Generation of HLA-deficient platelets from hematopoietic progenitor cells

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**BACKGROUND:** Exposure to allogeneic blood products often leads to the development of human leukocyte antigen (HLA) antibodies. Refractoriness to platelet (PLT) transfusion caused by alloimmunization against HLA Class I antigens constitutes a significant clinical problem.

**STUDY DESIGN AND METHODS:** We developed an RNA interference (RNAi)-based approach to silence the expression of HLA Class I molecules on PLTs derived from CD34+ progenitor cells. A lentiviral-based system was used to express short-hairpin RNA (shRNA) targeting β2-microglobulin (β2m) transcripts in CD34+ progenitor cells. Differentiation to PLTs was performed by incubating progenitor cells in the presence of thrombopoietin and interleukin-3.

**RESULTS:** The transduction of RNAi cassettes containing the sequences for shRNAs targeting β2m caused up to 85% reduction of progenitor cells HLA Class I antigen expression, which was maintained in the culture-derived PLTs. The HLA-deficient PLTs derived from HLA-silenced CD34+ cells proved to be fully functional in vitro tests when compared to peripheral blood-derived PLTs.

**CONCLUSIONS:** Our data show that in vitro generating HLA Class I–deficient PLTs from hematopoietic progenitor cells prove to be feasible. As malignancy risks associated with insertional mutagenesis are not to be expected in anucleated PLTs, provision of HLA-deficient PLTs from large-scale production units may become reality in the management of patients suffering from PLT transfusion refractoriness.

**ABBREVIATIONS:** β2m = β2-microglobulin; eGFP = enhanced green fluorescent protein; Flt3-L = Fms-like tyrosine kinase 3-ligand; FSC = forward scatter; GP(s) = glycoprotein(s); RNAi = RNA interference; shβ2m = shRNAs specific for β2m; shNS = nonsense short-hairpin RNA; shRNA(s) = short-hairpin RNA(s); SSC = side scatter; TPO = thrombopoietin.

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that those antibodies target few epitopes of HLA Class I molecules.\textsuperscript{7} Two validated strategies to select effective PLTs for alloimmunized refractory patients are the selection of HLA-matched PLT donors from HLA-typed donor registries and the use of manual or automated PLT cross-matching. Both strategies, which require significant organizational and financial resources,\textsuperscript{3} can provide successful PLT support in approximately two-thirds of trans-fusions. However, they may not work in cases with highly alloimmunized patients presenting with HLA Class I antibodies of broad specificity. In these situations, it would be desirable to have PLT units in which the antigenicity of HLA Class I has been eliminated from the PLT surface.

Hematopoietic stem cells and progenitor cells have emerged as an essential cell type in regenerative medicine. PLTs have been successfully generated from human cord blood and marrow CD34\textsuperscript{+} cells.\textsuperscript{8,9} CD34\textsuperscript{+} cell--derived PLTs exhibited features quite similar to peripheral blood--derived PLTs in terms of morphology and function. The culture systems used to expand and differentiate hematopoietic progenitor cells (HPCs) appear to be applicable for large-scale generation of PLTs for future clinical use. In addition, recent reports about in vitro production of functional PLTs by differentiated mouse embryonic stem cells suggest the potential utility of embryonic stem cell--derived PLTs as a substitute for PLT transfusion.\textsuperscript{10,11} Combined with the genetic manipulability of stem cells and HPCs, these approaches offer the opportunity to generate genetically engineered PLTs for clinical transfusion.

RNA interference (RNAi) has become a powerful tool in molecular medicine. RNAi is an evolutionarily conserved mechanism for silencing gene expression by targeted degradation of mRNA. Short double-stranded RNAs, known as small interfering RNAs, are incorporated into an RNA-induced silencing complex that promotes the degradation of RNA containing a homologous sequence. Lentiviral-based vectors have proved to be effective vehicles for the delivery of RNAi cassettes into mammalian cells, which allow a stable silencing of the target gene expression.\textsuperscript{13-15} Previously, we have shown the feasibility to regulate cell surface expression of HLA Class I molecules using a RNAi-based method.\textsuperscript{16} In this study, we used an RNAi-based approach to permanently silence the expression of HLA Class I molecules on PLTs derived from CD34\textsuperscript{+} progenitor cells and demonstrated in vitro that these HLA Class I--deficient PLTs are functionally equivalent to blood-derived PLTs.

