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CASE REPORT

Transplantation of haploidentical TcRαβ-depleted hematopoietic cells allows for optimal timing and sustained correction of the metabolic defect in children with infantile osteopetrosis

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ABSTRACT

In osteopetrosis, osteoclast dysfunction can lead to deafness, blindness, bone marrow failure and death. Hematopoietic cell transplantation (HCT) is currently the only curative treatment, but outcome remains disappointing. Although a rapid progression towards HCT is detrimental to prevent further progress of disease manifestations, 70 percent of cases lack an HLA-matched sibling and require alternative stem cell sources. We present two cases of osteopetrosis that successfully received an HCT with haploidentical TcRαβ-depleted cells from one of the parents. These cases showed no further disease progression, had restoration of functional osteoclasts and illustrate this approach to enable prompt HCT with ready available parental donors and rapid and sustained hematological, including osteoclast, recovery.

INTRODUCTION

Osteopetrosis (OP) is a genetic disorder characterized by improper bone resorption[1] that in most cases is caused by dysfunctional, hematopoietic stem cell (HSC)-derived osteoclasts. Most clinical phenotypes require hematopoietic cell transplantation (HCT) as the only current curative treatment.[1,2] In malign infantile OP (MIOP), performing HCT without delay is required to restore rapid osteoclast function to prevent progression of irreversible damage, including bone marrow failure, blindness and deafness.[1] Also in more slowly progressing OP cases terminal bone marrow failure often urges for HCT.[1] Still, the outcome of HCT in OP remains disappointing. The overall survival, at best, is 60–65%[1,3,4] in all OP cases and below 50% for OP cases who do not have an HLA-identical sibling donor (approximately 70% of cases). Graft failure is the most common cause of death.[3]

Autosomal recessive OP is overrepresented in ethnic groups with high parental consanguinity,[1] of which many have a low coverage within the bone marrow donor registries. Therefor alternative donors are to be considered when no HLA-identical healthy sibling is available. Moreover, even if a register donor is identified, a donor search is time consuming and justifies the use of alternative, ready available donors. As such, cord blood transplantation has been used in the context of OP but was associated with higher transplant-related morbidity and mortality.[3,5] HCT in MIOP using a parental haploidentical donor is seemingly attractive because the parents typically are readily available. A number of reports have described haploidentical transplantation (Haplo-HCT) using CD34-enriched mobilized peripheral blood stem cells (PBSC).[6-9] However, complications such as delayed hematological and immunological recovery, graft rejection, and, or, the need for a stem cell boost were frequently observed.

Generally in Haplo-HCT, the use of a T cell receptor alpha beta (TcRαβ)-depleted graft, as opposed to CD34 enrichment, confers rapid hematological and immunological reconstitution,[10,11] most likely because of the presence of highly proliferative, CD34-negative progenitor cells alongside the HSC in the graft. Also, the high numbers of TcRγδ cells, NK cells and monocytes in the TcRαβ-depleted graft may act to facilitate engraftment. The use of a TcRαβ-depleted graft has already been reported as successful in three OP cases, [12] but not in the context of a haploidentical donor. Together, we hypothesize that TcRαβ-depleted Haplo-HCT is an attractive approach to i) perform HCT without delay, ii) induce a fast recovery of osteoclast function in OP cases and iii) possibly accelerate the immunological recovery. To our knowledge, the published literature mentioned only one example of an OP case who received TcRαβ-depleted Haplo-HCT; this case experienced subsequent graft rejection.[13] Here we report successful TcRαβ-depleted Haplo-HCT in two OP cases, one a newborn MIOP case with an aggressive, rapidly progressing disease and one a 2-year-old who experienced OP-related bone marrow failure.

