Hodgkin lymphoma (NHL). However, long-term outcomes of auto HCT for HL and NHL have not been well described. We conducted a retrospective cohort study to evaluate the probability and risk factors for long-term survival and late relapse in this population. Recipients of auto HCT for HL (N = 407) and NHL (N = 960) between 1990 and 1998 reported to the CIBMTR and in continuous complete remission (CR) for at least 2 years post-HCT were included in this study . The median followup was 104 (range, 25–203) months for HL and 107 (range, 25–198) months for NHL. NHL subtypes were follicular (30%), diffuse large cell (45%), mantle cell (6%) and other (7%). The median age of HL patients was 31 years compared to 47 years for NHL; more HL patients were <20 years (15% vs 5%) while NHL patients were >50 years (6% vs 40%). Patients were predominantly White (87%), male (60%), with chemosensitive disease (82%),and KPS score ≥90 (67%). A greater proportion of HL patients had history of B symptoms (52% vs 34%) and were ≥12 months from diagnosis (90% vs 59%) while use of total body irradiation (TBI) was more prevalent for NHL (94% vs 64%). The probability of overall survival (OS) and cumulative incidence of relapse and non-relapse mortality (NRM) at 10-years after HCT was 76% (95% CI, 73–79%), 17% (13–22) and 13% (9–17%) for HL. The 10-year rates of OS, relapse and NRM for NHL were 77% (72–82%), 26% (23–30%) and 11% (9–13%). On multivariate analysis, OS (Table) was significantly associated with histology, age at HCT and time from diagnosis to HCT. Factors predictive for relapse included histology (compared to HL, relative risk (RR) 1.5 (1.0–2.2) for follicular, 1.4 (0.9–1.9) for diffuse large cell, 1.4 (0.8–2.8) for lymphoblastic/Burkitts, 4.6 (2.8–7.6) for mantle cell and 1.5 (0.9–2.4) for other NHL), male gender (RR 1.3 (1.0–1.6)), use of TBI (RR 1.6 (1.2–2.1)), use of growth factors (RR 1.5 (1.1–2.0)) and time since diagnosis of ≥12 months (RR 1.9 (1.5–2.6)) while KPS score of ≥90 at HCT decreased relapse risk (RR 0.8 (0.6–1.0)). Recipients of auto HCT for HL and NHL who remain in remission for at least 2 years have a very favorable subsequent long-term survival. However, they remain at risk for late relapse and NRM. Mantle cell histology, older age at HCT and increasing time between diagnosis and HCT are predictive of adverse long-term survival.

Multivariate analysis for overall survival in patients surviving in remission for at least 2 years after auto HCT

Variable	N	RR of death (95%CI)	P-value
Histology			
HL	399	1.00	<0.01
Follicular NHL	282	0.91 (0.63-1.32)	0.61
Diffuse large cell NHL	422	0.92 (0.64-1.31)	0.63
Lymphoblastic/Burkitts NHL	69	1.28 (0.66-2.48)	0.47
Mantle cell NHL	57	2.87 (1.70-4.87)	<0.01
Other NHL	117	0.95 (0.58-1.57)	0.85
Age at HCT			
<20 years	101	1.00	<0.01
20-49 years	843	0.94 (0.54-1.66)	0.84
≥50 years	402	2.23 (1.24-4.00)	<0.01
Time from diagnosis to HCT			
<12 months	426	1.00	
≥12 months	920	2.23 (1.62-3.09)	<0.01

Other variables considered: gender, race, KPS score, CR status, stage, B symptoms, chemosensitivity, pre-HCT radiation, pre-HCT chemotherapy regimens, HCT year, TBI use, graft source, graft purging, growth factor use and post-HCT radiation.

