PB0368

Development and First Human Transfusion of Allogeneic iPSC-Derived Platelet Product (MEG-002)

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CONCLUSIONS

- The first-in-human (FIH) study of MEG-002, an induced pluripotent stem cell-derived platelet (iPSC-PLT) product for allogenic use, was successfully initiated based on robust preclinical efficacy and safety data.
- Results from the first transfusion to a thrombocytopenic patient have suggested that MEG-002 is well-tolerable and safe at least at a single dose-regimen of 3 units.
- An increase in platelet counts was observed after the transfusion to the patient. Moreover, circulation of iPSC-PLTs was confirmed by flow cytometric analysis.

INTRODUCTION

Although platelet concentrates are essential to treat or prevent bleeding in patients with thrombocytopenia, maintaining an adequate supply is challenging due to their short shelf-life and demographic changes toward an aging society.

To overcome these issues, the methodology of producing iPSC-PLTs has been developed[1]. Recently, iPLAT1, a clinical trial on autologous transfusions of iPSC-PLTs was completed[2]. Here we report development of MEG-002, iPSC-PLTs for allogeneic use.

The aims of the current research were;

1. To establish safety and efficacy of MEG-002 in preclinical studies, enabling initiation of clinical trials.
2. To examine tolerability and safety as well as to estimate efficacy of MEG-002 in patients with thrombocytopenia.

METHODS

- Master cell bank (MCB) of an immortalized megakaryocyte cell line (imMKCL) was established following published method[1] from an iPSC line, YZWJs513 which is a homozygous of the most frequent HLA class I haplotype among Japanese population[3]. Working cell bank (WCB) was prepared from the MCB as the starting material for the manufacturing of each batch of MEG-002. MEG-002 was produced using turbulent-flow bioreactors according to the previously published method[4,5].
- Electron microscopic observations and in vitro functional assays were carried out according to published methods[4,5].
- Hemostatic test using a rabbit model of thrombocytopenia and blood circulation test in splenectomized rabbits were performed according to published methods[6].
- Preclinical safety studies were also conducted including a single dose toxicity study in immunocompromised NOG (NOD/Shi-scid, IL-2RγKO) mice. Male and female mice were infused either vehicle or MEG-002, and in-life assessments were conducted. Mice were sacrificed at 1- or 2-week post-infusion, and additional safety assessments were performed. No animal died and no toxicologically relevant finding was noted. (Data not shown.)
- The clinical trial notification (CTN) for the FIH study was submitted to Japan's Pharmaceuticals and Medical Devices Agency (PMDA) following multiple consultation meetings with the PMDA. The clinical trial was also reviewed and approved by Kyoto University Hospital Institutional Review Board.

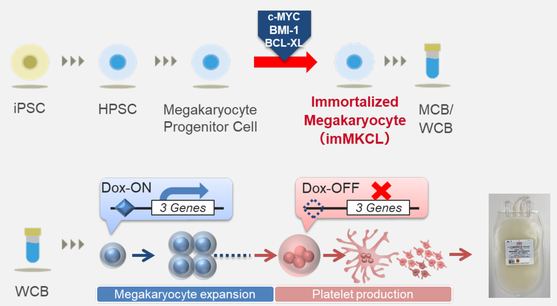
ACKNOWLEDGEMENTS

We thank Dr Koji Eto for his continuous supports for the entire project. We also thank Data and Safety Monitoring Committee members, Drs Tadashi Matsushita, Masanori Matsumoto and Akiyoshi Takami.

The iPSC stock YZWJs513 for regenerative medicine was established and provided to Megakaryon Corporation by CiRA Foundation.

RESULTS

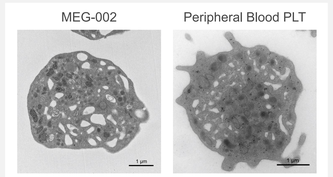
Mass Production of iPSC-PLTs



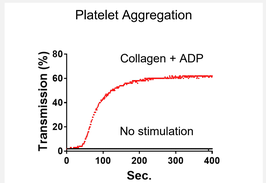
Establishment of imMKCL MCB/WCB
An imMKCL was established from human iPSCs by introducing three doxycycline (Dox) inducible transgenes, c-MYC, BMI-1, and BCL-XL. The imMKCL can be stored semi-permanently as MCB/WCB. Thorough safety tests were performed for the MCB to confirm sterility and absence of a wide spectrum of viruses.

Manufacturing of iPSC-PLTs
A cryopreserved WCB stock was thawed and expanded by culturing with Dox (Dox-ON). Platelets were produced upon maturation of imMKCL by turning off the transgenes through the depletion of Dox (Dox-OFF). Platelets were isolated, washed and packed into polyolefin bags. The final product was then X-ray irradiated to eliminate tumorigenic potential.

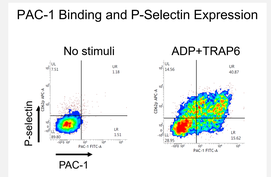
Electron Microscopic Morphology and In Vitro Functional Tests



Transmission electron microscopic images showed that ultrastructure of MEG-002 and human peripheral blood platelets are similar.

