Bone Marrow Transplantation (2021) 56:1452–1454
https://doi.org/10.1038/s41409-020-01196-4

CORRESPONDENCE

Irradiation-free re-conditioning in children following graft failure of a T cell-depleted graft from a haploidentical parent

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Received: 16 July 2020 / Revised: 30 November 2020 / Accepted: 8 December 2020 / Published online: 8 January 2021

To the Editor:

Haplo-identical stem cell transplantation (hHSCT) is an option for patients who require a stem cell transplantation, but lack an HLA-identical sibling or HLA-matched unrelated donor [1–3]. Various modes of hHSCT are currently employed [4]. Here, we focus on transplantation of T cell-depleted HLA-haploidentical stem cells (TCD hHSCT) from a parent to their child. This maneuver is associated with a risk of graft failure in 10% of these procedures. Graft rejection represents a life-threatening complication and requires timely re-transplantation [5]. Teltschik et al. reported on a cohort of 16 pediatric patients who underwent a second TCD hHSCT using a reconditioning regimen containing total nodal irradiation prior to the re-transplantation [6]. Although localized irradiation represents a toxicity-reduced approach, various reasons argue to avoid irradiation in pediatric transplant. In the present retrospective study, we identified nine patients with graft failure after first TCD hHSCT who received a salvage TCD hHSCT from the other parent preceded by an irradiation-free lymphodepleting reconditioning regimen. All of these patients achieved sustained engraftment and complete donor chimerism.

We report on a cohort of nine children who underwent T cell-depleted HLA-haploidentical stem cell transplantation from a parent between 2011 and 2019. Median age at first transplantation was 2.3 years (range 3 months to 10 years). We reviewed seven female and two male patients, whose indication for the transplantation was a malignancy in four cases (two cases of myelodysplastic syndrome, one case of each, acute lymphoblastic leukemia and neuroblastoma). In five patients, indication for HSCT was based on non-malignant diseases, i. e., mucopolysaccharidosis Type I Hurler in three cases and hemophagocytic lymphohistiocytosis in two children. Five patients had secondary graft failure and four patients showed non-engraftment after their first TCD hHSCT. In all cases, one parent donated for the first transplantation, and the other parent for the re-transplantation.

The conditioning regimen in the first haplo-transplantation included fludarabine and thiotepa. All but two patients also received anti-thymocyte globulin (ATG, Grafalon®), one patient thymoglobulin® and one of the HLH patients alemtuzumab. The fourth and last conditioning component varied, with treosulfan and melphalan employed in 4 patients each, and TDM busulfan in one patient. All nine patients received immunoglobulins intravenously on days 1, 3, 7, 14, 21, and 28. G-CSF was started intravenously in eight patients on day +5 post-transplantation until leukocyte counts exceeded 10^9/l. GvHD prophylaxis in all cases consisted of mycophenolate mofetil only, at a daily dose of 1200 mg/m^2, started on day −1 pre-transplantation.

For both transplantations, the stem cells were harvested from GCSF-mobilized peripheral-blood from a parent donor. In seven cases, CD34 positive selection was utilized, and CD3/CD19 depletion for the remaining two cases. All patients received between 2.9×10^6 and 22.1×10^6 CD34$^+$ stem cells/kg patient bodyweight (mean: 8.6×10^6/kg bw) and 14.7×10^3 to 80.0×10^3 T cells/kg patient bodyweight. An overview for the first transplantation is shown in Supplementary Table 1.

After the first (and ultimately unsuccessful) transplantation, three of the nine patients formally fulfilled leukocyte engraftment criteria, four patients showed detectable leukocyte counts from 0.2 to 1.2 10^9/l for a period of 2 to

Supplementary information The online version of this article (https://doi.org/10.1038/s41409-020-01196-4) contains supplementary material, which is available to authorized users.

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Table 1 Patient characteristics and overview for the re-transplantation.

Patient ID	Donor	Day of re-transplantation (days after rejection)	Day of engraftment	Acute GvHD	Lansky (day 100)	Last follow-up	Status at last follow-up	Chimerism at last follow-up
1	Father	15	10	No	90%	109	Alive	cc
2	Father	6	9	II	90%	80	Alive	cc
3	Father	9	12	No	90%	11	Alive	cc
4	Mother	10	11	II	80%	63	Alive	cc
5	Father	26	15	No	80%	4 (t.o.d.)	Dead	
6	Father	16	11	I	80%	26	Alive	cc
7	Father	16	12	No	90%	14	Alive	cc
8	Father	22	14	I	100%	11	Alive	cc
9	Mother	26	12	I	100%	15	Alive	cc

Patient ID identical to Supplementary Table 1.

cc complete chimerism.