**MATERIALS AND METHODS**

**CD34\textsuperscript{+} cell isolation and enrichment**

Peripheral blood CD34\textsuperscript{+} progenitor cells were isolated from leukapheresis samples of eight healthy individuals selected as donors for allogeneic stem cell transplanta-

**Lentiviral constructs and vector production**

A lentiviral vector was used for stable short-hairpin RNA (shRNA) expression. shRNA expression cassettes (Table 1) were cloned into the lentiviral expression vector pLVTHm/si containing enhanced green fluorescent protein (eGFP) as the reporter gene. Lentiviral particles for β2-microglobulin (β2m) silencing were produced by transfecting 5 × 10\textsuperscript{6} HEK293T cells with 10 μg of plasmid encoding for β2m-specific shRNA, 9 μg of plasmid pSPAX2 containing gag and pol proteins, and 3 μg of pMMD2G encoding for VSV-G protein. pLVTHm/si, pSPAX2, and pMMD2G were provided by D. Trono (Lausanne, Switzerland). A vector encoding a nonsense shRNA (shNS) was used as control. After 16 hours, the cells were washed and incubated with complete Dulbecco’s medium supplemented with 1% sodium pyruvate (C.C. Pro, Neustadt, Germany). After 24 hours, the lentiviral vector containing supernatant was collected, filtered (0.45 μm), and concentrated overnight by centrifugation.

**TABLE 1. Schematic representation of the shRNA sequence targeting β2m transcripts (shβ2m)**

| shRNA sequence | shβ2m |
|----------------|-------|
| 5' GGATGGAGAGAGATTGAA | TCA |
| 3' TTCTTACCTCTCTTTAACTTA | GAG |

**GENERATION OF HLA-DEFICIENT PLTs**
at 22,000 × g. The virus pellet was resuspended in complete medium.

**Lentiviral vector transduction**

Relative infectious titers of the lentiviral vectors were calculated by quantifying the percentage of eGFP-positive cells in HeLa cells transduced with diluted volumes of the concentrated viral supernatants. CD34+ cells (4 × 10^5/well) were seeded in fibronectin (RetroNectin, TAKARA Bio Inc., Shiga, Japan)-coated 24-well plate and infected with a multiplicity of infection of 10 in the presence of 8 μg/mL protamine sulfate (Sigma-Aldrich, Steinheim, Germany), SCF (100 ng/mL), Flt3-L (100 ng/mL), IL-6 (50 ng/mL), and TPO (100 ng/mL). After 8 hours, the CD34+ cells were washed using fresh complete medium. Transduction efficiency was calculated by considering the percentage of GFP-expressing cells.

**Suspension cultures for the generation of PLTs**

After transduction, 5 × 10^4 nontransduced, shNS-expressing progenitor cells or HLA Class I silenced progenitor cells, respectively, were cultured in IMDM supplemented with 5% AB serum, 20 ng/mL Flt3-L, 50 ng/mL TPO, and 50 ng/mL IL-3 for 5 days. CD34+ progenitor cells expressing eGFP+ as a marker for successful transduction were isolated by a flow cytometry–based cell sorting system (FACSAria, BD Bioscience, San Jose, CA). The GFP-positive cells were then maintained in culture in the presence of 50 ng/mL TPO and 50 ng/mL IL-3 for 16 days to allow the differentiation into megakaryocytes and the release of PLTs.9

**Morphologic analyses of megakaryocytes**

Megakaryocytes expanded in culture were identified with a fluorescence microscope (Keyence, Tokyo, Japan) 3, 6, 12, 17, and 21 days after transduction, based on shape and adherence properties.

