vs APB DC including surface molecules HLA-DOβ1(4F), HLA-
DRα5(2F), CD80(9F), CD86(5F), CXCL10 (10F), CX3CL1 (5F),
CXCR4 (6F), and immuno-regulatory gene ISG20 (11F), T\textsc{nf}sF10 (4.5F). The proteomic results indicated several zinc
finger proteins (292, 221) (2-5F) and interferon-γ (7.7F) were
expressed higher in APB vs CB DC. We then compared CB vs APB DC
antigen presentation activity to APB CD8 T cells by ELISPOT
assay for interferon-r (IFNr) production (BD Pharmingen).
Briefly, CD8 T cells (MHC HLA A2) were incubated with CB or
APB DC that were loaded without or with influenza peptide onto
ELISPOT plate. Influenza peptide loaded CB DC significantly
reduced the ability to induce CD8 T cells to produce IFN\textsc{r} com-
pared with APB mDC (3.5F). We postulate that decreased expres-
sion of specific surface molecules and other genes and proteins
resulting in lower surface protein expression in CB DC may in part
be responsible for the lack of initiation of cell surface signaling
events to trigger CB-DC to induce activation of CD8 T cells.
Furthermore, these significantly decreased expressed genes and
proteins in LPS-CB vs APB DC may also partially be responsible for
differential innate and adaptive immune function and proper-
ties of CB vs APB.

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EX VIVO ACTIVATED AND TRANSPLANTED HUMAN T CELLS GENERATE
LETHAL GVHD IN A MOUSE MODEL, AND ARE EFFICIENTLY ELIMI-
NATED IN VIVO WITH SUICIDE GENE THERAPY

Neretz, B.1, Retting, M.P.1, Ritchey, J.K.1, Wang, H.L.1, Baner, G.2,
Walker, J.3, Boneyhadi, M.L., Bevrson, R.J.2, Herbrich, P.E.1,
Hess, D.1, Nolda, J.A.2, DiPerio, J.F.1.1 Washington University
School of Medicine, St. Louis, MO. 2. Xyte Therapies, Inc., Seattle,
WA.

No in vivo models exist to consistently examine the effect of
ex vivo manipulation of human T cells (huT) on T cell function.
NOD SCIDβ2m\textsuperscript{-/-} mice (B2) were conditioned with 250 cGy
TBI on day−1 (n = 34), or 500 cGy on day 0 (n = 21). 10\textsuperscript{7} naïve
or CD3/28 bead activated (Xyte\textsuperscript{TM} Dynabeads\textsuperscript{®}) huT (Act)
were injected retro-orbitally (ro). Engraftment of huT was evalu-
ated weekly by FACS and euthanasia was performed if mice
lost >20% body weight. B2 mice receiving 250 or 500 cGy +
naïve, or 300 cGy + Act developed 60%, 75%, and 100% lethal
GVHD respectively. The huT engraftment was 20 ± 15%, 33 ±
21%, and 59 ± 19% respectively. Infiltration of murine tissues
was greatest in mice receiving 500 cGy + Act huT (spleen, liver,
lung, kidney: ±60%). Of interest, serum human IFN\textsc{γ} levels increased
over time in all mice who developed lethal GVHD (P < 0.05).
Importantly, histological examination of the target
tissues revealed changes consistent with human GVHD.
We developed a chimeric suicide gene consisting of human CD34
and full length HVS-tk and showed that the fusion protein
4CD34-TK conferred ganciclovir (GCV) sensitivity and pro-
vided a surface marker for selection of transplanted cells (Td). To
generate Td cells we used CD32/38 heads + IL-2, and after 2
days were incubated with 293 GPG-derived VSV-G
psudotyped CD34-tk oncoretrovirial supernatants for 6h at
37°C. Td cells were then expanded for 2 more days and isolated
by MACS (Miltenyi Biotech) and purified to >99% by CD34
(VarioMACS). To evaluate the Td GVHD potential, B2 mice
received 300 cGy and were injected with 10\textsuperscript{7} Act (n = 7) or Td
(n = 4) huT. Both groups developed similar engraftment with
49 ± 23% and 49 ± 10% huT respectively (P = NS); had
important infiltration in target organs (≥55% in the liver), and
developed lethal GVHD. Subsequently, we evaluated the ability of
GCV (days 1-7) to prevent GVHD after infusion of Td, B2
mice (n = 8) conditioned with 300 cGy were injected ro with
10\textsuperscript{7} Td and 50% received GCV. We efficiently eliminated Td
huT with GCV (day 3 ± 0.06; day 24 0.1 ± 0.1 Td cells). In
summary, we developed a xenogeneic model of lethal GVHD
where naïve, ex vivo activated, or Td selected CD34-tk huT
injected into intrathymetically irradiated B2 mice engraft, expand in
vivo, infiltrate target tissues sharing the major characteristics of
human GVHD, and causing the death of mice. Interestingly, Td
T cells could be efficiently eliminated in vivo by treatment with GCV,
meaning we could potentially control human GVHD with the suicide system.

