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Lab Resource: Stem Cell Line

## Generation and characterization of human iPSC line MML-6838-Cl2 from mobilized peripheral blood derived megakaryoblasts



Marten Hansen<sup>a</sup>, Eszter Varga<sup>a</sup>, Tatjana Wüst<sup>a</sup>, Nelleke Brouwer<sup>d</sup>, Hugues Beauchemin<sup>b</sup>, Clemens Mellink<sup>e</sup>, Anne-Marie van der Kevie-Kersemaekers<sup>e</sup>, Tarik Möryö<sup>b,c</sup>, Bert van der Reijden<sup>d</sup>, Marieke von Lindern<sup>a</sup>, Emile van den Akker<sup>a,\*</sup>

<sup>a</sup> Sanquin Research, Dept. Hematopoiesis, Amsterdam, The Netherlands and Landsteiner Laboratory, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands

<sup>b</sup> Institut de recherches cliniques de Montréal (IRCM), Département de microbiologie, infectiologie et immunologie, Université de Montréal, Canada

<sup>c</sup> Division of Experimental Medicine, McGill University, Montréal, QC, Canada

<sup>d</sup> Department of Laboratory Medicine, Laboratory of Hematology, Radboud University Medical Center, Radboud Institute for Molecular Life Sciences, Nijmegen, The Netherlands

<sup>e</sup> Academic Medical Centre Amsterdam, Dept. Clinical genetics (cytogenetics laboratory), Amsterdam, The Netherlands

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### ABSTRACT

Mobilized peripheral blood (MPB) CD34+ cells were cultured to CD41+/CD34+ megakaryoblasts. Cells were sorted to obtain a pure megakaryoblast population that was reprogrammed by a hOKSM self-silencing polycistronic vector using lentiviral delivery. The generated induced pluripotent stem cell (iPSC) lines were tested for silencing of the reprogramming construct by flow cytometry. Pluripotency of MML-6838-Cl2 iPSC line was confirmed by expression of associated markers and by in vivo spontaneous differentiation towards the 3 germ layers. The genomic integrity of iPSC line was shown by karyotyping. The MML-6838-Cl2 iPSC is, to our knowledge, the first to be generated from megakaryoblasts.

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### Resource table.

Name of Stem Cell line	MML-6838-Cl2
Institution	Sanquin blood bank, Amsterdam, The Netherlands
Person who created resource	Marten Hansen
Contact person and email	e.vandenakker@sanquin.nl
Date archived/stock date	January, 2014
Origin	Human megakaryoblasts
Type of resource	Induced pluripotent stem cell (iPSC); derived from mobilized peripheral blood donation.
Sub-type	Induced Pluripotent Stem Cells (iPSC)
Key transcription factors	OCT4, SOX2, KLF4, C-MYC
Authentication	Purity of iPSC was confirmed by: Flow cytometry, ALP staining, karyotyping and in vivo spontaneous differentiation potential.
Link to related literature	N/A
Information in public databases	N/A
Ethics	Informed consent was given in accordance with the

Declaration of Helsinki and Dutch national and Sanquin internal ethic boards.