CASE REPORTS

Case one. This case is a male offspring from consanguineous parents carrying a mutation involving the *TCIRG1* gene. He was diagnosed with OP at age 3 months while showing signs of progressing anaemia, hypogammaglobulaemia and esotropia/exophthalmia. He was blind, had impaired hearing, multiple skeletal malformations (Figure 1A) and displayed abnormal bone and bone marrow architecture (Figure 1B). Within 3 weeks, a fully myeloablative conditioning regimen was initiated consisting of anti-thymocyte globulin (ATG)-Fresenius (30 mg/kg), i.v. busulfan (TDM; AUC 90 mg ± 5 mg*h/L), fludarabine (160 mg/m^2), and thiotepa

(15 mg/kg). Since the risk of graft rejection seems to be correlated with the intensity of the conditioning regimen, he received a busulfan dose typically recommended for malignant diseases, although data addressing the optimal busulfan dose for OP cases is scarce. There is some evidence that replacing cyclophosphamide by fludarabine may increase survival, probably by reducing the risk of pulmonary and hepatic veno-occlusive disease (VOD).[14] Still, VOD was observed among cases conditioned with a combination of busulfan and fludarabine an as our cases received a high target busulfan AUC it was decided to give defibrotide as VOD prophylaxis, 25mg/kg/d from start of conditioning therapy until day +30. Maternal G-CSF-mobilized PBSC were harvested and TcRαβ+ cells were depleted from the graft (CliniMACS). The case was transplanted with a single graft including 10.9×10^8 TNC cells/kg, 44.8×10^6 CD34+ cells/kg, 48.6×10^6 TcRγδ+ cells/kg, and 0.0011×10^6 residual TcRαβ+ cells/kg. At day +1 the case received a single dose of rituximab 375 mg/m^2 as EBV-PTLD prophylaxis. Platelet ($>50 \times 10^9$/L) and neutrophil ($>0.5 \times 10^9$/L) recovery was reached at day +14 and day +15 post-HCT, respectively. The case required immunoglobulin substitution until day +99 and CD4+ T cells exceeded 200×10^6/L at day +181. Immunosuppression consisted of mycophenolate mofetile and methylprednisolone, which were discontinued after 4 and 6 weeks, respectively, without any signs of graft-versus-host disease. No significant transplantation-related morbidity was observed. Peripheral blood T cell chimerism showed spontaneous conversion from initially mixed chimerism (up to 40% of autologous cells) to 100% donor chimerism at 1 year post-transplant. OP characteristics showed no obvious progression following HCT, and at 3 years post-transplantation, the case is in a stable general condition with a good quality of life. He is blind and has decreased but stable hearing performance with no signs of further deterioration. The case has normal bone

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marrow function and experienced normalization of the phenotypic facial and skeletal characteristics (Figure 1C), indicative of osteoclast function recovery. He displays a physical and mental development similar to other vision impaired children.

Case two. This case, also the son of consanguineous parents, was admitted from Saudi Arabia to our hospital at 2 years of age. At age 6 months, a progressing dysmorphia of the facial bones and growth retardation characteristic of OP were observed, as well as progressing and persistent anaemia. Acoustic and visual function and neuroimaging were normal at age 18 months, but typical OP-related splenomegaly, skeletal malformations (Figure 2A) and pathological bone and bone marrow histology (Figure 2B) were observed. He had a weight-for-age z score of -2.5 SD (standard deviation) and a height-for-age z score of -2 SD. No known mutations in OP-associated genes were found. Because the boy experienced secondary bone marrow failure in the context of OP in the absence of a matched unrelated donor, he was scheduled for a parental Haplo-HCT. The conditioning regime and VOD prophylaxis was similar to that in the previous case, except for an ATG dose of 60 mg/kg, and was complicated only by transient ATG-associated serum sickness. The ATG dose in this case was increased due to a larger risk of rejection as a consequence of higher age and regular blood transfusions. This case received paternal G-CSF–mobilized, TcRαβ-depleted PBSC, followed by rituximab at day +1. The graft composition consisted of 21.6×10^8 TNC cells/kg, 43.3×10^6 CD34+ cells/kg, 50.2×10^6 TcRγδ+ cells/kg, and 0.018×10^6 residual TcRαβ+ cells/kg. Platelet and neutrophil recovery was observed at day +13. The case required immunoglobulin substitution until day +123 and CD4+ T cells exceeded 200×10^6/L at day +207. At day +11, the case developed fever while peripheral blood CD3+ T cell chimerism levels showed 40% autologous signal. Because of suspicion of rejection (non-infectious fever and

mixed T cell chimerism), the case received immunosuppression with cyclosporine A and steroids, which was stopped at day +159 with complete donor chimerism of myeloid cells and stable mixed chimerism (21% autologous) of CD3+ cells. As previously described[15] and as a consequence of potent allogeneic osteoclast recovery, the case developed hypercalcaemia that required regular treatment with the RANKL-inhibitor denosumab at least until the last day of follow-up, day +308, of which the first 225 days are illustrated in Figure 2C. The case showed normalization of the phenotypic facial characteristics in the absence of graft-versus-host disease.