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GENETIC MODIFICATION OF ANTIGEN-SPECIFIC CYTOTOXIC T LYMPHOCYTES (CTLS) RESTORES THEIR ABILITY TO RESPOND TO INTERLEUKIN-7 (IL-7)

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Adoptive transfer of tumor-specific cytotoxic T lymphocytes (CTLs) can induces tumor regression in patients with several types of lymphomas. Clinical responses appear to require the *in vivo* expansion and persistence of the infused CTLs so that IL-2 has frequently been used to support CTL therapies. Although effective, IL-2 is associated with significant toxicity and the expansion of regulatory T cells (Treg). Thus alternative cytokines able to sustain the expansion of CTLs but avoiding the toxic and negative effects of IL-2 are highly demanded. IL-7 plays a crucial role in T-cell homeostasis, appears well tolerated, and does not influence Treg numbers or function. Unfortunately, IL-7 may fail to expand tumor-specific CTLs in vivo because these cells lack IL-7Ra expression. To determine whether forced expression of transgenic IL-7Ra improves CTL survival and expansion we used Epstein-Barr-Virus specific-CTLs (EBV-CTLs) which we transduced with a retroviral vector encoding IL-7Ra. After transduction, IL-7Ra was detectable on 58–76% of EBV-CTLs. The transgenic IL-7Ra molecule was functional since addition of IL-7 induced STAT5 phosphorylation only in EBV-CTLs/IL-7Ra+. These EBV-CTLs/IL-7Ra+ and control CTLs expanded equally in response to IL-2, but only EBV-CTL/IL-7Ra+ significantly proliferated in the presence of IL-7 (5 ng/mL) [from 1×10^6 to 1.03×10^8 cells/ml (range, $0.38–2.9 \times 10^8$)] over five weeks of culture. As anticipated, the EBV-CTL/IL-7Ra+ subset of cells were selected on exposure to IL-7 and so formed an increasing proportion of the total T cell population. Importantly, IL-7 expanded EBV-CTLs/IL7Ra+ retained their ability to respond to other common-g-chain cytokines such as IL-2 and IL-15, and did not modify their antigen specificity measured by tetramer staining and by IFNg release. These CTLs also remained polyclonal, retaining both their effector-memory profile and MHC-restricted killing of autologous LCLs. Antigen or cytokine withdrawal abrogated CTL expansion. These in vitro characteristics were replicated in a xenograft mouse model, in which EBV-CTLs/IL7Ra+ expanded in response to IL-2 or IL-7 and maintained their antitumor effects. In conclusion, we have developed a novel approach to safely expand adoptively transferred CTLs using IL-7. This approach can be beneficial for CTL therapies in patients with lymphomas and other malignancies.

PEDIATRIC DISORDERS

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A PILOT STUDY OF REDUCED INTENSITY CONDITIONING (RI) WITH BUSULFAN (BU), FLUDARABINE (FLU), AND ALEMTUZUMAB FOLLOWED BY ALLOGENEIC STEM CELL TRANSPLANTATION (ALLOSCT) TO INDUCE SUSTAINED MIXED DONOR CHIMERISM IN PATIENTS WITH SYMPTOMATIC SICKLE CELL DISEASE (SCD)

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SCD can be cured by HLA matched family donor AlloSCT (Walters et al, NEJM, 1996). Patients undergoing myeloablative AlloSCT are at risk for many complications post SCT including death, graft versus host disease (GVHD), and life threatening infections, thus making RIC regimens an attractive alternative. Having an unaffected, HLA-matched identical sibling is another major obstacle for most SCD patients to proceed to AlloSCT(Bhatia et al, BMT, 2008). Umbilical cord blood (UCB) has been proven to be an excellent alternate donor source that can safely reconstitute hematopoiesis after AlloSCT(Cairo et al, BBMT, 2008). In this study, we report the results of RIC and AlloSCT from matched family and UCB donors in 14 patients (12M:2F) with symptomatic SCD (HbSS = 8, HbSC = 3, HbSßThal = 3). Conditioning was Bu (4mg/kg × 4d ≤ 4 yrs and 12.8mg/ kg × 4d > 4 yrs), Flu (30mg/m^2 × 6d), and Alemtuzumab (2mg/m^2 × 1d, 6mg/m^2 × 2d, and 20mg/m^2 × 2d). Median age was 6.15 yrs (1.5–16). Donor sources: 6-6/6 HLA-matched sibling bone marrow (BM), 2-6/6 sibling UCB, 3-4/6 and 3-5/6 unrelated UCB. Bone marrow recipients received median cell doses of $5.4 \pm 3.5 \times 10^8$ TNC/kg and $4.98 \pm 1.8 \times 10^6$ CD34/kg while cord blood recipients received median cell doses of $4.1 \pm 2.0 \times 10^7$ TNC/kg and $2.2 \pm 1.8 \times 10^5$ CD34/kg. All received tacrolimus and mycophenolate mofetil as GVHD prophylaxis and phenytoin or keppra as seizure prophylaxis for 180 days post SCT. Median neutrophil and platelet recovery was day 27(0–47) and day 43(0–86), respectively. One patient had primary graft failure at day +60 post