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PERSISTENT MIXED CHIMERISM IN PLASMA CELLS FOLLOWING AL-
LOGENEIC STEM-CELL TRANSPLANTATION (SCT) IN PATIENTS WITH
ACUTE LEUKEMIA IS A SURVIVAL FACTOR FOR POST-SCT GRAFT-
VERSUS-HOST DISEASE (GVHD)

Shimoni, A., Trakhtenbrot, L., Idoes, G., Hardan, I., Shen-Tze, N.,
Rechavi, G., Amariuglio, N., Nagler, A. Chaim Sheba Medical Center,
Tel-Hashomer, Israel.

Chimerism within cellular subsets following allogeneic SCT has
been studied extensively, yet there is only limited data on chimer-
ism kinetics within plasma cells (PC) and its prognostic signifi-
cance. In this study we prospectively analyzed the relative ratios
of recipient and donor-derived PC in pts with acute leukemia at serial
time-points following SCT from a sex-matched donor in relation
to SCT outcomes. Bone-marrow (BM) preparations were eval-
uated by a Duet combined cyto genetic/morphologic analysis
system (Bioview Ltd, Israel). The system scans BM preparations
and saves all cell coordinates. PC are detected and marked by their
morphology. The stain is then removed and FISH for
mRNA expression (n = 22) or reduced-intensity condi-
tioning (n = 28). Thirty-six pts (72%) had recipient PC detected early
after SCT, constituting 0.01-1.6% of BM cells. This was often
associated with low level recipient chimerism (<1%) in lympho-
cyes. Early detection of recipient PC was not related to donor
type, conditioning regimen or acute GVHD, and had no prognos-
tic significance. The median time to disappearance of recipient PC
was 12 months. In 16 of the 56 pts with recipient PC they persisted
beyond 6 months (and up to >18 months), in 10 they disappeared
by this time period, 6 died before 6 months with recipient PC and
had insufficient follow-up. Persistence of recipient PC beyond 6
months was not associated with mixed-chimerism in other subsets
at this stage. BM tests beyond 6 months are available in 30 pts of
all 50 pts. The outcome of 16 pts with recipient PC persisting
beyond 6 months was significantly inferior to 14 pts with no
recipient PC at this stage; 8 patients in the first group relapsed
compared to only 1 pt in the second. The 2-year DFS was 35%
(7-62), and 91% (74-100), respectively (P = .02). Donor derived
PC were detected during the course in 27 pts. The estimated
time to first detection of donor PC was 6 months (1-15).
Engraftment kinetics of donor PC had no relation to SCT out-
comes. In conclusion, recipient PC may persist for long durations
after allogeneic SCT and are relatively resistant to conditioning
and to allogeneic responses. Persistence of recipient PC beyond 6
months is a surrogate marker for ineffective GVL, even in pts with
GVHD, and is therefore associated with an increased risk for
leukemia relapse.

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INTENTIONAL INDUCTION OF IMMUNE-HEMATOPOIETIC MIXED CHI-
MERISM AS A PLATFORM FOR EARLY CELLULAR THERAPY IN PEDIAT-
RIC LEUKEMIA PATIENTS AFTER ALLOGENEIC TRANSPLANTATION:
ENHANCING GVL EFFECT WHILE AVOIDING GVHD

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Garcia-Castro, J., Madero, L. Hematopoietic Stem Cell Transplantation
Unit, Hospital “Niño Jesus,” Madrid, Spain.

To maximize graft-versus-leukemia (GvL) effect while mini-
imizing transplant-related mortality and morbidity, we designed
a study of allogeneic PBSC CD34+ selected transplantation
followed by DLI. PBSC CD34+ selection was performed by Clin-iMACS device. Between June 2004 and July 2005, sixteen
consecutive patients (4 females and 12 males) aged between 1-12
years (median 6 years) diagnosed with AML 6, ALL 10 were condition-
ed with fludarabine 30 mg/m\textsuperscript{2}/day × 4 days and mel-
phalan 140 mg/m\textsuperscript{2}/day × 1 day. Status at transplantation was 1st
CR 10, 2nd CR 5 and 3rd CR 1. GvHD prophylaxis consisted of
**Table 1. Results**

<table>
<thead>
<tr>
<th>Dog #</th>
<th>Pentostatin?</th>
<th>PBMC before DLI</th>
<th>% Donor Chimerism in PBMC after DLI</th>
<th>Duration of Mixed Chimerism in wks (Final % of Donor Chimerism in PBMC)</th>
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<td>9</td>
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*4 days after DLI.*