### 1. Resource details

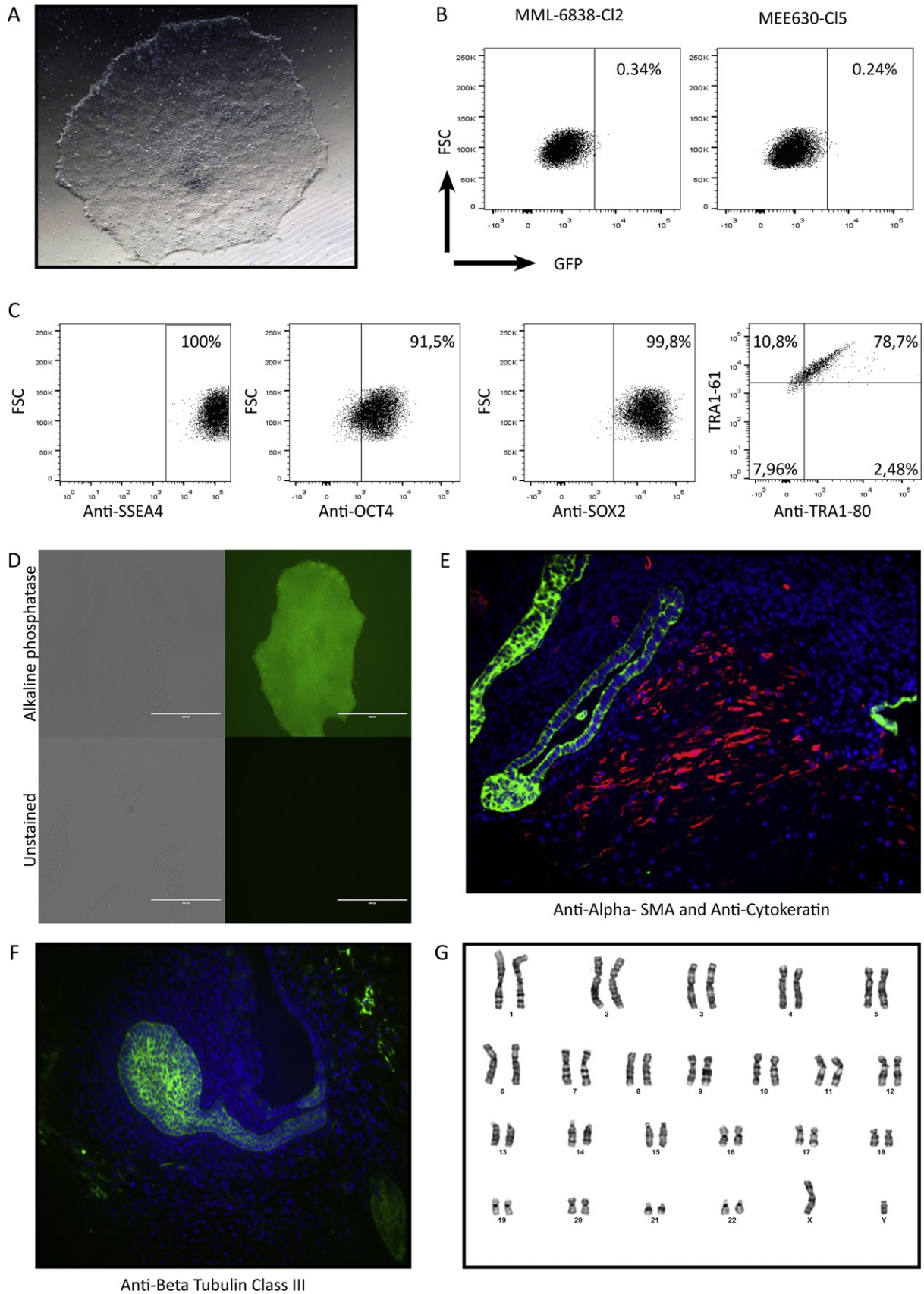
MPB CD34+ cells of a male donor were isolated and differentiated to the megakaryocytic lineage as described before (Heideveld et al., 2015) and FACS sorted to obtain pure CD34<sup>+</sup>/CD41<sup>+</sup> megakaryoblasts. Sorted Megakaryoblasts were transduced with the self-inactivating pRRL.PPT.SF.hOKSMco.GFP.preFRT lentiviral vector (Warlich et al., 2011; Voelkel et al., 2010). Reprogramming was performed on an irradiated mouse embryonic fibroblast (iMEF) feeder layer. The iPSC-like colonies were individually picked 14–20 days post-transduction and based on morphology criteria MML-6838-Cl2 iPSC was chosen for further examination (Fig. 1A).

Background green fluorescence was detected in the lentiviral reprogrammed MML-6838-Cl2 iPSC line and a control episomal (non-GFP) reprogrammed iPSC, indicating silencing (Fig. 1B). Pluripotency was confirmed by detecting expression of SOX2, OCT4, SSEA4, TRA1-61 and TRA1-80 pluripotency-associated markers by flow cytometry and alkaline phosphatase staining (Fig. 1C, D).

The spontaneous differentiation potential of MML-6838-Cl2 iPSC line was shown in vivo by analyzing teratomas formed in NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG). Three mice were injected and 2/3

\* Corresponding author.

E-mail address: [e.vandenakker@sanquin.nl](mailto:e.vandenakker@sanquin.nl) (E. van den Akker).



**Fig. 1.** Characterization of MML-6838-C12 iPSC line. A) Morphology of MML-6838-C12 iPSC line, 4× enlargement. B) Flow cytometry analysis of MML-6838-C12 iPSC to show the silencing of the reprogramming cassette measured by flow cytometry as the absence of green fluorescent protein (GFP; left); an episomal reprogrammed iPSC was used as negative control (right). C) Expression of pluripotency associated markers SSEA4, OCT4, SOX2, TRA1-61 and TRA1-80 by flow cytometry all isotype controlled. D) Alkaline phosphatase staining (top) and unstained control (bottom), showing bright field (left) and green fluorescence channel (right). E) Teratomas stained for mesoderm (Alpha-SMA, red), endoderm (cytokeratin, green) and F) ectoderm (Beta Tubulin Class III, green) and dapi (blue). G) Representative G-banded karyogram of MML-6838-C12.

formed teratomas. The commitment of the iPSC line to all three germ layers was confirmed by immunohistochemistry staining in which endodermal (PAN-CYTOKERATIN), mesodermal ( $\alpha$ -SMA) and ectodermal ( $\beta$ III-TUBULIN) markers were detected (Fig. 1E, F).

The Genomic integrity of MML-6838-CL2 iPSC line was determined by Giemsa-banding, proving normal diploid 46XY karyotype, without any detectable abnormalities (Fig. 1G).

## 2. Materials and methods

### 2.1. Experimental procedures

The chemicals were purchased from Sigma-Aldrich (Munich, Germany) and all the culture reagents from Thermo Fisher Scientific (Waltham, Massachusetts, USA), unless otherwise specified.

