of immunotherapy treatment. B7-H1 antibody administration significantly improved long-term survival after immunotherapy (40% vs 0% for controls), indicating that the B7-H1/PD-1 pathway inhibits development of myeloma-specific immunity. We are addressing that possibility that other immune suppressive mechanisms can be targeted to further improve immunotherapy efficacy, and several candidates have been identified including $CD4^{+}Foxp3^{+}$ regulatory T cells. These results support the use of B7-H1/PD-1 blocking strategies to increase tumor immunity in myeloma patients.

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BINARY CONTROL OF TUMOR-SPECIFIC CYTOTOXIC T LYMPHOCYTES BY TRANSGENIC IL7 AND IL7 RECEPTOR EXPRESSION

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While adoptive transfer of cytotoxic T lymphocytes (CTLs) engrafted with chimeric antigen receptors (CARs) has produced objective clinical responses in vivo, the infused cells usually fail to persist long-term, limiting benefit. We have recently demonstrated that CTL persistence can be improved by engineering cells to express the IL7 receptor alpha chain (IL7Ra) which is physiologically absent on CTLs, followed by infusion of IL7 cytokine. However, this approach requires access to clinical grade cytokine, and biodistribution of cytokine at tumor sites may be insufficient. To circumvent these problems we have prepared two CTL products; one expressing a tumor-specific CAR in combination with IL7Ra (product #1), and a second engineered to co-express the same CAR and produce IL7 cytokine (product #2). In this way both products have anti-tumor activity, mediated through the CAR, while cytokine produced from CTL#2 should support the survival and persistence of CTL#1 expressing the IL7Ra. A binary system such as this should be intrinsically safer than incorporating a positive feedback loop of both cytokine and receptor in a single cell.

As proof of principal we used the SFG-CAR that targets the kappa light chain, expressed on B cell malignancies. We made two retroviral vectors, SFG-CAR/IL7Ra-GFP (#1) and SFG-CAR/IL7cyto-mOrange (#2) and transduced EBV-CTL from 3 donors with each vector. FACS analysis indicated that all the transgenes were expressed at approx. equivalent levels; CTL#1 (CAR, IL7Ra and GFP; 58%±15, 53%±18, 57.8%±12) and CTL#2 (CAR and mOrange; 54%±18 and 52%±20). The modified CTL were functional, and cells transduced with either vector were able to kill the Kappa+ B cell tumor Daudi in Cr^{51} assay (72%±13 and 69%±25, respectively) at an R:S of 40:1. We confrmed the function of IL7Ra (#1) by measuring pSTAT5 and cell proliferation after IL7 administration (67,648±2703 CPM vs 8,764±793 CPM when cells were cultured in media). In addition, we were able to measure IL7 cytokine from product #2 by ELISA. IL7 production was dependent on the intensity of antigenic stimulation as demonstrated using different ratios of CTL:EBV-LCLs (4:1, 2:1, 1:1 and 1:2 which induced 3.7, 20, 99, and 192pg/ml IL7, respectively). Finally to demonstrate that product #1 could sustain #2, we mixed the cultures at a 1:4 ratio. Over 3 weeks, CTL#1 progressively increased (14% to 87%), while the frequency of CTL#2 progressively declined over the same period (63% to 5%).

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HIGH-YIELD OF CD34+ CELLS WITH BORTEZOMIB-BASED MOBILIZATION REGIMEN IS ASSOCIATED WITH SPECIFIC GENOMIC EXPRESSION PATTERNS, DECREASE IN SDF-1 PLASMA LEVELS AND UP-REGULATION OF CXCR4 IN MULTIPLE MYELOMA (MM) PATIENTS

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Typical stem cell mobilization regimens in MM include G-CSF alone or in combination with plerixafor or high-dose cyclophosphamide (CTX). Given the known *in vitro/in vivo* synergy between bortezomib (VELCADE, Vel) and alkylating agents, we investigated the potential for concurrent cytoreduction by adding Vel to the mobilization regimen. Eligible patients (pts) had symptomatic, Durie-Salmon stage II/III MM. All pts received six 21-day cycles of Vel/dexamethasone ± liposomal doxorubicin. Cycle 7 only, mobilization (Vel-mob) comprised Vel 1.3 mg/m^2 on days 1, 4, 8, and 11, CTX 3 g/m^2 on day 8, and Filgrastim 10 ug/kg (rhG-CSF) for 10 days from day 9. At data cut-off, 25 pts have been mobilized. In comparison to pts receiving just G-CSF alone, there was a significant increase in the median CD34+ collection (22.6×10^6/kg vs 10.6 10^6/kg). In addition, the number of CD34+ cells/kg collected far exceeded the study goal of 10X10^{6}cells/kg which is typical of using CTX and/or GCSF alone in