**Characterization of megakaryocytic lineage using anti-CD41**

Five days after transduction, cells were collected and centrifuged at 450 × g for 5 minutes and resuspended in PBS. Megakaryocytic lineage cells were identified and quantified by flow cytometry analysis (FACSCanto, BD Bioscience Immunocytometry Systems) after staining with phycoerythrin (PE)-conjugated anti-CD41 (glycoprotein [GP]IIb-IIIa; Serotec, Dusseldorf, Germany; and BD PharMingen, Heidelberg, Germany). A PE-conjugated IgG was used as isotype control (BD PharMingen). The data were processed using software (FACSDiva, BD Bioscience Immunocytometry Systems). Two variables, the percentage of antigen-expressing cells and the mean fluorescence intensity as a relative measure for the amount of antigen expression on the cell surface, were used to describe the expression results.

**Quantification of PLTs derived from CD34+ progenitor cells**

Twenty-one days after transduction, PLT-containing supernatant was collected and centrifuged at 201 × g to

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Fig. 1. Microscopic analysis of cell morphology and GFP expression. Representative microscopic images of (A) megakaryocytes, 6 days after transduction (40x); (B) a GFP-expressing, pro-PLT–bearing megakaryocyte, 6 days after transduction (100x); and (C) GFP-expressing progenitor cells expressing shβ2m 3 days after transduction (20x).
remove large cells. The supernatant was further centrifuged at 1800 \( \times \) \( g \) for 5 minutes to obtain a PLT pellet. The PLTs were resuspended in PBS, labeled with PE-conjugated anti-CD61 (GPIIIa) (Sero tec) or anti-CD42a (GPIX; BD PharMingen), and measured by flow cytometry. The PLT gate was fixed in the forward scatter (FSC) and side scatter (SSC) profiles of blood-derived PLTs from healthy volunteers.

**Analysis of HLA Class I expression**

An allophycocyanin-labeled W6/32 antibody (Sero tec) that recognizes an antigenic determinant shared among all HLA-A, -B, and -C proteins was used to detect the expression of HLA on the cell surface by flow cytometric analysis. A gate was fixed in the FSC and SSC dot plot with the use of peripheral blood PLTs from healthy donors.

Total RNA was isolated from cells derived from the CD34\(^+\) progenitor cells (RNeasy mini kit, Qiagen, Hilden, Germany) 1 and 2 weeks after transduction. The isolated RNA was then reverse transcribed to cDNA using the high-capacity cDNA reverse transcription kit (Applied Biosys tems, Darmstadt, Germany). Real-time polymerase chain reaction (PCR) for \( \beta \)2m was performed using the oligonucleotide pairs and MGB-TaqMan probes as described elsewhere.\(^{16} \) The thermal cycling conditions used on a real-time PCR system (ONEStep Plus, Applied Biosystems) were 50°C for 15 minutes and 95°C for 10 minutes followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. The constitutively expressed \( \beta \)-actin gene was amplified as the reference standard for normalization of mRNA levels. All real-time PCR analyses were performed in triplicate.

**PLT activation assay with adenosine 5’-diphosphate and thrombin**

PLTs generated from CD34\(^+\) progenitor cells or blood-derived PLTs as controls were stimulated with 25 \( \mu \)mol/L adenosine 5’-diphosphate (ADP) plus 1 U of thrombin for 10 minutes and immediately fixed with fixation solution (Cytotix, BD PharMingen). To assess PLT activation by flow cytometry, PLTs were stained with PE-conjugated anti-CD62P (P-selectin, BD PharMingen) or purified anti-activated GP IIb-IIIa antibody (PAC-1, BD PharMingen) for 30 minutes. In the latter case, a PE-conjugated anti-mouse IgG (BD PharMingen) was used as the detection antibody.

