for the CD19 molecule (CD19.CAR) present on most B-ALL blasts. Six multivirus-specific CTL lines were generated from mononuclear cells (MNCs) obtained from the peripheral blood (PB) of healthy donors and umbilical cord blood (UCB). MNC were stimulated weekly with dendritic cells and EBV-lymphoblastoid cell lines transduced with an adenovector encoding the CMV pp65 protein (Ad5/f35pp65). After the 3rd stimulation, CTLs were transduced with a retrovirus encoding the CD19.CAR, and then further expanded. Growth kinetics was unchanged from control multivirus CTLs (NT-CTLs). CD19.CAR expression was 65 ± 14% for UCB-CTLs and 62 ± 16% for PB-CTLs and co-expression was seen in virus specific CTL populations visualized using pp65, hexon and EBV MHC multimers. CAR-modified CTLs retained native receptor function against viral targets as assessed by ^{51}Cr release and IFNg Elispot assays; producing the same level of cytotoxic activity against autologous PHA-blasts loaded with pp65 pepmix (PB-CTLs-69%±34% and UCB-CTLs-39%.±17%) as NT-CTLs (PB-CTLs-74%±8% and UCB-CTLs-38%±15%). CD19.CAR+ and control PB-CTLs produced IFNg in response to pp65 (832 ± 53 vs 869 ± 57 SFC/10^5 cells, respectively), hexon (285 ± 29 vs 125 ± 4) and EBV antigens (323 ± 14 vs 309 ± 46). CD19.CAR+ and NT-UCB-CTLs responded to pp65 (100 ± 14 vs 180 ± 19 SFC/10^5 cells, respectively) and hexon (130 ± 28 vs 52 ± 20) pepmix. CD19.CAR+ PB-CTLs and UCB-CTLs lysed CD19+ target cells including Raji (73%±10% and 63%±12%, respectively, at a 40:1 E:T ratio), and primary B-ALL cells (68%±1% and 68%±6%, respectively), but not the CD19- target HDLM-2 (10%±4% and 8%±4, respectively). In contrast <10% cytotoxic activity against CD19+ targets was observed for control CTLs. CD19+ targets were completely eliminated on co-culture with CD19.CAR+ CTLs. Hence CD19.CAR can be effectively transferred to multivirus-specific CTLs generated from both PB and UCB, potentially providing transplant recipients with a single T cell product that possesses both anti-viral and anti-leukemic properties.

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OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) FOR ADVANCED PHASES OF CHRONIC MYELOID LEUKEMIA (CML) IN THE EARLY IMATINIB MESYLATE (IM) ERA

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Despite advances in supportive care, allografting patients in advanced phases of CML remains associated with poor outcomes. We analyzed CIBMTR data of these patients transplanted between 1999–2004, to assess the impact of pre-transplant IM on outcomes and identify prognostic factors. The cohort included 449 patients in second chronic phase (CP2, n = 184), accelerated (AP, n = 185), and blast phase (BP, n = 80) CML who received HLA-identical sibling (27%), related (3%), and matched or mismatched unrelated donor (70%), peripheral blood (47%) or bone marrow (53%) HSCT after myeloablative (78%) or non-myeloablative (22%) conditioning. GVHD prophylaxis consisted of CSA/MTX (52%), FK506/MTX (23%) or other (25%). 52% (96/184) of patients in CP2, 49% (91/185) in AP, and 46% (37/80) received IM for median of 7 (1–60), 11 (1–54), and 8 (1–36) months, respectively, prior to a planned transplant (43%) or transplantation for IM intolerance (6%), or failure (51%). Last dose of IM was administered within 15 days of the start of conditioning in 117 (52%), 15–30 days in 17 (8%), 30–90 days in 37 (16%) and >90 days in 53 patients (24%).

