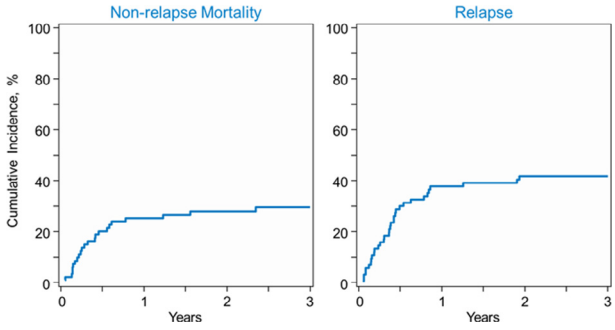

Figure 1.

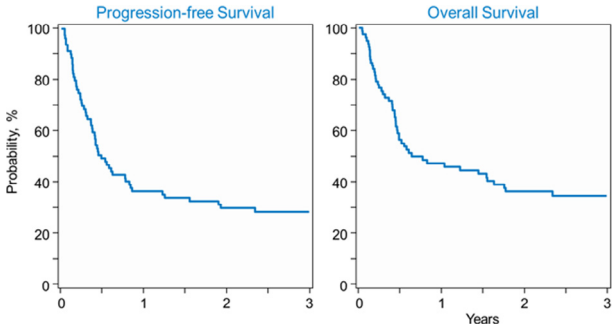

Figure 2.

Conclusions: This CIBMTR study, largest and only study to include Caucasians, shows that alloHCT provides durable remission in a subset of ENKL. Race, PET status and conditioning intensity did not affect outcomes. While no relapses were seen beyond 2 yrs, disease relapse was the most common cause of death underscoring need for novel relapse prevention strategies after alloHCT.

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Adoptive Transfer of Multi-Tumor Antigen Specific T Cells as Treatment for Patients with Multiple Myeloma

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Allogeneic hematopoietic stem cell transplant (HSCT) remains the only curative immunotherapy for patients with multiple myeloma (MM). However, high rates of transplant-associated mortality (up to 30%) limits the applicability of this approach. Thus, to separate the beneficial post-HSCT "graft versus myeloma" effect from the antecedent toxicities, we developed a strategy to enrich autologous MM-specific T cells *ex vivo*by stimulating patient PBMCs with overlapping peptide libraries spanning 5 MM expressed tumor associated antigens (TAAs), PRAME, SSX2, MAGEA4, NY-ESO1 and Survivin. To test the safety and efficacy of these cells we have initiated a phase I/II study (NCT02291848) with 2 arms: >90 days (Group A) or <90 days (Group B) post-autoHSCT. To date, we have enrolled 9 patients (pt's) and completed multiTAA T cell manufacture for 8. The lines were polyclonal: CD4+ (mean 21.59 ± 4.7%) and CD8+ (61.36 ± 6.3%) T cells that recognized 2 to 5 of the targeted TAAs: SSX2 (range 1-120.5 IFNγ spot forming cells (SFC)/2×10^5 T cells on an ELIspot assay), Survivin (0-63), NY-ESO-1 (4-107), PRAME (2-235) and MAGEA4 (1-382). None of the lines exhibited auto-reactivity against non-malignant cells (mean 2 ± 2.1% lysis, E:T 20:1). Thus far, 7 pt's (median of 3 prior therapies) have been infused with multiTAA T cells (.5 to 1×10^7 cells/m^2). Three who were in remission when infused, remain in remission 3-10 mo's post-infusion, while 3 of 4 pt's treated for active disease, have derived a clinical benefit. This includes 1 stable disease (ongoing at 11 mo's post-infusion), 1 partial response (ongoing at 10 mo's), and 1 complete remission (ongoing at 9 mo's). In each case clinical benefit coincided with an expansion in the circulating frequency of T cells directed against both TAAs targeted in the T cell line as well as against non-targeted TAAs, indicating antigen spreading. We also detected enrichment of TAA-specific T cells in the marrow of pt's with active disease, indicating tumor infiltration. Finally, our only non-responding patient was treated for refractory MM after failing 5 prior therapies, including 2 autoHSCT's. Post-infusion, initially there was a decline in monoclonal IgG*k* (2.2 to 1.6 g/dl within 2 mo's), but by 6 mo's post-infusion the patient progressed (IgG*k*: 6.5 g/dl). To investigate escape mechanisms we analyzed the frequency of tumor-specific T cells over time as well as performing IHC on serial marrow biopsies. This analysis demonstrated the capacity of the infused T cells to recognize the antigens expressed on the tumor and showed how this profile evolved as a mechanism of immune escape. In summary, we have demonstrated the safety of multiTAA T cells in patients with MM and early evidence of clinical benefit co-incident with the *in vivo* expansion of tumor-specific T cells. Furthermore, we highlight the importance of simultaneously targeting multiple tumor-expressed antigens for clinical benefit.

CLINICAL CELLULAR THERAPY

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Safety and Efficacy of Donor T Cells Engineered with Herpes Simplex Virus Thymidine-Kinase Suicide Gene (TK Cells) Given after T-Cell Depleted (TCD) Haploidentical Hematopoietic Transplantation (Haplo-HSCT): Results of a 14-Year Follow-Up in 45 Patients

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T-cell engineering is increasingly used in cancer immunotherapy, with long-term safety being a major issue. Since August 2002,we used TK cells after TCD haplo-HSCT to hasten immune reconstitution (IR), while controlling GvHDby suicide gene induction with ganciclovir (GCV).

