Differentiation of Hematopoietic Progenitors from hPSCs

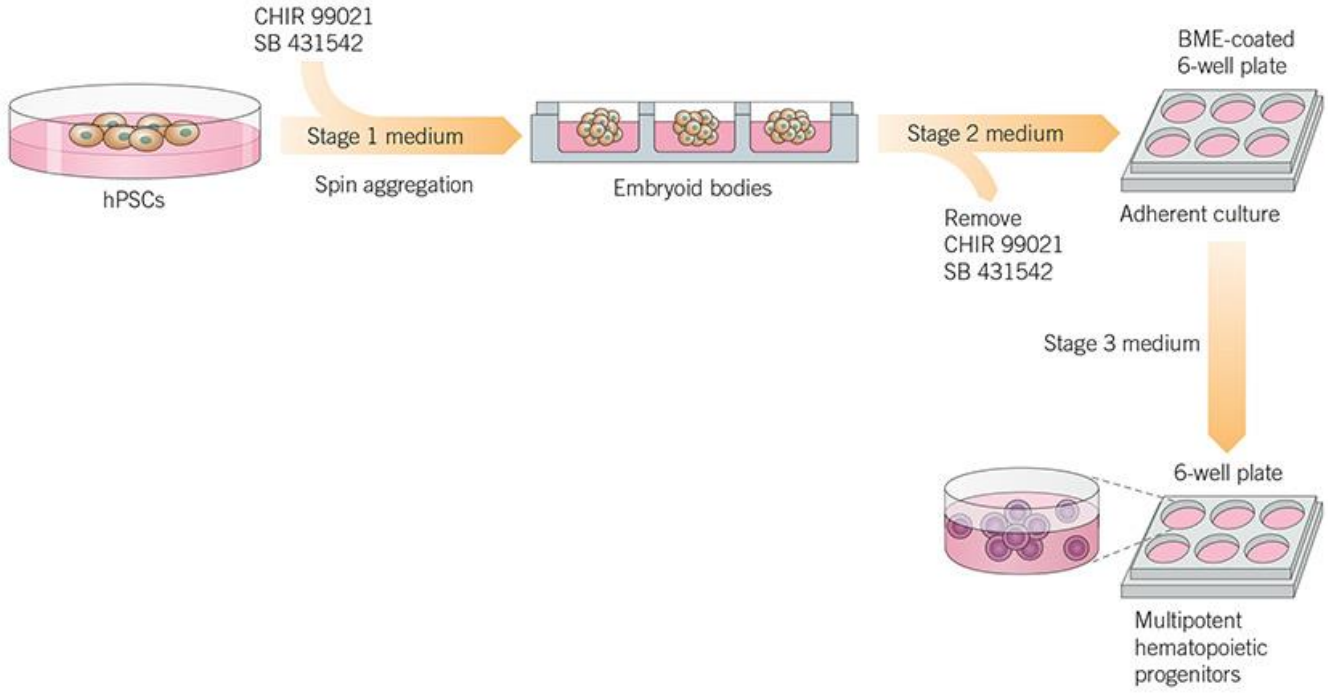
This is intended as a guide only; for full experimental details please read the reference provided.

In Brief

Nafria *et al.* describe a 3-stage procedure for the differentiation of human pluripotent stem cells (hPSCs) to multipotent hematopoietic progenitors in 14+ days using a cocktail of small molecules and growth factors.

Briefly, hPSCs are co-cultured with feeder cells for 1 week, after which the hPSCs are harvested, dissociated and seeded in non-adherent round-bottom 96 well plates in stage 1 differentiation medium. On day 0 plates are centrifuged and cells aggregate forming embryoid bodies (EB; 1 per well). On day 1, SB 431542 and CHIR 99021 are added to the Stage 1 differentiation medium to direct the mesoderm toward the definitive hematopoietic program. On day 4 the medium is exchanged for Stage 2 medium without small molecule inhibitors. At day 8 of differentiation EBs are transferred to Basement Membrane Extract (BME, e.g. Cultrex™) coated 6-well plates containing stage 2 medium. The next day (Day 9) the medium is removed and replaced with stage 3 medium. Then around day 13-15 Stage 3 medium is replaced with Stage 3 Specific medium to promote proliferation and differentiation of hematopoietic lineage cells.