**PLT aggregation assay after ADP and thrombin stimulation**

A mean of 1.8 \( \times \) \( 10^6 \) PLTs generated from CD34\(^+\) progenitor cells or blood-derived PLTs suspended in 100 \( \mu \)L of PBS were incubated with 40 \( \mu \)mol/L ADP plus 1 U thrombin for 10 minutes. Aggregation was then analyzed by flow cytometry analysis. In the stimulated PLTs, particles with higher FSC and SSC are observed, indicating aggregate formation.

**PLT aggregation assay after collagen stimulation**

A mean of 2 \( \times \) \( 10^7 \) PLTs generated from CD34\(^+\) progenitor cells or blood-derived PLTs resuspended in 200 \( \mu \)L of plasma were stimulated with 10 \( \mu \)g collagen (Nycomed, Linz, Austria). Aggregation was measured using an
RESULTS

Generation of genetically modified megakaryocytes and PLTs from CD34+ progenitor cells

Megakaryocytes were identified based on their morphology and CD41 (GPIIb-IIIa) expression. There was no difference in the phenotype of the progenitor cell–derived megakaryocytes between the cultures in which transduced and those in which nontransduced CD34+ cells were used for expansion and megakaryocyte lineage differentiation (Fig. 1A). The megakaryocytes exhibited long beaded projections, so-called pro-PLTs, from which PLTs were produced (Fig. 1B).17 There were no differences in the proportions of generated megakaryocytes between the cultures based on transduced and those based on nontransduced CD34+ cells. Five days after transduction, the proportions of CD41+ cells ranged from 25% to 53% (mean, 40%-42%; Fig. 2). Megakaryocytes yields tended to be higher for CD34+ progenitor cells transduced with shRNA-encoding vectors than for the nontransduced progenitor cells, although the differences were not significant. Interestingly, the highest number of megakaryocytes were obtained from the progenitor cells that had been treated with vectors encoding for β2m-specific shRNAs: after 1 week of culture in the presence of TPO and IL-3 for PLT differentiation, 5 \( \times \) \( 10^3 \) CD34+ progenitor cells produced a mean of 25.7 \( \times \) \( 10^3 \) \pm 11.2 \( \times \) \( 10^3 \) CD41+ cells (Fig. 3A).

PLTs were identified based on the scatter properties of peripheral blood PLTs from healthy donors and the PLT-specific antigens CD61 (GPIIIa) and CD42a (GPIX). The first PLTs were observed around Day 8 of cell culture. Flow cytometry analysis verified that culture-derived PLTs exhibited a similar but broader size distribution than freshly isolated blood PLTs (data not shown).18 After 21 days, the mean percentages of CD34+ progenitor cell–derived PLTs were similar under different treatment conditions, ranging from 55 to 65% when CD61 was used as the marker for PLT identification and from 25 to 30% when CD42a was used (Fig. 4). The imbalance in CD61 and CD42a expression on progenitor cell–derived PLTs compared to the expression profile of peripheral blood–derived PLTs suggests that the culture protocol used in this study may not completely mimic the natural conditions in the marrow.18 According to the megakaryocyte yield results, cultures with CD34+ cells treated to express β2m-specific shRNAs tended to produce larger numbers of PLTs than the cultures with nontransduced progenitor cells or progenitor cells expressing nonspecific shRNAs (Fig. 3B). On Day 21 of cultivation the concentration of CD61+ PLTs released by CD34+-derived megakaryocytes reached up to 475 \( \times \) \( 10^3 \) \pm 320 \( \times \) \( 10^3 \)/mL of culture.