DISCUSSION

We present here two cases with osteoclast-rich OP (Figures 1B and 2B) that, as opposed to osteoclast-poor OP, can profit from HCT as curative treatment modality. Both cases successfully received a parental haploidentical TcRαβ-depleted transplantation, following full myeloablative conditioning and high-dose serotherapy with ATG. Both cases showed transient mixed chimerism before converting to 100% donor chimerism. Importantly, neither case rejected the graft or required a stem cell boost due to graft failure, as often occurs following HCT in OP.[1,3] Also, despite the intensive conditioning regimens no VOD or severe viral infections were observed. The first case represents a MIOP population, who require rapid HCT and recovery of osteoclast function to stop further progression of the disease. The approach presented here illustrates the feasibility of TcRαβ-depleted Haplo-HCT in such a case, with HCT and hematopoietic recovery both commencing within only a few weeks of the OP diagnosis. In support of this early intervention, no further phenotypic progression of the disease was observed. The second case illustrates that TcRαβ-depleted Haplo-HCT in cases with slower disease progression but who ultimately progress to bone

marrow failure is also an effective means of restoring osteoclast function, when an HLA-identical sibling or unrelated donor is not available. We speculate that the preservation of large numbers of CD34-negative myeloid progenitors (the direct ancestor of the osteoclast lineage) contained in the graft following TcRαβ-depletion, as opposed to a CD34 enrichment that depletes most of the CD34-negative cell fraction, allows for the desired and rapid osteoclast recovery in OP cases.

Overall, we conclude that Haplo-HCT using TcRαβ-depleted cells is an encouraging approach in malignant infantile OP that enables i) prompt HCT with immediate availability of a parental donor and ii) rapid and sustained hematological recovery. Both cases show objective signs of osteoclast recovery, with normalisation of radio imaging in case one (Figure 1C), and hypercalcaemia in case two (Figure 2C).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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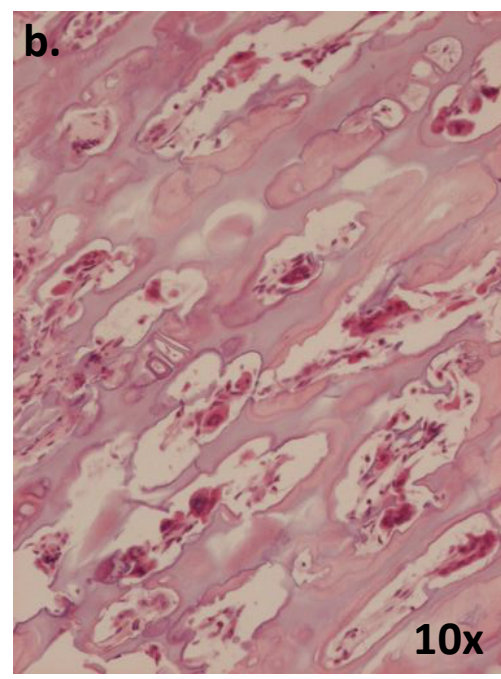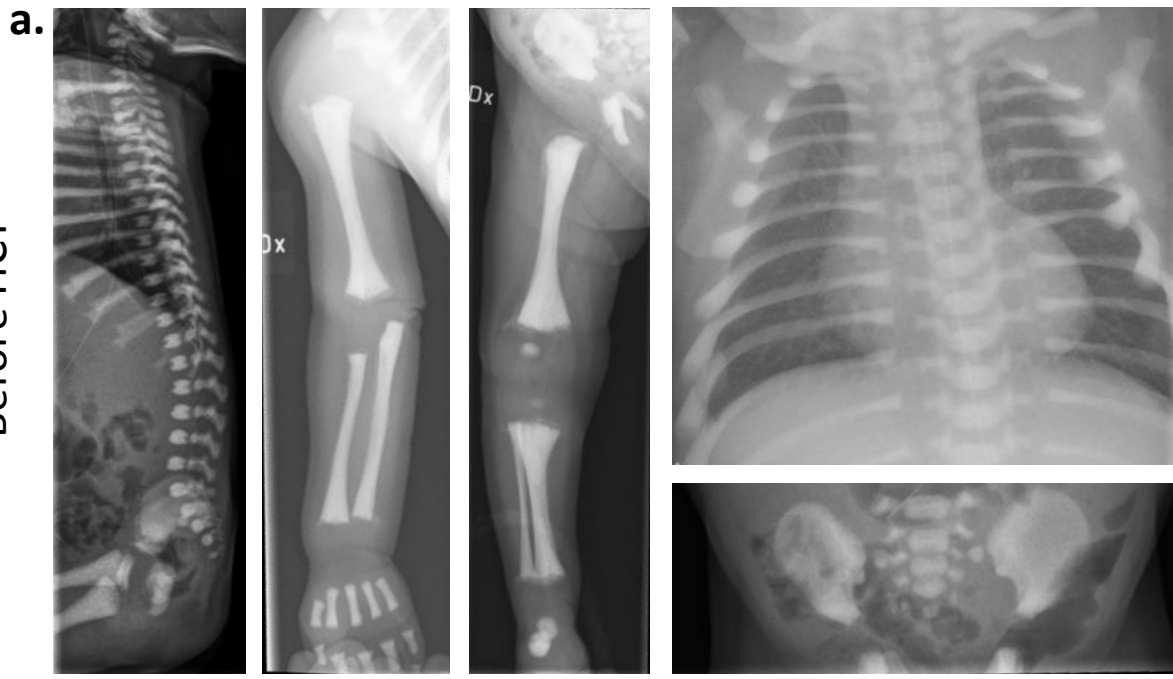   Transplant. 2012;16(5):E140-5.