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**Early Donor Lymphocyte Infusion (DLI) Does Not Consistently Prevent Graft Rejection in Dogs Given DLA-IDENTICAL Marrows After 1 Gy Total Body Irradiation (TBI)**

**Background:** Secondary graft rejections may occur after non-myeloablative conditioning for hematopoietic cell transplantation (HCT), particularly in patients with chronic myeloid leukemia given unrelated grafts. We have shown that low (≤50%) donor T-cell and NK cell chimerism levels on day 14 after HCT predicted graft rejection. Here we investigated in a pre-clinical canine model of HCT, whether pre-emptive DLI could prevent secondary graft rejection that was the rule in dogs given DLA-identical marrow after suboptimal conditioning with 1 Gy TBI and postgrafting immunosuppression immunosuppression with cyclosporine (CSP) combined with either mycophenolate mofetil (MMF) or rapamycin. In 11 of 11 dogs experienced graft rejection by wk 12. Methods: 9 dogs were given DLA-identical marrow grafts after 1 Gy TBI followed by postgrafting immunosuppression with MMF (days 0-27) and CSP (started on day −1 and discontinued 1 day before DLI). A single DLI (containing 1.0-2.3 × 10⁷ T-cells/kg) was given 28-36 days after HCT in each dog. In addition, 5 of 9 dogs received pentostatin, 4 mg/m² given 2 days before DLI (n = 3); or 4 mg/m²/day on days 4-2 before DLI (n = 2) (Table 1). Presence of donor cells in peripheral blood mononuclear cells (PBMC) was assessed by VNTR-PCR. The endpoint of the study was persistence of mixed donor/host chimerism at 30 wks. Results: Marrow cell numbers were similar in DLI and historical dogs (medians 3.6 × 10⁹ and 4.0 × 10⁹ nucleated cells/kg, respectively, P = 0.2). Two of the first 4 dogs given DLI only were mixed donor-host chimeras at 30 wks, while 2 rejected their grafts on wks 10 and 15, respectively. We then investigated in 6 dogs whether host immunosuppression with pentostatin prior to DLI could overcome the pending rejection and increase the efficacy of DLI. Three of the 5 dogs (including 1 of the 2 given 3 doses of pentostatin) rejected their grafts on wks 8, 12 and 16 after HCT, respectively, while 1 was a stable mixed donor/host chimera 24 wks after HCT, and 1 was too early to be evaluated. The 30-wk probability of sustained donor engraftment was 37.5% in dogs given DLI, versus 0% in historical dogs (P < 0.1). Conclusions: DLI, with or without preceding pentostatin, was effective in one third of dogs studied to prevent graft rejection. The model might be useful to develop further strategies aimed at preventing graft rejection in patients with low chimerism (Table 1).

**Ex Vivo Expanded T Regulatory (Treg) Cells Block the Breaking of Tolerance in Mixed Chimeras**

Nonmyeloablative allogeneic hematopoietic cell transplantation (HCT) consisting of 2 Gray (Gy) total body irradiation (TBI), dog leukocyte antigen-identical marrow, and short-term postgrafting immunosuppression with cyclosporine and mycophenolate mofetil was developed in the dog model as an effective way to establish long-term, stable mixed hematopoietic chimerism. We hypothesized that Treg cells are crucial to maintaining stable mixed chimerism. All dogs in the current study were stable mixed chimeras established as described. Previously, we showed in 8 mixed chimeras that naive DLI did not change the percent donor chimerism. We asked if reconditioning mixed chimeras with a nonmyeloablative dose of 2 Gy TBI followed by DLI could increase donor chimerism. Seven mixed chimeras were re-conditioned with 2 Gy TBI followed by DLI (median dose, 1.5 × 10⁹CD3+ cells/kg). Within 4 weeks after DLI, conversion to 100% donor chimerism was seen in 5 of 7 dogs and 2 dogs had a >50% sustained increase in donor chimerism. A control group of 3 mixed chimeras reconditioned with 2 Gy TBI without DLI had no change in donor chimerism. These results show that reconditioning with 2 Gy TBI followed by DLI can break the tolerance mechanism established in mixed chimeras. Next, we asked if CD4+CD25+ Treg cells obtained from mixed chimeras prior to reconditioning could block the increase in donor chimerism following 2 Gy TBI and DLI. Peripheral blood mononuclear cells (PBMC) were activated with mitogenic anti-CD3 antibody. After 10 days, CD4+CD25+ Treg cells had expanded a median of 23-fold and inhibited MLR. To date, Treg cells were generated from 4 mixed chimeric dogs and were infused back into the respective mixed chimeras. The model might be useful to develop further strategies aimed at preventing graft rejection in patients with low chimerism. **Table 1. Results**

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