pleted cells and 8 patients were treated at 10^4 cells/kg/dose and 8 patients received 10^5 cells/kg/dose. Patients receiving 10^5 cells/kg/dose showed significantly improved T-cell recovery at 3, 4 and 5 months post-SCT compared with those receiving 10^4 cells/kg/dose ($P < .05$). Accelerated T-cell recovery occurred as a result of expansion of the effector memory ($CD45RA^-CCR\text{-}7^-$) population ($P < .05$), indicating derivation from the infused allodepleted cells and that protective T-cell responses are likely to be long-lived. Using tetramer and ELISPOT assays, we have observed CMV and EBV-specific responses in 4/6 evaluable patients at dose level 2 as early as 2-4 months post-transplant, whereas such responses were not observed until 6-12 months in dose level 1 patients. The incidence of significant acute (2/16) and chronic GVHD (2/13) was low. At a median follow-up of 25 months, only 1 patient has died from infection and 8 of 16 of these high-risk patients are disease-free. These data demonstrate that allodepleted donor T-cells can be safely be used to improve T-cell reconstitution after haploidentical SCT and that total cell doses of 3×10^5/kg are sufficient to confer useful anti-viral immunity. This approach may broaden the applicability of haploidentical SCT by reducing infection-associated mortality (Table 1).

Table 1. *Comparison of CD3 +ve T-Cell Recovery between Dose Levels by Month post-SCT*

Months Post-SCT	Dose Level 1 (n)	Dose Level 1 (Median)	Dose Level 2 (n)	Dose Level 2 (Median)	*P* Value
1	7	0	6	4	.215
2	7	2	6	20	.216
3*	7*	2*	6*	616*	.016*
4*	6*	112*	6*	747*	.017*
5*	6*	175*	6*	900*	.04*
6	5	488	5	1421	.129
9	3	1047	2	1450	.787

*Significant increases in T-cell numbers in dose level 2 compared to dose level 1.

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PERIPHERAL $CD4^+CD25^+$ REGULATORY T CELLS (T_{reg}) BLOCK ALLOREACTIVE HOST ANTI-DONOR T CELLS IN CANINE MIXED HEMATOPOIETIC CHIMERAS

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$CD4^+CD25^+$ T_{reg} cells may be important regulators for the maintenance of immune tolerance after allogeneic hematopoietic cell transplantation (HCT). In the canine model of nonmyeloablative HCT, stable mixed hematopoietic chimerism persists following 2 gray total body irradiation, dog leukocyte antigen (DLA)-identical HCT and short-term postgrafting immunosuppression with mycophenolate mofetil and cyclosporine. We hypothesized that $CD4^+CD25^+$ T_{reg} are an important component of the cellular mechanism maintaining stable mixed chimerism. We asked if depletion of $CD4^+CD25^+$ T_{reg} from peripheral blood mononuclear cells (PBMC) from dogs with stable mixed chimerism could generate minor histocompatibility antigen (mHAg)-specific, host anti-donor cytotoxic T lymphocytes (CTL). Responder PBMC from 4 mixed chimeric dogs were cultured in MLC with irradiated, $CD34^+$ derived dendritic cells (DC) from the respective DLA-identical HCT donor. On day 4 of MLC, $CD4^+CD25^+$ T cells were depleted by either immunomagnetic selection (Miltenyi) using the CD25 monoclonal antibody (ACT-1) or the addition of 10^{-11} M denileukin diftitox (DAB_{389}IL-2, Ontak). DAB_{389}IL-2 is a fusion protein of the ADP-ribosyltransferase domain of diphtheria toxin and IL-2. Depletion of $CD4^+CD25^{bright+}$ was 60%-66% with immunomagnetic selection and 70%-77% with DAB_{389}IL-2. A secondary MLC was established with $CD4^+CD25^+$ depleted responder cells and DC from the respective DLA-identical HCT donor. There was a 54%-95% increase in proliferation of cells depleted of $CD4^+CD25^+$ T cells with immunomagnetic selection and a 121%-133% increase in proliferation of DAB_{389}IL-2 treated cells compared with non-depleted responder cells. We evaluated mHAg-specific CTL activity of the $CD4^+CD25^+$ depleted responder T cells by ^{51}Cr release assay. There was 74%-82% specific lysis of the respective HCT donor cells by the $CD4^+CD25^+$ depleted responder T cells, while the non-depleted control responder cells had 4%-6% specific lysis. PCR analysis with informative microsatellite markers confirmed the alloreactive CTL were of host origin. Depletion of $CD4^+CD25^+$ T_{reg} from PBMC of mixed chimeras permitted the emergence of host anti-donor mHAg-specific CTL. The data suggest (1) mHAg-specific host anti-donor T cells persist in stable mixed chimeric dogs, (2) peripheral $CD4^+CD25^+$ T_{reg} prevent alloreactive immune responses, (3) mixed hematopoietic chimerism established without T cell depletion relies on peripheral immune tolerance mechanisms.

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PR1 VACCINE AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: PR1 peptide has been established as a human myeloid leukemia-associated antigen. We studied PR1 peptide vaccine in a phase I/II clinical trial in HLA-A2+ patients with AML, MDS and CML. There were concerns that prior hematopoietic stem cell transplantation (HSCT) might inhibit an immune response to PR1 vaccine due to impaired immune reconstitution. To address this question we studied the outcome in 19 patients who received PR1 vaccine after a HSCT. **Methods:** Nineteen patients (AML/MDS 13 and CML 6) were vaccinated a median of 10 months after one of the following HSCT: allogeneic related 12, allogeneic unrelated 3, autologous 3, and syngeneic 1. At the time of vaccination, 7 patients were in CR, 5 had relapsed or refractory CML and 7 had relapsed or refractory AML. Patients were off systemic immunosuppressive therapy for at least 2 weeks, and without active acute or chronic GVHD. The vaccine was given subcutaneously every 3 weeks for a total of 3 injections at one of three dose levels: 0.25, 0.5 and 1.0 mg. Immune response to the vaccine (IRV) was defined as a doubling of PR1-specific cytotoxic T lymphocytes by PR1/HLA-A2 tetramer assay. **Results:** After a median follow-up of 23 months, toxicity was limited to grade I/II injection site reactions, with no exacerbations of acute or chronic GVHD. PR1-vaccine induced immune responses were observed in 9/18 (50%) evaluable patients. Of the 9 patients with PR1-specific immune response, 6 remained in continuous complete remission (CCR), 2 patients with measurable disease achieved a complete remission and 1 patient had hematologic improvement. Of the 9 patients without an immune response, 6 had no clinical response/disease progression, 2 remained in CCR and 1 had hematologic improvement. There was a significant association between the development of PR1-specific immune response and a clinical response ($P = .03$). Median progression-free survival (PFS) in IRV+ vs. IRV− patients was 23.4 months vs. 4.1 months, respectively ($P = .36$). On univariate and multivariate Cox proportional hazards analysis, minimal residual disease ($P = .009$) was associated with a lower risk of progression. **Conclusions:** PR1 vaccine can produce a PR1-specific immune response in patients after HSCT. This immune response is associated with a better clinical response and a trend towards longer progression-free survival.

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ACTIVATION OF LMP1- AND LMP2-SPECIFIC T-CELLS FOR THE IMMUNOTHERAPY OF EBV POSITIVE MALIGNANCIES WITH AN ADENOVIRAL VECTOR ENCODING FULL LENGTH LMP1 AND LMP2

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Background: LMP1 and LMP2 are potential targets for the immunotherapy of EBV-positive malignancies such as nasopharyn-