6 days, whereas the remaining two patients displayed no measurable peripheral blood leukocytes at all (Supplementary Fig. 1). Non-engraftment was defined as leukocyte count continuously $<10^9$/l in combination with bone marrow aplasia and/or a donor chimerism below 5%. Secondary graft failure was defined as leukocyte count $<10^9$/l after initial engraftment (i. e., three consecutive days with leukocyte count $>10^9$/l) in combination with bone marrow aplasia and/or donor chimerism <5%.

Of note, fever was manifest in eight of the patients, while one patient with non-engraftment showed no fever. Elevated D-dimers were seen in all patients. No acute GvHD was observed after the first transplantation.

The second transplantation (i. e., the re-transplantation) took place between 6 to 26 days (median: 16 days) after graft failure. Although conditioning regimens were heterogeneous in the initial hHSCT, all patients were conditioned with fludarabine (at a dosage of 40 mg/m^2 on days –6 to −4.; one patient under 1 year of age received 3 × 1.6 mg/kg), thymoglobulin with 4.5 mg/kg in three doses on days −8 to −6, and thiotepa with 3 × 5 mg/kg in 3 patients and 4 × 5 mg/kg in 6 patients (Table 1) on days −4 to −3. In contrast to the previously reported cohort, no radiation was administered. T-cell depletion of PBSC was employed in six grafts by CD34$^+$ selection and in three with CD3/CD19 depletions. In comparison to the first transplantation, the median of transplanted stem cells for the re-transplantation was higher with 19.3×10^6 CD34$^+$ stem cells/kg patient bodyweight (range $5.7–26.48 \times 10^6$ CD34$^+$ stem cells/kg patient bodyweight). The median of transplanted T cells remained the same with 60×10^3 CD3$^+$ cells/kg patient bodyweight (range $50–87 \times 10^3$ CD3$^+$ cells/kg patient bodyweight).

Leukocyte engraftment was achieved in all patients 9–15 days post transplantation (median: day 12). The leukocyte levels over time for the second transplantation are depicted in Supplementary Fig. 2.

Acute GvHD was rated according to the Glucksberg criteria [7]. Four of the nine patients exhibited no symptoms of GvHD, three patients had grade I aGvHD, two patients had grade II aGvHD, which was limited to the skin. By d100 GvHD had resolved in all patients. At last follow-up no patient showed symptoms of a GvHD.

The last follow-up date included for this report was on May 15, 2020, resulting in a median follow-up time ranging from 11 months to 9 years (median: 15 months). At the time of this analysis, eight of the nine patients were alive, one patient died 4 months after the re-transplantation due to a leukemia relapse with subsequent multi-organ failure as the cause of death. Of the eight surviving patients, one patient had a relapse of the neuroblastoma and is in palliative treatment, lost to follow-up. One had a severe episode of autoimmune hemolytic anemia (AIHA), this patient was also treated with two donor-lymphocyte infusions after the second transplantation. The patient suffered from a prolonged CMV reactivation after both, the first and second transplantations, as well as a BK-virus-associated hemorrhagic cystitis. At last follow-up this patient was in good health, with medication for her AIHA. In total, four patients had a CMV reactivation post re-transplantation, one suffered from a transfusion-related acute lung injury shortly after transfusion. At last follow-up, complete donor chimerism was found in all patients. Of the eight surviving patients, one patient received transplantation-related medication and seven were in good health, one is in palliative care. The last transplanted patient is at day 462. The Lansky Score for the 8 patients who reached day 100 was between 80 and 100% ("active, but gets tired more quickly" to "fully active, normal", median: 90%). The reconstitution of lymphocytes, CD3+, CD4+, CD8+, and CD19+ cells is depicted in Supplementary Fig. 3.

With a mean follow-up of 15 months, 8 out of 9 patients are alive with complete donor chimerism and no case of

transplant-related mortality. So, the overall outcome after irradiation-free re-conditioning is favorable. Omitting irradiation spares side effects and the need to transfer the patient out of the isolation room during this vulnerable period of prolonged aplasia. It is difficult to assess risk factors for non-engraftment or rejection in this limited cohort. One observation was, that 4 of the 9 patients still had measurable levels of leukocytes at day 0. Underlying disease and lack of chemotherapeutic pre-treatment may have also favored rejection in the patients with metabolic diseases or hyperimmune syndromes such as HLH.

Schlegel et al. had reported on alternative irradiation-free concepts for re-transplantation after failing variable primary conditioning based on OKT-3, which is no longer clinically available [8]. At the moment, there is no standardized procedure for patients after rejection or non-engraftment. The conditioning regimen in this cohort was identical for all nine patients, with the only variance in the dosage of thiotepa. Thiotepa seems to be of some relevance in reduced conditioning settings to accomplish complete donor chimerism [9, 10]. With all patients showing a robust engraftment post-transplantation, and only few complications, this conditioning seems to be a promising regimen, notwithstanding the small cohort size. Nevertheless, these results may help in clinical decision making in this rare, but life-threatening situation.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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