The table below summarizes outcomes at 3 years

	CP2	AP	BP
	Probability (95%CI)	Probability (95%CI)	Probability (95%CI)
OS	36 (29–43)	43 (35–50)	14 (8–23)
Relapse	34 (27–41)	26 (20–33)	36 (26–48)
LFS	27 (20–34)	37 (30–44)	10 (4–17)

Cox proportional hazards regression models were constructed to assess factors that affected overall survival (OS), leukemia-free survival (LFS), TRM, relapse, acute and chronic GVHD. Time from diagnosis to transplant, KPS, and degree of HLA matching independently predicted OS, relapse and LFS; while GVHD prophylaxis, CMV serostatus, sex mismatch, conditioning regimen, and degree of HLA matching affected GVHD and TRM. Pre-transplant IM had neither a positive nor a negative impact on transplant outcomes. Reported reason to proceed with allografting (IM resistance, or planned transplant) and duration of IM therapy prior to transplantation did not impact on any outcomes. Causes of death included disease recurrence (CP2 33%, AP 26%, BP 43%) and complications of GVHD (CP2 48%, AP 54%, BP 35%). The retrospective nature of this study, absence of information on disease status at time of initiation of IM, and changes in disease status while on IM were limitations of this study. We conclude that conventional prognostic indicators remain the major determinants of transplant outcomes in advanced phases of CML. CP2 and AP patients appear to have similar outcomes while BP patients do very poorly. The administration of imatinib prior to HSCT was not associated with improved outcomes.

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SIROLIMUS (SRL)-BASED GVHD PROPHYLAXIS AFTER ALLOGENEIC HSCT IN PEDIATRIC ALL PATIENTS: LOW NRM, LOW INCIDENCE OF VOD, AND HIGHER THAN EXPECTED EFS: RESULTS OF A MULTI-INSTITUTIONAL PILOT STUDY

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Relapse and NRM after allogeneic HSCT remain significant barriers to success in treating very high-risk ALL. Sirolimus (SRL) has been shown to have cytotoxic activity against human ALL at serum levels used for immune suppression. We hypothesized that the addition of SRL to a tacrolimus (TAC)/methotrexate (MTX) GVHD prophylaxis regimen would decrease relapse after HSCT for ALL. This multi-institutional pilot trial included a preparative regimen of TBI (1200cGy), thiotepa (5mg/kg/dx2) and cyclophosphamide (60mg/kg × 2). Pts received IV TAC (start d-2, target 5–10ng/mL), PO SRL (start d0, target 3–12ng/mL), and IV MTX (5mg/m^2, d1, 3, and 6 plus d11 for UD BM/PBSC). TAC was tapered between d+42–96 for MRD and d+100–180 for others. SRL was tapered over 4 wks starting 6m after transplant. The study enrolled 58 pediatric pts (med age 9 (1–22)) with a med f/u of 22m (2–63m). Immunophenotypes included 47 B-lineage and 11 T-lineage. Risk groups included 17 pts in high risk (HR) CR1, 14 in HR CR2 (BM relapse <36m from dx), 16 in intermediate risk (IR) CR2 (5 isolated extramedullary (IEM) relapse, 11 late BM relapse ≥36m), and 11 in CR3. Stem cell sources included 25 MRD, 28 UCB, and 5 UD. Results: 2yr EFS was 71% (SE 6.4) and did not differ by stem cell source. 2yr EFS by risk groups is outlined in the table. CIBMTR anticipated OS for pediatric ALL in early phase (CR1) is 48–60% (UD vs MRD), intermediate phase (CR2) 39–52%, and late phase 17–29%; by comparison our EFS outcomes exceed these projections by 20–30%. Toxicity with the regimen was low. One recipient of CB failed to engraft, a second relapsed prior to engraftment, and remaining pts engrafted at a median of 20d (13–31) and 29d (16–62) for MSD/UD and CB, respectively. Acute GVHD grade II-IV and III-IV occurred in 41 and 21% of patients, respectively, while cGVHD occurred in 8 and 37% of RD and UD recipients. NRM occurred in 6 pts (10%: 5 CB, 1 MSD). Significant toxicities included VOD (5pts (8%), fatal in 2 cases), non-fatal HUS (2pts), and non-fatal IPS (1pt). In summary, SRL-based GVHD prophylaxis after TBI/TT/Cy allogeneic HSCT results in high rates of engraftment, low NRM, and improved 2yr EFS in all risk groups. Though numbers are small, as opposed to recent reports in adult trials, sirolimus/mtx does not appear to increase the risk of VOD in children (anticipated rates 11–20%). A phase 3 trial in the COG for ALL patients comparing TAC/MTX with TAC/MTX/SRL after TBI/TT/Cy allogeneic HSCT is ongoing.

Two Year EFS after Sirolimus-Based HSCT

ALL Risk Group	ALL HR CR1	ALL HR CR2	ALL IR CR2	ALL CR3
2yr EFS (±SE)	85% (±10)	57% (±13)	85% (±10)	48% (±17)