Pharmacokinetics of Alemtuzumab (CAMPATH-1H) used for in vivo and in vitro T cell Depletion in Allogeneic Transplants: Relevance for Early Adoptive Immunotherapy and Infectious Complications.

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Running Title: Pharmacokinetics of alemtuzumab (CAMPATH-1H) in stem cell transplantation

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ABSTRACT

Persistence of alemtuzumab (CAMPATH-1H) at lympholytic concentrations after reduced-intensity conditioning allogeneic stem cell transplants (RITs) could impair immune reconstitution and reduce donor T cell mediated graft versus leukemia/lymphoma (GVL) effects, derived from the graft or subsequent adoptive immunotherapy. We have studied the pharmacokinetics of alemtuzumab in two different groups: RIT (100 mg alemtuzumab in vivo over five days) and myeloablative allografts (20 mg alemtuzumab added in vitro to the stem cells prior to return). Alemtuzumab concentrations in RIT patients were in excess of that required to kill infused donor CD52+ cells at the time of transplant and remained at potentially lympholytic levels (> 0.1 ug/ml) for approx 56 days post-transplant, 26 days longer than for the myeloablative group. Total lymphocyte counts were significantly lower in the RIT group persisting beyond 6 months post-transplant (p=0.005) and median absolute CD4 counts > 200 x 10^6/l were delayed until 9 months post-transplant.

INTRODUCTION

Reduced-intensity conditioning allogeneic stem cell transplants (RITs) depend on immunosuppression to facilitate donor hematopoietic and lymphoid engraftment in the context of minimal recipient myeloablation, allowing patients previously considered unfit for a conventional allograft to benefit from the putative graft versus leukaemia/lymphoma (GVL) effect. Many varied conditioning regimens are used with excellent engraftment rates, although acute graft-versus-host disease (GVHD) occurs in up to 50% of patients in published series 1-3.

We have used a RIT conditioning regimen incorporating the monoclonal antibody alemtuzumab- a humanised IgG1 monoclonal antibody directed against the CD52 antigen expressed on lymphoid cells and cells of monocyte lineage - to achieve in vivo T cell
depletion, that is associated with very low rates of acute and chronic GVHD and low early TRM.

Current dosing of alemtuzumab is based on previous work using the homologous rat IgG2b CAMPATH-1G, which is reported to have a shorter half-life than alemtuzumab. Slower clearance of alemtuzumab could thus impair immune reconstitution, affect rates of viral reactivation and limit efficacy of the donor T cell mediated GVL effect.

In this study we have measured the pharmacokinetics of alemtuzumab given in two different regimens- RIT with high dose antibody in vivo for five days pre-transplant and conventional TBI-based T depleted allografts with a single, lower dose added to the stem cells in vitro. The serum alemtuzumab levels, half-life and levels at up to 28 days post transplant were determined. The effect of alemtuzumab on the immune system was measured by analysis of absolute lymphocyte, CD4 and CD8 counts, rate of viral reactivation, other infective complications and expansion of adoptive T cells transferred (where appropriate).

STUDY DESIGN

Ten patients were studied in the RIT group and five patients in the myeloablative transplant group (table 1).

Conditioning in the RIT group consisted of intravenous infusions of alemtuzumab 20mg/day from day –8 to –4 (total 100mg), fludarabine 25mg/m² daily from day –7 to –3 and melphalan 140mg/m² on day –2. Cyclosporin A (CsA) was also administered from day – 1 at 3mg/kg/day. On day 0, patients received granulocyte colony-stimulating factor (GCSF)-mobilised unmanipulated allogeneic peripheral blood stem cells (7) or bone marrow (3). CsA was tailed from 2-3 months post-transplant.

Patients in the myeloablative group received cyclophosphamide 60 mg/kg on days –6 and –5 followed by total body irradiation on days –4 to –1 (14.4 Gy). Fludarabine 30mg/m² was
given from day –9 to –7 to the three patients receiving MUD allografts. Alemtuzumab (20mg) was added to the stem cells at room temperature 30 minutes prior to their reinfusion. CsA was administered as above.

Antiviral prophylaxis was with aciclovir. Weekly surveillance for cytomegalovirus (CMV) infection by quantitative PCR was performed, with two consecutive positives triggering treatment. Anti-viral and *Pneumocystis carinii* prophylaxis were continued until the absolute CD4+ T cell count was greater than 200 x 10^6/l. Fungal prophylaxis consisted of intravenous itraconazole until neutrophil regeneration.

RESULTS AND DISCUSSION

Median total lymphocyte counts < 0.05 x 10^9/l (0.00 – 0.6) in the RIT group and < 0.01 x 10^9/l (0.00– 0.05) in the myeloablative allograft group occurred 24 hours after the first infusion of alemtuzumab, with values eight weeks post transplant of 0.15 x 10^9/l (0.0 – 0.21) and 1.06 x 10^9/l (0.25– 2.66) respectively (Figure 1a) (p = 0.005). Significant differences in longer term lymphoid recovery are also shown, with a five month delay in achieving sustained total lymphocyte counts > 1 x 10^9/l for the RIT group.
Early and long term lymphoid recovery. Median absolute total lymphocyte counts with 12 months follow up for the high dose \textit{in vivo} (RIT) group and 6 months follow up for the low dose \textit{in vitro} (myeloablative allograft) group.