LEGENDS

Figure 1. Characteristics of Case One. **a)** From left to right, X-ray imaging of the
skeletal structures of the spine, right arm, right leg, thorax (upper) and pelvis (lower)
before transplantation at age 3 months. The radiographs show generalized
osteosclerosis, with reduced cortico-medulllary differentiation. In the spine the
vertebra have a sandwich vertebra appearance due to bone accumulation in the
endplates. In the extremities fraying of the metaphysis is seen as well as widening of
the metaphyseal ends of the bones and periosteal new bone formation, which indicates

superimposed rickets. **b)** Diagnostic trephine biopsies were obtained from the iliac crest, fixed, decalcified and after paraffin embedding, the samples were sectioned and stained with hematoxylin eosin for microscopic examination, indicated magnification x100. The bone marrow cavity is replaced by spongiotic unmineralized bone matrix surrounding some cores of cartilage. There are numerous osteoclasts in the remaining narrow bone marrow spaces. **c)** From left to right, X-ray imaging of the thoracic spine, upper and lower right arm, and upper and lower right leg, 3 years after transplantation. All the changes seen previously have disappeared and the radiological appearance of the skeleton is normalized.

Figure 2. Characteristics of Case Two. **a)** From left to right, X-ray imaging of the skull (upper), pelvis (lower), thoracic and lumbar spine, thorax and the upper and lower right arm, prior to transplantation at 2 years of age. The osseous structures have overall increased density, in particular within the medullary portion. The skull bone is thickened and the vertebral bodies have a sandwich vertebra appearance, with a bone-within-bone phenomena. The bone-within-bone phenomena is also seen in the extremities. In addition, the metaphysis of the extremities have an abnormal appearance, with an Erlenmeyer flask deformity and multiple dense metaphyseal bands. The ribs are widened anteriorly. **b)** Diagnostic trephine biopsies from the iliac crest, indicated magnification x100. Most of the bone marrow cavity is replaced by thick spongiotic unmineralized bone matrix surrounding islands of mineralized woven bone harboring some osteocytes. Some osteoclasts were identified (insert). **c)** Peripheral blood plasma concentrations of calcium (upper, round-dotted line) and calcium ion (lower, square dotted line), respectively. Dots indicate time point of blood

analysis. Gray shadowed areas indicate normal range. Asterisks (*) indicate time point of treatment with RANKL-inhibitor denosumab.

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a.

Before HCT

b.

10x

c.

3 years post-HCT

a.

b.

10x

40x

c.

mmol/L

Calcium
Calcium ion

4

3

2

1

* * * * * *

-50   0   50   100   150   200   250

Days post transplantation