Patient	Days Required for Collection	Days to Collection	CD34+ Stem Cells (million/kg)	Stem Cells infused (X10^6/kg)	Viability (%)	Day of neutr. engraftment	Day of plt. engraftment
1	1	18	21.2	5.78	85	14	20
2	1	18	47.4	13.22	80	11	13
3	1	19	22	9.87	60	13	22
4	1	18	17.9	9.03	90	10	15
5	4	19	40.6	5.44	97	11	21
6	1	18	19.9	9.24	94	10	16
7	3	19	294.2	17.30	91	10	17
8	2	17	13.8	6.32	80	13	24
9	5	18	9.25	4.25, 2.74	80, 94	11	18
10	2	17	21.4	9.05	93	16	21
11	1	24	50.0	no transplant	no transplant		
12	2	19	66.12	12.83	85	11	11
13	1	18	30.4	7.38	93	11	11
14	2	16	43.6	10.02	92	12	14
15	1	19	51.0	12.72	87	13	11
16	1	17	15.56	5.31	93	11	13
17	1	17	6.8	6.66	92	12	13
18	1	17	31.67	9.2	95	11	20
19	1	17	7.8	7.40	92	10	11
20	1	16	43.9	11.06	98	10	21
21	1	18	23.2	no transplant	no transplant		
22	1	17	14.0	3.47	90	16	16
23	2	17	2.946	3.96	94	12	12
24	1	16	32.8	7.92	93	11	14
25	1	20	11.5	5.63	97	10	14
median	1	18	22	8.48	92	11	15

23 of 25 patients (92%), see Table 1 for patient data summary. In order to gain insight into the mechanism of this enhanced mobilization, a pilot study investigating gene expression analysis was performed on CD34+ cells purified from frozen control apheresis samples, comparing samples from Vel –mob (n = 7)vs. G-CSF + CTX(n = 8). A significant change in the expression of genes associated with the following canonical pathways was observed: docosahexaenoic acid signaling, angiopoietin signaling and the molecular mechanisms of cancer. In relationship to SDF-1 signaling, there was a 1.44 fold increase ($p \leq 0.05$) in CXCR4 mRNA isolated from CD34+ cells in pts treated with vel-mob when compared to CD34+ cells isolated from GCSF + CTX treated pts. SDF-1 peripheral blood plasma levels were then measured in vel-mob treated pts (n = 7) and compared to the levels in GCSF alone (n = 8) treated pts. The mean SDF-1 level in vel-mob treated pts was significantly decreased (p = 0.0012) when compared to GCSF alone treated pts (1319 pg/ml vs 2225 pg/ml). Vel-mob is a novel mobilization regimen that produces very high and predictable CD34+ yields. Vel-mob modulates SDF-1 protein levels in plasma, CXCR4 gene expression in CD34+ cells. We have also identified important canonical pathways that may be associated with improved mobilization.

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PREVENTING REJECTION IN PRIMARY IMMUNODEFICIENCY PATIENTS WITH DONOR LYMPHOCYTE INFUSIONS

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Rationale: The post transplant infusion of donor lymphocytes (DLI) has been used to increase alloreactivity against residual host hematopoiesis in malignant diseases after stem cell transplantation (SCT). DLI can treat impending rejection, prevent relapse in the face of decreasing donor chimerism or even treat overt relapse in some forms of leukemia. However the risk of inducing GVHD after DLI has limited its use after allogeneic SCT for non malignant disorders such as primary immunodeficiency diseases (PID).

Methods: PID patients with decreasing donor chimerism despite discontinuation of immunosuppression were treated with DLI. The initial dose of $CD3^+$ cells per kg body weight was up to $5*10^5$ for matched sibling donors (MSD), $1*10^5$ for matched unrelated donors (MUD) and $2.5*10^4$ for haploidentical family donors (MMFD) in the absence of GVHD. Patients were to receive additional DLI at 4-6 week intervals in increasing doses until either GVHD or a stabilization/reversal of mixed chimerism was observed.

