

Depletion of TCR alpha/beta+ T-lymphocytes from grafts for haplo haematopoietic CELL transplantation (HCT) in children

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ABSTRACT

This study presents the results of haplo HCT where TCR alpha/beta positive T-lymphocytes and B lymphocytes with the capacity of causing GvHD and PTLD are removed. The graft was harvested from one of the parents. The donor with most B KIR motifs was used. If no difference between the parents the mother was used. Grafts were processed using CliniMACS Prodigy (Miltenyi Biotec ®) and T lymphocytes with TCR alpha-beta was removed to at least <50000/kg preferably to <25000/kg. We aimed at administering at least 10 x 10E6 CD34/kg positive stem cells. Pre transplant conditioning consisted of ATG (Grafalon) 30mg/kg, Fludarabin 150mg/M² (or Clofarabin 200mg/M²), Thiotepa 10 mg/kg and Melphalan 120mg/M^2 . No GvHD prophylaxis were given if alpha/beta cells administrered was below 25000/kg. If more than 25000/kg alpha/beta cells were given the patient received a short course of mycophenolate mofetil. 23 children received TCR alpha/beta depleted haplo HCT or CD3 depleted haplo HCT (n: 3), 20 of these patients being transplanted for leukemia, NHL or MDS. Twelve of the 15 patient with acute leukemia were in 2nd or later CR. The patients with non-malignant diseases suffered from primary immune deficiency (N:2) or metachromatic leucodystrophy (N:1). The KM estimate for 5-yers overall survival was 53% for the whole group and 47% for clonal diseases. Severe acute GvHD was observed in only one patient (grade III). Immune reconstitution was slow following HCT, with half the patients reaching normal CD3 and CD4 counts between 0.5 and 1.0 year after the HCT NK cell counts were the first to reach normal levels. The present study shows that haplo HCT using TCR alpha/beta depleted grafts is possible and can be used for high risk HCT patients.

Keywords: TCR alpha/beta, T-lymphocytes, haplo haematopoietic cell transplantation

INTRODUCTION

The use of haploidentical donors for HCT in children is increasingly used. The reason for this being at least two factors: 1) the almost 100% and rapid availability of a haplo donor in children and 2) the possibility for very strong allo reactivity in the case donor leucocyte infusion is needed. An additional beneficial factor is the lack of cost in relation with a donor search if a matchingfamily donor is not available. Haplo HCT may be performed using two principally very different methods: 1) Using whole graft infusion followed by post-transplant cyclophosphamide eliminating allo reacting cells and 2) Using manipulated graft infusion where only cells believed to cause GvHD are specifically removed. The last

option (2) has the theoretical advantage of allowing residual lymphocyte subsets providing graft versus leukemia activity as well as anti-viral activity in the graft to be preserved.

In this study we present the results of haplo HCT whereTCR alpha/beta positive T-lymphocytes (and B lymphocytes) with the capacity of causing GvHD are removed from the graft whereas gamma/delta T lymphocytes, NK cells and other unspecified lymphocyte subsets are retained.

MATERIAL AND METHODS

The graft of mobilized peripheral blood stem cells was normally harvested from one of the parents but occasionally the haplo graft may be provided from a sibling. Normally the haplo donor with most B KIR motifs was used.¹ If no difference between the parents were found, the mother was most often used.

Grafts were processed using either the CliniMACS Plus or the CliniMACS Prodigyand T lymphocytes with TCR alpha-beta was removed to at least $< 50\ 000/kg$ preferably to $< 25\ 000/kg$ patient weight. With the aim of reducing the risk for EBV related PTLD following the transplant also CD19 positive B lymphocytes were removed (by in vivo Rituximab and/or by the Prodigy collum). We aimed at administering at least 10 x 10⁶ CD34/kg positive stem cells.