Vascular structures and hemogenic endothelium expressing the SOX17 marker are generated from day 8 to 14 and from day 14 onward, SOX17- CD34+ RUNX1C-/+ hematopoietic progenitors are generated within the SOX17+ endothelium displaying similarities to human aorta-gonad mesonephros (AGM) and adult hematopoietic stem cells.



Cocktails

Stage1 Differentiation Medium		Stage 2 Differentiation Medium		Stage 3 Differentiation Medium		Stage 3 Specific Medium	
STAPEL medium		STAPEL medium		STAPEL medium		STAPEL medium	
CHIR 99021 Cat.No 4423	0.5 µM	BMP-4 314-BP/CF	20 ng/mL	BMP-4 314-BP/CF	10 ng/mL	SCF 7466-SC/CF	100 ng/mL
BMP-4 314-BP/CF	20 ng/mL	VEGF 293-VE/CF	50 ng/mL	SCF 7466-SC/CF	100 ng/mL	Flt3 308-FK/CF	100 ng/mL
VEGF 293-VE/CF	25 ng/mL	SCF 7466-SC/CF	50 ng/mL	Flt3 308-FK/CF	100 ng/mL	TPO 288-TP/CF	50 ng/mL
SCF 7466-SC/CF	25 ng/mL	IGF-2 292-G2	10 ng/mL	TPO 288-TP/CF	50 ng/mL	IL-6 7270-IL/CF	25 ng/mL
Activin A 388-AC/CF	7.5 ng/mL	FGF-2 3718-FB	10 ng/mL	VEGF 293-VE/CF	50 ng/mL	IL-3 203-IL/CF	25 ng/mL
FGF-2 3718-FB	10 ng/mL			IL-6 7270-IL/CF	25 ng/mL	Penicillin/Streptomycin B21210	Optional
From Day 1				IL-3 203-IL/CF	25 ng/mL		
CHIR 99021 Cat.No 4423	3 µM			IGF-2 292-G2	10 ng/mL		
SB 431542 Cat.No 1614	3.8 µM			FGF-2 3718-FB	10 ng/mL		
				Penicillin/Streptomycin B21210	Optional		

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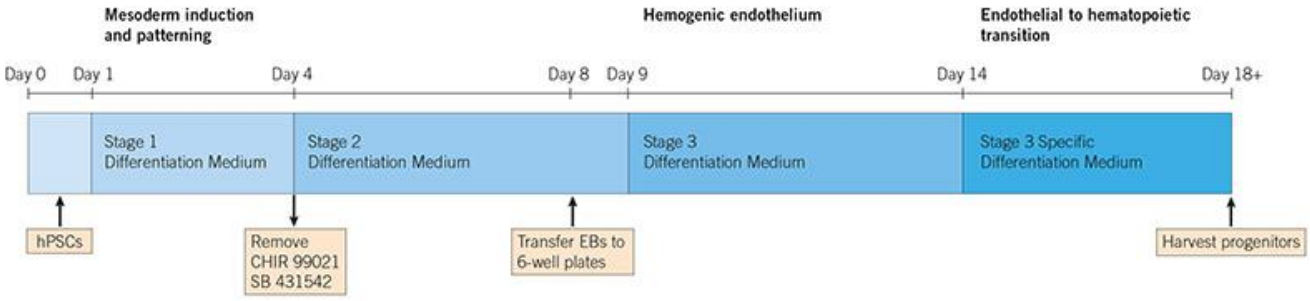

Reference

Nafria *et al.* (2020) Protocol for the generation of definitive hematopoietic progenitors from human pluripotent stem cells.STAR Protoc **1** 100130 PMID: 33377024