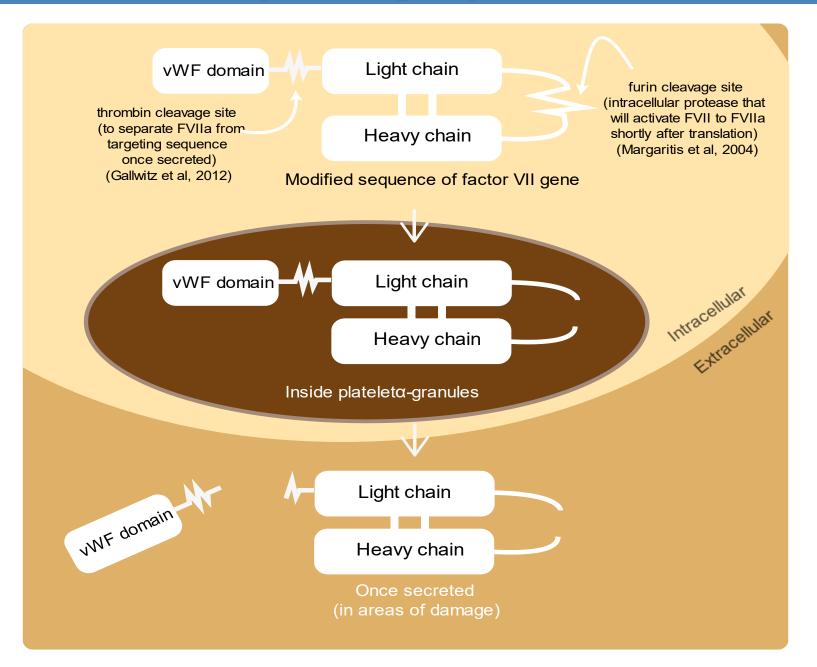


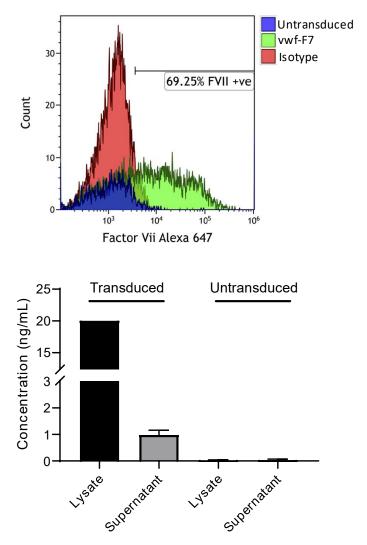


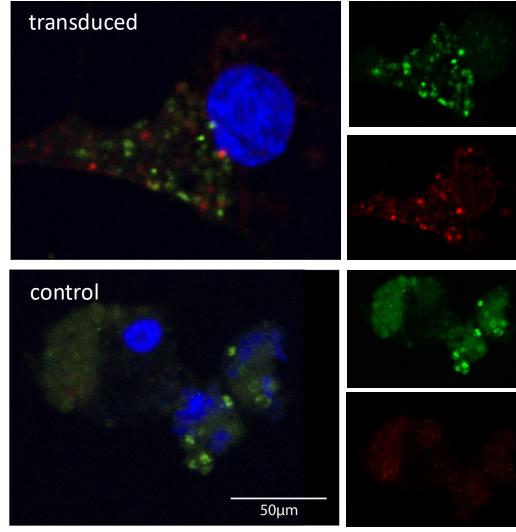


 Targeting FVIIa to the platelet αgranules:

- Novoseven is an IV injectable recombinant FVIIa with proven benefit for protracted bleeding
- Packaging into the platelet granules would remove thrombotic side-effects inherent to high systemic concentrations when using Novosevem