Pre- transplant conditioning consisted of ATG (Grafalon) 30 mg/kg, Fludarabin 150 mg/m² (or in some cases Clofarabin 200 mg/m²), Thiotepa 10 mg/kg and Melphalan 120 mg/m². No GvHD prophylaxis were given if the number of alpha/beta cells administrered was below 25 000/kg. If more than 25 000/kg alpha/beta positive T cells were given the patient received a short course of mycophenolate mofetil for 28 days.

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MAIN RESULTS

At the time of this study a total of 23 children had received TCR alpha/beta depleted haplo HCT or CD3 depleted haplo HCT (n: 3), 20 of these patients being transplanted for leukemia, NHL or MDS.Nine children suffered from ALL, 6 patients from AML, 2 patients from NHL,2 patients from JMML, and 1 patient from MDS. Twelve of the 15 patient with acute leukemia were in 2nd or later CR. The patients with non-malignant diseases suffered from primary immune deficiency (N:2) or metachromatic leucodystrophy (N:1).

Patients with malignant diseases had a marrow examination performed at day +30 and at day 100 post HCT. After this time point a marrow examination was performed every 3 months for the first year and then at 1.5 year.

The Kaplan Meier estimate for 5-years overall survival was 53 % for the whole group and 47 % for the patients with clonal diseases. All 3 patients with non-malignant diseases are long time survivors.

Acute GvHDof more than gade I was observed in only one patient (grade III) who also had viral infection with adenovirus in the gastrointestinal tract and a positive CMV pcr in the blood. In one patient who received donor leukocyte infusion for relapse at day +90 severe acute GvHD was observed resembling Steven Johnsons disease. The DLI infusion and the induced strong allo reaction caused the minimal residual disease of this AML patient to disappear however, the patient later relapsed 9 months after the first 4 doses of DLI and was now irresponsible to DLI which induced neither GvHD nor remission.

Immune reconstitution in the patients was slow following HCT, with approximately half the patients reaching normal CD3 and CD4 counts between 0.5 and 1.0 year after the HCT (figure).

The concentrations of CD8 lymphocytes reached close to normal levels earlier between 0.5 and 1.0 year. NK cell counts were the first to reach normal levels with normal levels often reached after few weeks (figure).

Viral (CMV, EBV, ADV, BKV and occasionally HHV6 and 7) reactivation and infection was investigated in the blood with PCR methods every week for the first 3 months and every 1-3 weeks after 3 months. After this time point viral infection was examined for based on clinical suspicion. Respiratory virus was examined in nasal swaps on clinical suspicion. Likewise, rotavirus, ADV and noro virus were looked for by PCR in case of diarrhea. 3



Figure. A: Immune reconstitution in the patients. B: Concentrations of CD8 lymphocytes reached.

Viral reactivation was in most cases not a major problem possibly due to the faster reconstitution of T cells with gamma/delta phenotype and the rapid reconstitution of NK cells following HCT. Also, viral reactivation was treatable with antiviral drugs such as ganciclovir, foscarnet, cidofoviror brincidofovir. In two patients virus infection (CMV) was prolonged and clinically significant and both these patients were treated with DLI in the form of CD45RA depleted T-cells (25 000 CD3/kg patient weight; corresponding to 25 000 CD3 RO cells/kg). In the first patient treated the infusion was effective in the sense that CMV infection resolved the second patient was treated recently and the effect is presently unknown.

CONCLUSIONS

The present study shows that haplo HCT using TCR alpha/beta depleted grafts is possible and can be used for high risk HCT patients such as patients with no available matching family donors or matching unrelated donors. Furthermore, this method may be justifiable as a second transplant in patients relapsing after a first HCT because treatment of minimal residual disease with DLI from a haplo mismatching donor may provide a strong anti-leukemia allo reaction. Finally, haplo HCT is a safe and rapid rescue treatment for non-engraftment or rejection. The advantage of haplo HCT in children is the rapid and easy presence of a donor source in almost all instances. The important question regarding haplo transplantation at the moment is whether the here described relative costly procedure used for processing the harvested graft is cost beneficial or if the much easier procedure of post HCT cyclophosphamide treatment has similar beneficial effects.

REFERENCES

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