Results: Ten patients (1 XLP, 2 SCID, 7 WAS) experienced decreasing donor chimerism at a median of 94 days (range 62-180 days) after myeloablative (n = 8, non-SCID) or reduced intensity (n = 2, SCID) conditioning SCT from MSD (n = 1), MUD (n = 7) or MMFD (n = 2). None of the patients had had pretransplant maternal engraftment. They received DLI from their original donors at a median of 120 days (78-366) after SCT. At a median follow up of 779 days (41-3807) after first DLI and a median of 5 DLI (2-14) we observed a stabilization of mixed chimerism in 5 patients and improvement in 5. None experienced complete rejection or returned to full donor chimerism. Of note, none of the patients experienced GVHD of any grade.

Conclusion: DLI after SCT for PID is feasible and may be able to halt impending rejection. This observation may be especially important in the light of an increasing use of reduced intensity conditioning regimes with presumably a higher incidence of graft failure and the fact that many PID can be considered cured even with stable, low level donor chimerism. We propose a graded dosing regimen of $CD3^+$ cells depending on donor/recipient HLA disparity to minimize the risk for DLI induced GVHD, which appeared to be safe and effective in our patients.

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OPTIMIZING THE BUSULFAN DOSING REGIMEN TO GET A MORE PREDICTABLE EXPOSURE: A DATA DRIVEN ANALYSIS

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Background: Therapeutic drug monitoring (TDM) guided dosing of busulfan is associated with higher event free survival rates due to less graft-failure/relapses and lower toxicity in hematological stem cell transplantation (HSCT). The observed differences in plasma exposures of intravenously administered busulfan to children may be related with age, or diseases like immune deficiencies. The question is whether a more predictable exposure can be obtained by individualization of busulfan dosing in pediatric HSCT. This study aims to predict the growth related developmental changes in the pharmacokinetics (PK) of busulfan, using an extensive covariate analysis, in order to design an individualized dosing regimen.
Methods: In this retrospective multicentre study, 245 patients were included, ranging between 1 month and 26 years of age. All patients intravenously received busulfan-based conditioning combined with therapeutic drug monitoring (TDM). Data were gathered from four pediatric centers. Demographics, diagnosis, concomitant medication, hematological and clinical chemistry parameters were used as covariates, using linear and allometric correlations. NONMEM was used for data and covariates analysis.
Results: Bodyweight significantly influenced the PK of busulfan in a nonlinear relation. The other evaluated covariates did not influence the pharmacokinetic parameters. An allometric scaling method was used to estimate the clearance of each individual, resulting in the function for clearance: $2.94\text{L/h} * (BW_i/15\text{kg})^{exp\ a}$. Based on objective function, diagnostics and root mean square error, the model with "exp a" as one single exponent was inferior compared to a bodyweight-dependent exponent, with a function; exp a = $1.96*BW^{-0.234}$. This model resulted in a scaling exponent of 1.51 in neonates (BW 3-5 kg) decreasing to 0.65 in young adults (BW 80-109 kg). The volume of distribution was allometrically scaled with one single exponent of 0.87. Based upon this final model a new dosing regimen was designed to obtain an AUC of 95 mg*h/L for myeloablative or 45 mg*h/L for non-myeloablative regimens.
Conclusions: This population PK model for busulfan in patients ranging between 1 month and 26 years of age, describes the PK parameters of busulfan using a continuous non-linear function depending on bodyweight. The model-derived individualized dosing regimen, which is expected to lead to a more predictable exposure of busulfan in each child, will be prospectively evaluated.

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LONG TERM ORGAN STABILITY AND OUTCOME IN CHILDREN AND ADOLESCENTS WITH SICKLE CELL DISEASE (SCD) FOLLOWING REDUCED TOXICITY CONDITIONING (RTC) ALLOGENEIC STEM CELL TRANSPLANTATION (SCT)

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Myeloablative (MA) SCT is curative of SCD, and children with stable donor engraftment no longer experience SCD symptoms (Walters et al, BBMT 2009), but quality of life may be diminished by MA SCT related organ toxicity. RTC regimens may potentially enhance preservation of organ function in patients with SCD. Between 8/27/2004 and 3/2/10, 18 (16M: 2F) patients with symptomatic SCD underwent RTC SCT. Conditioning was Busulfan (4mg/kg × 4d ≤ 4 yrs and 12.8mg/kg × 4d > 4 yrs), Fludarabine (30mg/m^2 × 6d), and Alemtuzumab (2mg/m^2 × 1d, 6mg/m^2 × 2d, and 20mg/m^2 × 2d). Donor sources: 8-6/6 related bone marrow, 2-6/6 related cord blood (CB), 1-6/6 unrelated CB, 4-5/6 unrelated