Silencing of HLA Class I expression

We constructed a VSV-G pseudotyped lentivirus vectors for the stable delivery of a β2m-specific shRNA or a nonspecific shRNA as control. These vectors also encoded for eGFP as reporter gene. A mean of 35 ± 20% GFP-expressing cells was obtained after transduction of the CD34+ progenitor cells (Fig. 1C). Five days after transduction, real-time PCR showed up to 95% reduction of β2m mRNA levels in progenitor cells treated for shRNAs specific for β2m (shβ2m) expression compared to levels in nontransduced cells or those expressing the nonspecific shRNA (Fig. 5A). Accordingly, a reduction in HLA Class I
protein expression by up to 85% was observed in the CD34+ cells expressing the β2m-specific shRNAs compared to those cells that had been transduced with the control construct (Fig. 5B). Up to 85% HLA Class I antigen reduction was observed in PLTs that derived from β2m-specific shRNAs expressing progenitor cells, suggesting that RNAi-mediated HLA deficiency was maintained during PLT maturation (Fig. 4). The degree of HLA Class I silencing on PLTs remained stable during the entire culture period (data not shown).

**Functional assessment of HLA Class I-silenced PLTs**

To explore the functionality of CD34+ progenitor cell-derived HLA Class I-deficient PLTs derived from, we used flow cytometry to perform PLT aggregation analyses and to examine GPIIb-IIIa activation and CD62P (P-selectin) antigen up regulation on PLTs after stimulation with the major PLT agonists thrombin and ADP. The expression of activated GPIIb-IIIa and CD62P on the CD34+ progenitor cell–derived PLTs was clearly evoked by this treatment in a fashion similar to those from healthy volunteers (Fig. 6). In particular, there was no difference in ADP- and thrombin-induced activation between the HLA Class I-silenced and the nonsilenced PLTs. A mean of 75% of HLA Class I-silenced PLTs upregulated CD62P expression and the expression of activated GPIIb-IIIa was increased by up to 35% on these PLTs. Activation with ADP and thrombin produced similar values in PLTs derived from both nontransduced CD34+ progenitor cells and PLTs generated from progenitor cells expressing nonspecific shRNAs (CD62P up regulation of 70 ± 27% and PAC-1 binding of 30 ± 17%). Blood-derived PLTs exhibited CD62P expression levels of 82 ± 17% and PAC-1 binding of 26 ± 7% after stimulation.
Our aggregation analysis also suggested that HLA Class I–silenced PLTs derived from HPCs were as functional as nonsilenced PLTs and PLTs from peripheral blood. There was no difference in thrombin-induced aggregation in these different types of PLTs (Fig. 7A). PLT aggregation was detectable in 36 ± 25% of the HLA-silenced PLTs. After stimulation, 35 ± 26% of nontransduced progenitor cell–derived PLTs and 30 ± 22% of shNS-expressing progenitor cell–derived PLTs formed aggregates. Similarly, stimulation of blood-derived PLTs

![Fig. 5. Silencing of HLA Class I expression in CD34+ progenitor cells. A lentiviral-based vector was used for the delivery of shRNAs specific for β2m or nonspecific shRNAs. (A) HLA Class I surface expression and (B) β2m transcript levels were measured by flow cytometry and real-time PCR, respectively, in nontransduced progenitor cells (negative control [NC]), progenitor cells transduced with the vector encoding for shNS, and progenitor cells transduced with the vector encoding for shβ2m 5 days after transduction and GFP-positive cell sorting.

![Fig. 6. Assessment of activated GPIIb-IIIa and CD62P (P-selectin) expression on CD34+ progenitor cell–derived PLTs after stimulation. Activated GPIIb-IIIa (A) and CD62P (B) were measured in the absence and presence of thrombin (25 μmol/L) and ADP (1 U) on PLTs derived from blood, progenitor cells transduced with the vector encoding for shNS, and progenitor cells transduced with the vector encoding for shβ2m. For assessment of activated GPIIb-IIIa the cells were stained with PAC-1, an antibody that mimics the specific fibrinogen binding to human GPIIb-IIIa in the activated state.](image-url)
A. Blood-derived PLTs  shNS  shβ2m