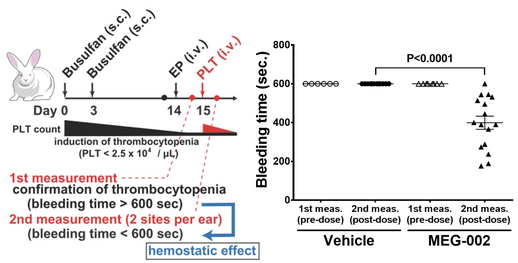


MEG-002 was stimulated with 5 μg/mL collagen and 5 μM ADP. Changes in light transmission was recorded by an aggregometer. MEG-002 demonstrated a competent response.



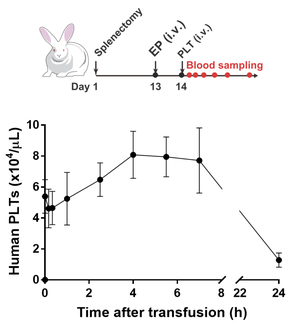
MEG-002 with or without stimulation by 100 μM ADP and 40 μM TRAP6 were analyzed by flow cytometry. P-selectin expression and PAC-1 binding were induced by the stimulation.

In Vivo Hemostatic Activity in Thrombocytopenic Rabbits



Thrombocytopenia was induced by s.c. injections of busulfan. One day before bleeding test, ethyl palmitate (EP) was administered to block the reticuloendothelial system, thereby inhibiting rapid clearance of MEG-002. Rabbits with platelet counts less than $2.5 \times 10^4/\mu L$ were selected for further experiment. To confirm prolonged bleeding time, an incision was made in the fine vein of ear, and the time to hemostasis was recorded. Rabbits demonstrating prolonged bleeding time (> 600 seconds) were then infused with either vehicle or MEG-002 (1×10^{10} PLTs/2.5 kg). Two additional incisions per rabbit were made, and time to hemostasis was recorded for each incision. Bleeding times greater than 600 seconds were recorded as 600 seconds.
In contrast to vehicle treated rabbits, most of the animals treated with MEG-002 showed hemostasis at both incisions within 600 seconds. Accordingly, the mean time to hemostasis was significantly lower in MEG-002 versus vehicle treated rabbits (p<0.0001, Mann-Whitney U test).

Circulation of MEG-002 in Splenectomized Rabbits



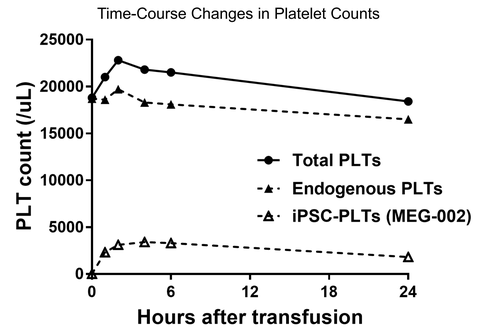
Splenectomized rabbits were administered with ethyl palmitate (EP) one day before infusion of MEG-002. Rabbits were infused with MEG-002 (2×10^{10} PLTs/2.5 kg) and blood samples were collected. Platelet counts were determined by flow cytometry. Mean human platelet count reached a maximum at 4 hours post-infusion. Human platelets persisted at similar levels to the 7-hour time point, after which the mean human platelet count fell substantially.

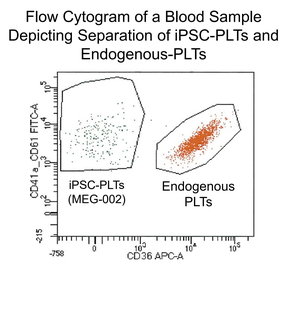
The First MEG-002 Transfusion to a Human Subject

A thrombocytopenic patient diagnosed with aplastic anemia (female, 54 years old) was transfused with MEG-002 at a dose of 0.6×10^{11} PLTs (3 units) after obtaining informed consent. The patient completed the study as planned without protocol deviation/violation.

No adverse event was reported. There were no abnormalities in vital signs and electro cardiograms. Clinically relevant changes were not observed for laboratory tests including blood chemistry, hematology and urinalysis. Bleeding episode was not reported throughout the study period.

An increase in platelet counts was observed after the transfusion of MEG-002. Moreover, circulation of iPSC-PLTs was confirmed by a flow cytometric analysis.





Platelet counts in blood samples were determined by flow cytometry using anti-hCD41-FITC, anti-hCD61-FITC and anti-hCD36-APC. CD41+/CD61+ population (defined as total PLTs) was gated for CD36+ and CD36− in order to separately count endogenous PLTs (i.e. patient-derived platelets) and transfused iPSC-PLTs (MEG-002), respectively. An increase in total-PLT counts was observed coincide with the increase in iPSC-PLTs.

Synopsis of FIH Study of MEG-002

Registration#	jRCT2053210068	
Study title	Exploratory clinical study on the tolerability, safety and efficacy of iPSC-derived platelets (MEG-002) in patients with thrombocytopenia	
Phase	I/II	
Study Design	Open-label, non-randomized, single dose study	
	Part 1 (Phase I)	Cohort 1: 3 units of MEG-002, 2 subjects Cohort 2: 10 units of MEG-002, 2 subjects
	Part 2 (Phase II)	Cohort 3: 10 units of MEG-002, 6 subjects
	• Study duration for each patient is 3 weeks (except for screening and follow-up periods). • Enrollment of patients into each cohort is staggered. • All safety data is checked and reviewed by Data and Safety Monitoring Committee before enrolling next cohort.	

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