SCT. Median whole blood donor chimerism on days 30, 60, 100, and 180 was 95%(9–100), 90%(61–99), 90%(60–99), and 91%(88–98), respectively. Median CD71 chimerism on days 30, 60, 100, and 180 was 98%(17–99), 88%(1–99), 90%(71–99), and 92%(80–96), respectively. Hb electrophoresis demonstrated median %HbS levels of 1.1±0.1, 0±0.1, and 1.0±0.1 on days 30, 60, and 100 respectively. Of evaluable patients, Grades II-IV acute GVHD was seen in 4/13(30.7%) patients and chronic extensive GVHD was seen in 1/9 (11.1%) patients. Three patients developed CMV pre-engraftment (CMV pneumonitis (n = 2), CMV viremia (n = 1)) and one developed CMV viremia post engraftment. OS is 100% (longest follow up 1491 days). In summary, RIC and AlloSCT in patients with SCD using both related and unrelated donors has been shown to be well tolerated and effective in inducing a high degree of sustained mixed donor chimerism.

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HIGH THROUGHPUT NON-VIRAL GENE TRANSFER OF T CELLS BY MICRO-ELECTROPORATORS TO GENERATE CD19-SPECIFIC CELLS FOR IMMEDIATE INFUSION

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Genetic modification of T cells to express a chimeric antigen receptor (CAR) is a promising therapy for the treatment of malignancies and we have implemented a clinical trial infusing T cells which express CAR after electro-transfer of DNA plasmid vector. However, generating CAR^+ T cells for diseases that are rapidly progressing, such as relapsed pediatric B-lineage acute lymphoblastic leukemia (B-ALL) after allogeneic hematopoietic stem-cell transplantation (HSCT) is challenging. To meet this challenge we have combined bioengineering with immunotherapy to develop a new platform technology for gene transfer of CAR into T cells. This approach sidesteps the problem associated with conventional viral and non-viral methods for gene transfer that rely on integrating expression vectors which are typically laborious (sometimes taking weeks of cell-culture to generate clinically-significant cell doses) and expensive. What is needed, and is provided here, is a cost-efficient approach to the genetic transfer of large numbers of minimally manipulated T cells that can be rapidly modified *ex vivo* and immediately infused for *in vivo* therapy. We have fabricated and tested a series of **high t**hroughput **micro-e**lectroporation **d**evices (HitMeds). The initial multi-electrode HitMed was designed to investigate the electroporation parameters in a channel with single-cell flow dynamics. Subsequently, a "wafer-type" HitMed was developed to handle a large number of T cells in short time. The area of the electrodes of wafer-HitMed is 3,600 mm^2 (commercial cuvette: 144 mm^2) with a gap, initially set at 400 μm, but which can be easily changed to enable large numbers of T cells to be synchronously electroporated. We have adapted the wafer-HitMed to the electro-transfer of mRNA to avoid genotoxicity associated with integration. The data in **Table 1** demonstrates that a CD19-specific CAR coded from introduced mRNA is efficiently electro-transferred into T cells. The expression of CAR transgene was 80% 24 hours after electroporation as determined by flow cytometry analysis. We recognize that the CAR expression is transient, but this can be compensated through repeated infusions of the CAR^+ T cells. We foresee the implementation of micro-electroporators to generate CAR^+ T cells for immediate infusion to achieve B-ALL *disease control*. As needed, these initial infusions may be followed by adoptive immunotherapy of T cells with stable CAR expression for *disease eradiation* and prevention.

