## *In vitro* modeling of megakaryopoiesis in patients cells with congenital bone marrow failures

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Project carried out in the Department of Pediatrics, Oncology and Hematology

"Oncolab" - Medical Laboratory for Pediatric Oncology and Hematology Immunopathology and Genetics Unit







**Cell cultivation** CAAATATCATG Single cells seeding

Stable Wild Type

**iPSC** primed

culture

Sanger Seq (single colony)



**Bulk Sanger Seq** 

(editing efficiency)

## Reprogramming



Epi5	
pCE-hOct3/4 - 0,6ug	
pCE-hUL (L-MYC i LIN28) - 0,6ug	
pCE-hSK (SOX2 i KLF4) - 0,6ug	
pCE-mp53DD - 0,6ug	
pCXB-EBNA1 (transient, EBV - 0,6ug	

#### .... Effciency: almost 1.3%

Walczak, M.P., Drozd, A.M., Stoczynska-Fidelus, E., Rieske, P., Grzela, D.P., 2016. Directed differentiation of human iPSC into insulin producing cells is improved by induced expression of PDX1 and NKX6.1 factors in IPC progenitors. J. Transl. Med. 14, 341. https://doi.org/10.1186/s12967-016-1097-0

## iPSC *ETV6* (NM\_001987.4:c.[641C>T];[=])



















### iPSC ETV6 (NM\_001987.4:c.[641C>T];[=])



## Selected causative mutations

Patient	Gene	Mutation	Protein	Clinical manifestation
Patient nr 1	ANKRD26	NM_014915.2:c.[-118C>A];[=]	-	Thrombocytopenia 2
Patient nr 2	<mark>ETV6</mark>	NM_001987.4:c.[641C>T];[=]	p.P214L	Thrombocytopenia 5
Patient nr 3	ETV6	NM_001987.5:c.1297C>T (p.Arg433Cys)	p.Arg433Cys	
Patient nr 4	ETV6	NM_001987.5:c.1157G>T (p.Arg386lle)	p.Arg386lle	
Patient nr 5	RUNX1	NM_001754.4:c.[806-2A>C];[=]	-	MDS
Patient nr 6	RUNX1	NM_001754.4:c.[1052delG];[=]	p.Gly351AlafsTer243	Thrombocytopenia
Patient nr 7	МҮН9	NM_002473.4:c.287[C>T];[=]	p.Ser96Leu	Macrothrombocytopenia and
	ient nr 7 <i>GATA1</i> NM	NM_002049.3:c.191[G>T];[0]	p.Arg64Met	Granulocyte inclusions with or without Nephritis or sensorineural Hearing Loss\GATA1-related X-linked cytopenia

## Differentiation protocol timeline

0.

1.

2.

3.

4a.

**4b** 



#### TPO vs TA-316 (Day 20 of diff protocol – MK maturation)



#### TPO vs TA-316 (Day 30 of diff protocol) – PTLs like particles



# TA-316 (Day 25 of diff protocol)

Early megakaryocytes

Megakaryocytes progenitors

## Tet-On system





## Projects involvement

Project	Subject	Role	Status
"RESET" (KPOD.07.07-IW.07-0153/24)	iBMF	Banking CD34+ from BM and iPSC generation from patients suffered from iBMF	Already granted
"INNOGENE" Horizon MSCA	VWD	Direct gene therapy (PrimeEditing), generation iPSC, differentiation to endothelial and megakaryocyte	Non-granted
"Daisy" (TRANSMED I: ABM/2024/8)	NBS	Gene replacement therapy for NBS and iPSC generation	Will be submitted on July 2025

## Thank you for your attention!