In addition, a median absolute CD4 count $> 200 \times 10^6/l$ (160–270) was reached at six months post transplant following myeloablative allograft, but not until nine months in the RIT group, median $210 \times 10^6/l$ (130–310) (figure 1b).
Recovery of T cell subsets. Median lymphocyte counts post transplant until 9 months post transplant up for the high dose in vivo (RIT) group and 6 months post transplant for the low dose in vitro (myeloablative allograft) group.

In previous studies, alemtuzumab doses as small as 10mg result in prolonged lymphopenia and high doses may not be required to prevent graft rejection. It has, however, been suggested that persistent elevated concentrations of alemtuzumab may delay engraftment by loss of the graft-enhancing effect of donor T cells. No graft rejection was seen in this study and median times to neutrophil and platelet recovery were 13 and 14 days respectively.

Despite other differences between the conditioning regimens it is likely that the differences in immune reconstitution are due principally to the persistence of alemtuzumab as RIT conditioning using fludarabine without alemtuzumab results in more rapid immune reconstitution but higher GvHD rates.
Regarding pharmacokinetics, in the RIT group the median peak level was 13.7 µg/ml (7.5-16.6), occurring 15 min after the final dose (figure 1c). At 28 days, the median level was 1.0 µg/ml and above the limit of quantitation (0.5 µg/ml) in 7 of 10 patients. The half-life from 4 to 32 days after the last infusion was 8 days, giving an estimated time to achieve levels below 0.1 µg/ml of 60 days.

**Figure 1c.**

**Median serum alemtuzumab levels.** Assays of alemtuzumab in patient serum: Serum samples for pharmacokinetic analysis were collected 24 hours prior to the first infusion of alemtuzumab, 15 minutes before and after each infusion, 4-hourly for 24 hours after the last infusion, daily for the next 7 days and then twice weekly until at least 28 days post the last alemtuzumab infusion. Sera were stored at -70°C until analysis. Before analysis complement was inactivated by heat incubation of the samples at 56°C for 30 minutes. Validation studies have previously confirmed that this step does not alter alemtuzumab activity. Alemtuzumab activity was measured by indirect immunofluorescence as described in detail elsewhere.\(^{12,13}\)

Previous studies have determined the following parameters for this assay: limit of detection 0.3 µg/ml, analytical range 0.5 – 20 µg/ml, linearity 0.999, overall precision 13%, bias +9%. There was no interference by a range of normal and patient control sera, and no reactivity with F(ab')\(_2\) fragments of alemtuzumab. The assay has been shown to be robust with respect to variations in sample treatment and storage conditions, and variations in assay procedures. All sera samples were tested in duplicate at a final dilution of 1:2. Patient weight, total
leukocyte count, underlying disease and disease status at time of transplant did not affect alemtuzumab levels or lymphocyte depletion.

In the myeloablative allograft group the median peak level was 3.2 µg/ml (1.0-5.0), occurring at 15 minutes following the infusion of stem cells containing alemtuzumab. By day +10, levels were below the limit of quantitation in three patients, just above (0.8 µg/ml) in one and not determined in one. The terminal half-life from 2 to 8 days post-infusion was 7 days, not significantly different from the RIT group. The predicted time required to achieve antibody levels below 0.1 µg/ml was 30 days.

Alemtuzumab activates complement and binds human Fc receptors, but complement mediated lysis alone is insufficient for depletion of lymphocytes in vivo and cell mediated killing (ADCC) is believed more important. Concentrations as low as 0.1 µg/ml are sufficient to opsonise lymphocytes for cell ADCC in vitro, therefore ongoing depletion of CD52+ cells might occur for approx two months post-transplant in the RIT group.

With respect to infection rates, 4 of 6 ‘at risk’ donor: recipient pairs (RIT group), and 2 of 2 ‘at risk’ pairs (myeloablative allograft group) reactivated CMV. Biopsy proven CMV colitis and prolonged PCR positivity was seen in one RIT patient. No other documented cases of CMV disease occurred. One patient (RIT) received donor-derived CMV CTLs on day +35 and cleared CMV, which is in keeping with published results with this regimen, where adoptive immunotherapy as early as day +29 resulted in sustained expansion of infused cells. Despite high levels of CMV reactivation, no difference has been observed in survival when alemtuzumab was compared with alternative GvHD prophylaxis in RITs.

Regarding GVHD, in the RIT group, two patients developed grade I and one grade II acute GvHD (one following further donor PBSC at day +35). Three of eight evaluable patients developed chronic GvHD (2 limited, 1 extensive). One patient developed extensive chronic GvHD following DLI.
In the myeloablative allograft group, two patients developed grade I acute GvHD, and both progressed to limited chronic cutaneous GvHD. One further patient developed limited chronic GvHD.

Thus, high dose in vivo alemtuzumab effectively prevents GvHD and allows engraftment, whilst circulating serum antibody levels are in excess of that required to be effective in target cell lysis and are still detectable at the time of return of CMV-specific CTLs. Ongoing dose-reduction studies will determine the optimal dose of in vivo alemtuzumab and may show more rapid immune reconstitution and perhaps improved disease free survival.

ACKNOWLEDGEMENTS

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REFERENCES


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**Abbreviations:** LG NHL low grade Non Hodgkin’s lymphoma; HD Hodgkin Disease; MM muliple myeloma; HG NHL high grade Non Hodgkin’s lymphoma; AML acute myeloid leukemia; RAEBT refractory anemia with excess blasts in transformation; CR complete remission; PR partial remission; VGPR very good PR; MUD matched unrelated donor; Sib sibling; BM bone marrow; PBSC peripheral blood stem cells; Recip recipient; Neg CMV seronegative; Pos CMV seropositive; * CMV reactivation.
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