Effects of TK cells on outcome were assessed in 45 pts receiving 1-4 monthly doses (.1-1.0 × 10^7/Kg; 21-49 days after HSCT) in a ph 2 trial (n = 30; *Lancet Oncol 2009; 10: 489*) and in the experimental arm of an ongoing ph 3 trial (n = 15; *NCT00914628*). Long-term safety was assessed yearly. Endpoints: 1-year NRM, OS, LFS, RI and acute (a) or chronic (c) GvHD. Median follow-up: 3.7 years (IQR 1.5- 8.5). A pair-matched analysis (PMA), supportive of conditional marketing authorization recently granted for TK cells (*www.ema.europa.eu / EPAR / Zalmoxis*), compared data from both TK trials with control data from contemporaneous HSCT (2000-2013).

34 pts (76%) had IR after a median of 2 TK-cell infusions (IQR 1-2), a median cumulative dose of 1.3 × 10^7/kg (1.0-2.4) and a median time from HSCT of 83 days (65-108). IR was not influenced by baseline risk factors or dose of TK cells, but was associated with improved NRM ($P < .0001$), LFS ($P = .005$) and OS ($P < .0001$). Grade 2-4 aGvHD (35%; grade 3-4: 7%) was unrelated to TK-cell dose. Only one patient had cGvHD. All GvHD events fully resolved (median 14 days; 10-27) after GCV (15 days; 13-16). RI (31%) did not differ by IR, but inversely correlated with TK-cell dose: RI of 60%, 33% and 0% with <1.0, 1.0-2.4 and >2.4 × 10^7/Kg, respectively ($P = .004$). The PMA compared (1:4 ratio) TK pts (n = 36) with controls alive and relapse free 21 days after HSCT (n = 139; 69 TCD, 70 T-cell replete + cyclo [TCR]), using diagnosis (AML/ALL/sAML), status (CR1-3, relapse), time from diagnosis (±3 months) and age (±3 years) as matching factors. TK pts vs controls had improvements in OS (51% vs 34%; $P = .007$), NRM (20% vs 46%; $P = .003$) and cGvHD (6% vs 23%; $P = .02$), which were further confirmed by 3 landmark analyses in pts alive and relapse free 4, 6 and 8 weeks after HSCT. There were no differences in LFS and RI. Main NRM events in controls were infection (54%) and cGvHD (20%). After further matching on more recent HSCT (2008-2013), outcomes were better in TK than in control groups using either TCD (OS: 49%% vs 23%, $P = .001$; LFS: 37% vs 22%, $P = .007$) or TCR (OS: 72% vs 43%, $P = .04$; LFS: 66% vs 37%, $P = .06$). Following GvHD onset, TK pts with grade 2-4 aGvHD treated with GCV had improved OS than controls with grade 2-4 aGvHD (67% vs 25%; $P = .009$). No mutational event was recorded in extended follow-up. Ex-vivo analysis of TK cells confirmed a stable transgene expression with functional sensitivity to GCV.

TK cells are a safe cell therapy tool in up to 14-year follow-up. Early IR, full aGvHD control, low cGvHD and dose-related antileukemic effects after TK cells translate in improved NRM and OS, which compare favorably with outcomes after current haplo-HSCT approaches.

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Direct Comparison of in Vivo Fate of Second and Third-Generation CD19-Specific Chimeric Antigen Receptor (CAR)-T Cells in Patients with B-Cell Non-Hodgkin Lymphoma (B-NHL): Reversal of Toxicity From Tonic Signaling

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Although 2nd-generation (2G) CD19-specific CARs containing CD28 or 4-1BB costimulatory endodomains show remarkable efficacy against B-NHL, the optimal choice of domains in these and other CARs remains controversial. Individual endodomains, such as CD28 (Long, Nat Med 2015), may be associated with deleterious ligand-independent tonic signaling in the transduced T cell, but it is unclear if tonic 4-1BB signaling may have such consequences as well, and if such effects can be reversed.

We therefore modeled tonic CAR signaling in T cells by transducing them with gammaretroviral vectors expressing 2G CD19.CAR constructs containing CD3-ζ and either the CD28 or 4-1BB endodomains. 4-1BB CD19.CAR-T cells (CARTs) expanded 70% slower, which was coupled with a 4-fold increase in apoptosis and a gradual downregulation of CAR expression. This was a consequence of 4-1BB-associated tonic TRAF2- dependent signaling, leading to activation of NF-κB, upregulation of Fas and augmented Fas-dependent activation induced T cell death. Because of the toxicity of 4-1BB in our CAR construct, we could not directly compare the in vivo fate of 4-1BB CD19.CARTs with that of CD28 CD19.CARTs. We found, however, that the 4-1BB toxicity could be overcome in a 3rd-generation (3G) CD19.CAR vector containing both CD28 and 4-1BB.

We thus compared the fate of that 3G vector with the 2G vector containing CD28 alone. Eight patients with refractory/relapsed diffuse large B-cell lymphoma received 2 cell populations, one expressing 2G and one expressing 3G vectors. To determine whether CD28 alone was optimal (which would suggest 4-1BB is antagonistic) or whether 4-1BB had an additive or synergistic effect contributing to superior persistence and expansion of the CD28-41BB combination, patients were simultaneously infused with 1-20 × 10^6 of both 2G and 3G CARTs/m^2 48-72 hours after lymphodepletion with cyclophosphamide (500 mg/m^2/d) and fludarabine (30 mg/m^2/d) × 3. Persistence of infused T cells was assessed in blood by qPCR assays specific for each CAR. Molecular signals peaked approximately 2 weeks post infusion, remaining detectable for up to 6 months. The 3G CARTs had a mean 23-fold (range 1.1 to 109-fold) higher expansion than 2G CARTs and correspondingly longer persistence.

Two patients had grade 2 cytokine release syndrome, with elevation of proinflammatory cytokines at the time of peak expansion. Of the 6 patients evaluable for response, 2 entered complete remission (the longest ongoing for 1 year), 1 has