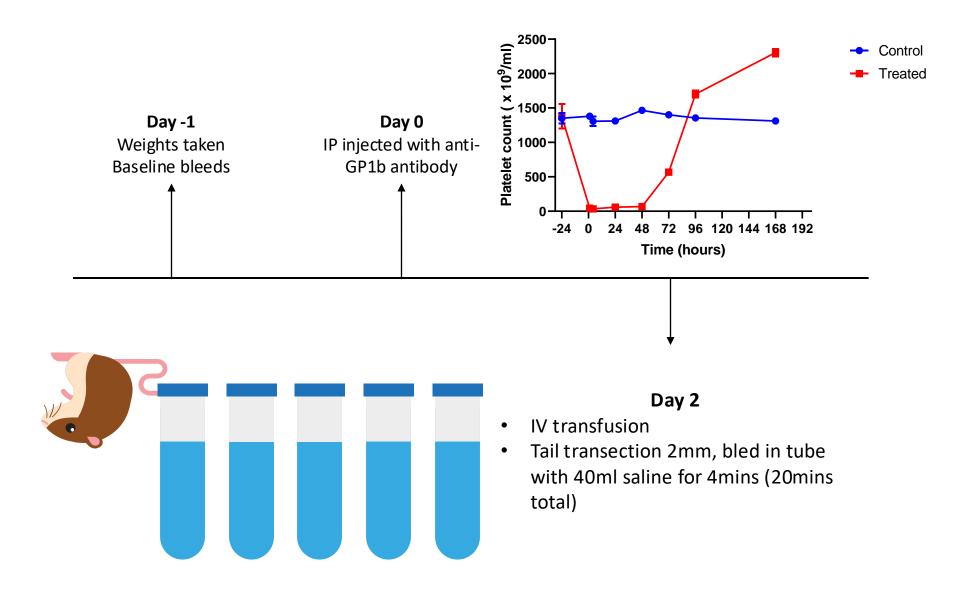






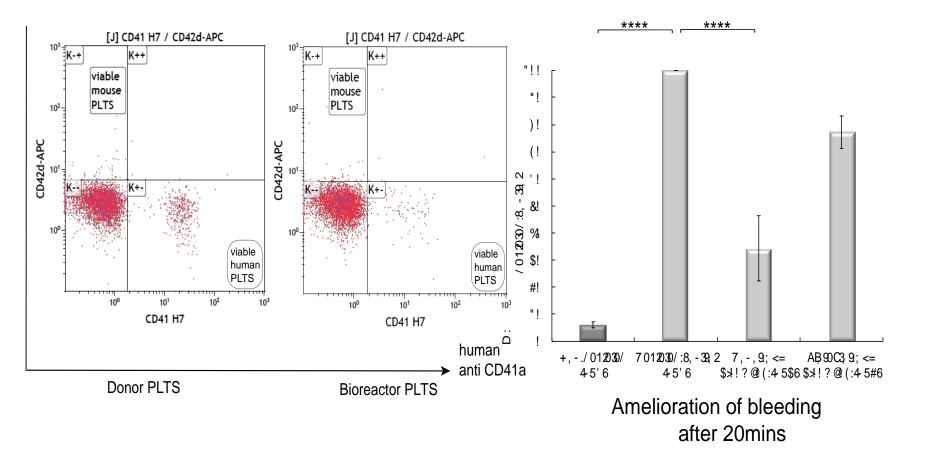
**FVII DAPI** THBS

#### Mouse model of haemostasis



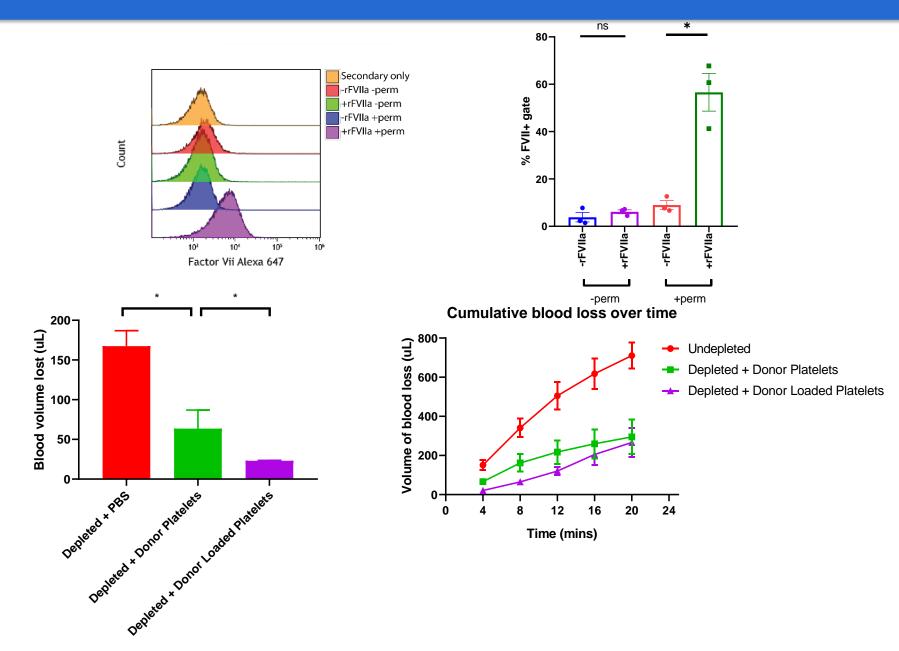
#### Evans et al, Blood Advances 2021

#### Mouse model of haemostasis



Evans et al, Blood Advances 2021

#### Platelets loaded with FVIIa show increase haemostasis in vivo



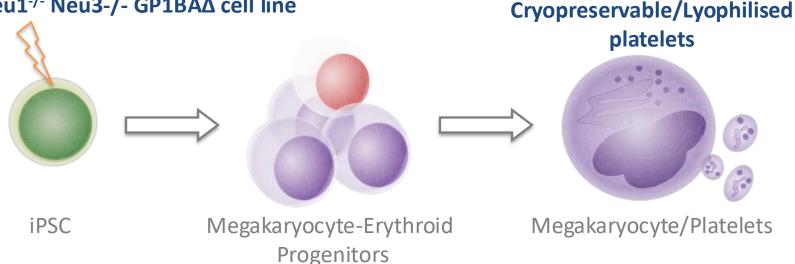
### Platelet that can be cryopreserved

Upon cooling/freezing platelets

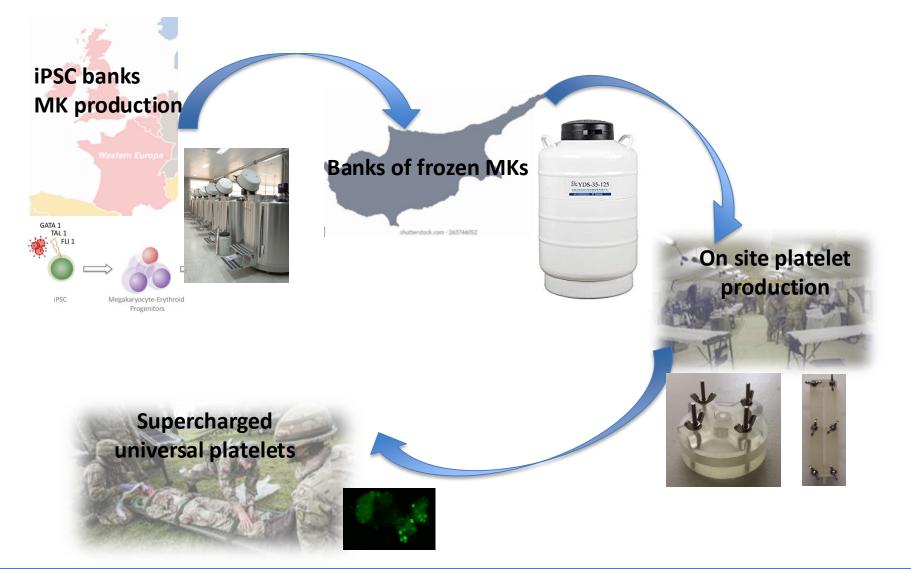
- Lose surface receptors due to cleavage driven by neuraminidases
- Lose CD42 expression (vWf receptor) due to cleavage at the hinge domain

- Shorter survival in circulation
- Loss of potency

# Gene editing by CRISPR/Cas9 to create a Neu1<sup>-/-</sup> Neu3-/- GP1BA∆ cell line



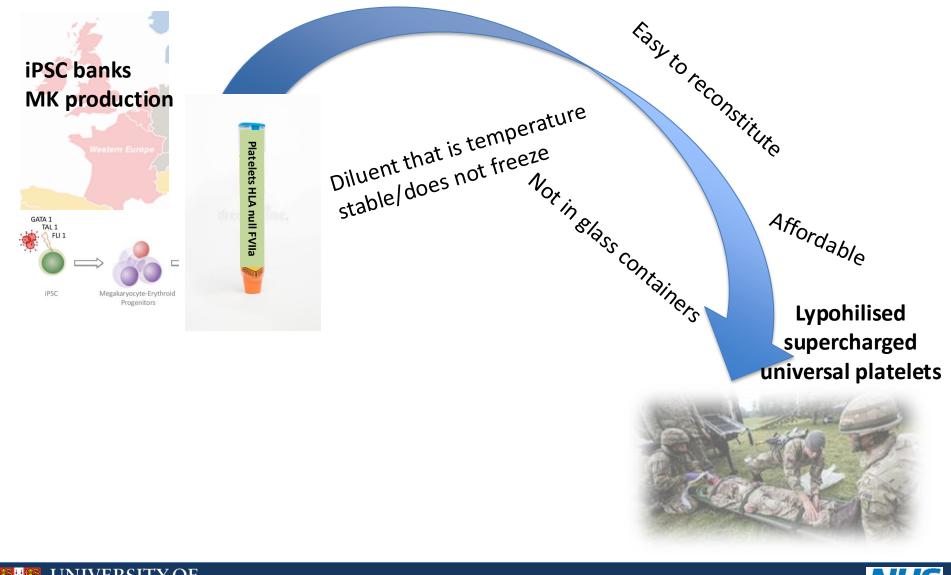
#### Platelets made in vitro – the journey to the front line







### Platelets made in vitro – the journey to the front line\_truly disruptive



Blood and Transplant



# **The Ghevaert Group**



#### NHSBT/ Haematology Cambridge Amanda Evans Holly Foster Dan Howard **Amie Waller** Moyra Lawrence Winnie Lau Nina Herbert Souradip Moookerjee James Warland **Rebecca Mc Donald** Samantha Mason Adam Pullen

<u>CSCI</u> Irina Mohorianu Susanne Bornelov Arash Shasavari

**Mark Kotter** 

James Baye Marion Perrin Maike Steindel Paula Jimenez Material Sciences Ruth Cameron Serena Best

Maike Paramor

#### Christopher Penfold







wellcometrust





UK Regenerative Medicine Platform

MRC Centre for Stem Cell Biology and Regenerative Medicine



**NHS** National Institute for Health Research