### 2.2. Cell culture

All cells were cultured at 37 °C in humidified atmosphere containing 5% CO<sub>2</sub>. The iPSCs were cultured on Matrigel (BD Biosciences, Breda, The Netherlands) in essential 8 medium (E8) following the manufacturer's instructions.

### 2.3. Lentiviral production

Reprogramming virus was produced in 293 T cells in Gibco DMEM, 1% Pen/Strep, 2 mM/ml L-Glut, 10% fetal calve serum. In Brief, cells were seeded and 24 h later transfected with the pRRL.PPT.SF.hOKSMco.GFP.preFRT lentiviral plasmid and lentiviral packaging constructs: pVSV, pRSV-REV and pMDI using calcium phosphate transfection. Lentivirus particle containing supernatant was collected 48 and 72 h after transfection then pooled. Cell debris was cleared by centrifugation at 3000g, 5 min and the supernatant was filtered using a 0.45  $\mu$ m filter. Lentiviral particles were immobilized by PEG (final concentration of 5%) and virus-PEG precipitates were spun down at 2000 g, 20 min, 4 °C and snap-frozen in liquid nitrogen and stored at –80 °C until use.

### 2.4. Isolation and culture of megakaryoblast from MPB and reprogramming

CD34+ hematopoietic stem and progenitor cells were isolated from MPB using CD34 Microbeads (Miltenyi Biotec, Leiden, The Netherlands) magnetic-activated cell sorting according to manufacturer instructions. CD34+ cells were cultured in IMDM (Biochrome, Merck, Berlin, Germany) supplemented with growth factors: 50 ng/ml stem cell factor (SCF), 50 ng/ml TPO, 1 ng/ml IL-3 and 20 ng/ml IL-6. 4 days later the growth factor mix was changed to 50 ng/ml TPO and 10 ng/ml IL-1 $\beta$  as described previously by Heideveld et al. (all from PeproTech, Pittsburgh, USA). Megakaryocyte cultures were sorted on CD34<sup>+</sup>/CD41<sup>+</sup> positivity to obtain a pure megakaryoblasts population (ARIA III, BD Bioscience). 1.5.10<sup>6</sup> megakaryoblasts were transduced by the hOKSM-carrying lentivirus and further cultured for one day in IMDM (Biochrome, Merck) supplemented with 50 ng/ml TPO, 10 ng/ml IL-1 $\beta$ , 10 $\mu$ g/ml polybrene and 2 mM valproic acid (VPA). Cells were

seeded on an iMEF (GlobalStem, Rockville, USA) layer with VPA, 50 ng/ml TPO and IL-1 $\beta$  for 7 days. Day 10 post-transduction the medium was changed to E8 and iPSCs-like colonies were individually isolated between day 14–20 post-transduction. iPSC were maintained on Matrigel (BD Biosciences) in E8 medium following the manufactures instructions.

### 2.5. Karyotyping

Cells were treated with Demecolcine solution (10  $\mu$ g/ml in HBSS), and processed with standard methods. Giemsa-banded karyotyping was performed and a minimum of 10 metaphases were analyzed. The chromosomes were classified according to the [International System for Human Cytogenetic Nomenclature \(ISCN\)](#), (2016).

### 2.6. Teratoma formation

Cells were harvested using Triple Select or trypsin. 1–3  $\times$  10<sup>6</sup> human iPSC and 1–2.10<sup>6</sup> OP9 cells were mixed together with Matrigel (BD bioscience) prior subcutaneous injection into 8–9 weeks old NSG mice (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ). The teratoma formation ability of iPSCs was recorded and upon reaching 15 mm diameter teratomas were excised and further processed for histopathology analyses. Teratomas were stained for pan-Cytokeratin,  $\alpha$ -SMA and  $\beta$ III-Tubulin all from eBioscience (San Diego, California, USA).

### 2.7. Alkaline phosphatase staining

Alkaline phosphatase (AP) staining was performed by alkaline phosphatase live stain (Thermo Fisher Scientific). In short, the cultured iPSCs were washed with DMEM/F-12 prior staining. E8 was added on plates with AP live stain (500 $\times$ ) and incubated for 30 min, then washed 2 $\times$  with DMEM/F-12 and visualized on EVOS-FL.

### 2.8. Flow cytometry

iPSC single cell suspensions were made using TrypLE Select, washed in PBS then re-suspended in PBS-BSA (0.1%). Anti-TRA1-80-FITC (1:100; Stem cell technologies) or anti-TRA1-61-APC (1:100; Millipore, Amsterdam, The Netherlands) were incubated with iPSC for 30 min at 4 °C. Intracellular staining for OCT4, SOX2, SSEA4 were performed according to manufacturer instructions (R&D Systems, Abingdon, United Kingdom). Cells were washed and measured on an LSR-II (BD Bioscience) and analyzed using Flowjo software (Flowjo, Ashland, USA).

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