Nonstimulated

SSC

CD62P

96% 78% 85%

Stimulated

B. Blood-derived PLTs  shNS  shβ2m

Nonstimulated

SSC

PAC-1

24% 19% 32%

Stimulated
resulted in aggregation of 42 ± 21% of the PLT population. In response to collagen, PLTs isolated from peripheral blood showed a mean of aggregation of 35 ± 8% and 80 ± 10% 30 seconds and 5 minutes, respectively, upon stimulation (Fig. 7B). Thirty seconds and 5 minutes after collagen stimulation, 24 ± 29 and 39 ± 24.9% of the PLTs derived from CD34+ progenitor cells expressing shNS were able to aggregate. PLT aggregation was detectable in 33 ± 11% and 46 ± 9% of the HLA Class I–silenced PLTs 30 seconds and 5 minutes, respectively, after collagen stimulation. For interpretation of the PLT aggregation results obtained by the PAP4 aggregometer it must be taken into account that due to their limited availability the cell culture–derived PLTs could not be used in the concentrations this assay has originally been validated for.

DISCUSSION

In recent years, an increasing number of studies reported culture methods for generation of human blood cells from progenitor cells. Functional red blood cells (RBCs) and PLTs were obtained from hematopoietic stem cells of diverse origins: peripheral blood, marrow, and cord blood.8,9,18-23 In addition, embryonic stem cells with their unlimited proliferative potential as well as induced pluripotent stem cells have been considered as a new source for in vitro hematopoiesis; culture systems have been developed that enable embryonic stem cells to efficiently develop into hematopoietic progenitors capable of releasing functional blood cells.10-12,24,25 The degree of large-scale expansion and maturation achieved appears very promising for the ex vivo generation of PLT and RBC units for clinical applications. Such methods of generating blood cells from embryonic stem cells or pluripotent stem cells could make it possible to produce a constant and safe supply of blood cells, thus eliminating the need to obtain blood cells from blood donation. This perspective has motivated research groups around the world as well as the US mili-
tary to launch “blood pharming” programs aimed at creating systems to produce RBCs derived from progenitor cells for use in blood transfusions.

The concept of using progenitor cells for mass production of blood cells holds the potential to genetically manipulate the starting material to produce designer blood cells for multiply transfused patients for whom it is difficult to resource compatible blood. Analogously to what has recently been shown for cell culture–derived RBCs, we were able to generate in vitro human PLTs with a target phenotype different from the original donor phenotype. We used a combination of lentiviral gene transfer and RNAi technology to silence HLA Class I expression in PLTs derived from CD34+ cells. The dimeric structure of the HLA Class I molecule, comprising the polymorphic HLA heavy chain and the conserved β2m molecule, was used to develop the strategy for Class I–specific HLA silencing. Target sequences within the coding region of β2m were selected to design shRNAs to silence all HLA Class I molecules on the cell surface. Stable reduction of HLA Class I antigen expression by up to 85% on progenitor cells and PLTs suggests that the expression of shRNA in hematopoietic stem cells was efficient and maintained during megakaryocyte differentiation, thus resulting in inhibition of targeted protein in PLTs. Moreover, the silencing strategy seemed to be satisfactorily efficient to inhibit de novo synthesis of HLA Class I molecules from residual mRNA in mature PLTs during storage in the culture medium. These data indicate that lentiviral-mediated RNAi is a powerful tool for continuous production of genetically engineered PLTs.