Expression of CAR and GFP (%)

	Cuvette	Cuvette	HitMed	HitMed
	CAR	GFP	CAR	GFP
DNA	31.05	44.04	23.95	36.74
mRNA	78.39	82.48	80.02	90.67

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ADENOVIRUS DNA POSITIVITY IN NASOPHARYNGEAL FLUID PRECEEDING HAEMATOPOIETIC STEM CELL TRANSPLANTATION: A VERY STRONG PREDICTOR FOR ADENOVIRUSREACTIVATION IN PEDIATRIC PATIENTS

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Objective: After paediatric haematopoietic stem cell transplantation (HSCT) adenovirus (HAdV) infection is a severe complication with high morbidity and mortality rate. By intensive surveillance patients at high risk for developing HAdV disease can early be identified in order to start preemptive therapy with antiviral medication in an early stage.

Methods: In a prospective study, we determined the predictive value of HAdV DNA positivity in nasopharyngeal fluid preceding HSCT to identify patients at risk for a plasma HAdV reactivation after HSCT. We weekly monitored Adenovirus DNA loads in plasma after HSCT. HAdV reactivation was defined as plasma viral DNA load >1000cp/mL. Secondly, the association between plasma HAdV reactivation and alloreactive disease (Graft-versus-Host disease and/or idiopathic pulmonary syndrome) was analyzed, using Cox proportional hazard models.

Results: A total of 62 patients were included: 37 (60%) recipients received bone marrow (17/37, 46%, matched family donor) or unrelated peripheral blood stem cells while 25 (40%) recipients received unrelated cord blood stem cells. 42/62 (68%) recipients received HLA-matched stem cells (for BM/PBSC high resolution typing, for CB intermediate: HLA-A and B on low and HLA-DR on high resolution). All patients received myeloablative conditioning and standardized Graft-versus-host disease prophylaxis. The median follow-up time was 47 (5–150) weeks. Prior to HSCT, HAdV DNA could be detected in nasopharyngeal fluid of 8/62 patients (13%). In all these patients (100%), plasma HAdV reactivation occurred during isolated hospitalization at a median time of 12.5 days (range 5–72 days) after HSCT. Additionally, 11 (18%) patients developed plasma HAdV reactivation at a median time of 40 (14–160) days after HSCT. In multivariate analysis HAdV DNA positivity in nasopharyngeal fluid was the only significant predictor for plasma HAdV reactivation after HSCT (HR 9.7; 95%CI 3.4–27.4; p = 0.000). Plasma HAdV reactivation was a predictor for allo-reactive disease (HR 2.6; 95% CI 1.2–5.4; p = 0.013).

Conclusions: HAdV positivity in nasopharyngeal fluid pre-HSCT is a very strong predictor for the development of plasma HAdV reactivation after HSCT. Prevention or early pre-emptive treatment with antiviral therapy might contribute to prevent HAdV disease and / or HSCT associated problems after HSCT.

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UNRELATED CORD BLOOD TRANSPLANTATION (UCBT) OF 30 CONSECUTIVE PATIENTS WITH TRANSFUSION-DEPENDENT -THALASSEMIA FROM A SINGLE CENTER

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UCBT offers a cure for thalassemia and has advantages as a stem cell source because of the less stringent requirements for an HLA match between donors and recipients. Previous reports of UCBT for thalassemia have yielded transplant related mortality (TRM) that appeared high. It is known that pre-freeze total nucleated cell (TNC) dose is critical for UCBT. With strategies that maximize TNC dose – using non-red cell reduced but plasma depleted (PD) CB, no post-thaw wash (NW), and double cord transplantation (DCT) when necessary – we have achieved promising results with UCBT in young patients with transfusion-dependent β-thalassemia. Between 10/2003 and 5/2008, 39 CB products were infused after Bu/Cy/ATG myeloablation into 30 pediatric thalassemia major patients (9 DCT) using NW PD CB exclusively. Patient status: 21 Pesaro class-1, 8 class-2, and 2 unknown. Median age was 5 years (range 1–14 years) with a median weight of 18 kg (range 11–37 kg). The data was audited by the transplant center (TC) and on-site by