The idea of providing HLA-reduced PLTs for heavily HLA Class I–alloimmunized patients is not new. Elution techniques with chloroquine or citric acid have been used to remove HLA antigens from PLTs. The first successful transfusion of HLA-eluted PLTs in an HLA-alloimmunized patient was reported by Shanwell and colleagues in 1991. They demonstrated that the degree of HLA reduction varies, which raised doubt about the application of HLA-eluted PLTs for transfusion purposes. The first successful transfusion of HLA-eluted PLTs in an HLA-alloimmunized patient was reported by Shanwell and colleagues in 1991. They demonstrated that the degree of HLA reduction varies, which raised doubt about the application of HLA-eluted PLTs for transfusion purposes. Further progress was made toward the standardization of HLA reduction by acid elution and although acid-treated PLT transfusions were successfully used on a small scale, PLT quality could become critically affected by the PLT preparation procedure. However, even if the clinical and in vivo survival data obtained with acid-treated PLTs are only based on case studies, they suggest that HLA-deficient PLTs may survive almost normally in the circulation and be functionally intact. This is to be expected in particular because HLA reduction on silenced PLTs (up to 85%) was similar to what was described for acid-treated PLTs and silenced PLTs are not subjected to any harsh preparation procedures. In previous studies, we could demonstrate in microcytotoxicity tests that RNAi-mediated silencing of the HLA Class I expression prevented antibody-mediated cytotoxicity. In addition, T-cell cytotoxicity and proliferation showed to be significantly reduced when allogeneic T cells were exposed to HLA-deficient target cells. Our observation that HLA Class I antigen expression on HLA-silenced PLTs was not up regulated after in vitro stimulation suggests that the posttransfusion increment in HLA-alloimmunized patients may not be impaired by de novo synthesis of HLA on activated PLTs. Nevertheless, studies in animals and humans are needed to determine the success rate and tolerability of HLA-silenced, culture-derived PLTs.

The functional role of HLA Class I on PLTs is not entirely clear. In blocking studies, Cosgrove and coworkers showed that both heavy and light chains of HLA Class I antigens might be involved in PLT aggregation and release reactions. HLA alloantibodies could induce the aggregation of human PLTs and selectively block the binding of some PLT agonists (e.g., collagen and adrenaline) but not of others (e.g., thrombin). Rubinstein and colleagues suggested that HLA Class I might synergize with the Fcy RI receptor to activate PLTs. Although a specific function of PLT membrane HLA antigens cannot be excluded, our in vitro data suggest that expression of PLT–specific membrane GPs and PLT function are not affected in HLA-silenced PLTs. The silencing strategy presented herein might be an attractive tool to be used in an autologous animal model to further determine the functional relevance of HLA Class I on PLTs.

Important safety concerns exist whenever gene transfer is used to manipulate progenitor cells for clinical purposes. The main difference to be considered in risk assessments between clinical trials using genetically engineered progenitor cells for transplant purposes itself or PLTs derived thereof in cultures for transfusion purposes is the complete absence of any proliferation potential of fully matured PLTs. This virtually eliminates any risk of malignant transformation of transfused PLTs making the development of leukemia in the recipient improbable. Moreover, standard blood preparation techniques are available to ensure that transfusion units contain only matured PLTs by eliminating or inactivating contaminating nucleated cells: leukoreduction filters could be used to effectively eliminate any contaminating undifferentiated progenitor cells and PLTs, as anucleate fragments of cytoplasm, can easily be irradiated before transfusion.

In conclusion, this study shows for the first time the feasibility of generating functional HLA Class I–deficient PLTs from CD34+ progenitor cells. Blood products containing HLA-deficient PLTs may become a potent tool to overcome clinical refractoriness in multiply transfused patients. At present, transfusion units from large-scale production of cultured blood cells appear to be a technological luxury, considering the costs to establish industrial production conditions compatible with transfusion requirements and the rules of good manufacturing...
practice. For example, current strategies to produce PLTs from HPCs include expensive cytokines continuously to be added to the culture media. However, the new technology presented here makes it possible to produce designer blood products that can be live-saving in certain clinical situations. The provision of specially designed blood units such as HLA-silenced PLT concentrates may help to improve the cost-benefit ratio of in vitro generated blood cells. Currently, the amount of PLTs generated in any experimental setting fails to satisfy the clinically required quantity for transfusion. The key challenge is the efficient and continuous expansion of progenitor cells that can differentiate into mature PLTs. Human embryonic or induced pluripotent stem cells, which have an unlimited proliferative potential, could become in the next years an attractive resource for blood cell engineering. However, the technical conditions for industrial development must still be created. In addition, a better understanding of the megakaryocytosis and the mechanism of PLT production is needed to optimize cell culture conditions.

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CONFLICT OF INTEREST

The authors declare that they have no competing financial interest.

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