However, malignant disease remained a predictor for lower OS. (HR =3.38(1.37 -7.3 at 95% CI, p = 0.007). In multivariate analysis of OS modeling, comparing mean values acquired only between day 0 to Day 365, more accelerated thymic recovery in the first year (>9% of circulating T cells with a RTE phenotype [CD62L+/CD45RA+]), was the only immune parameter that was associated with a reduced risk of death at any time point, HR 0.20 (0.07-0.60, 95%CI), p = 0.004.

Conclusion: Even among children with relatively better preserved thymus compared to adults, those with more rapid thymic recovery leading to more robust rise in RTE will have superior clinical outcome. These findings should spur further research aiming to enhance recovery of the central, thymic derived pathway of T cell generation.

SOLID TUMORS

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CMV-SPECIFIC HER2-REDIRECTED T CELLS FOR THE ADOPTIVE IMMUNOTHERAPY OF GLIOBLASTOMA

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Background: We demonstrated that T cells from GBM patients engineered to express a HER2-specific chimeric antigen receptor (CAR) kill autologous HER2 + GBM cells ex vivo, including the CD133 + GBM stem cell population. In vivo, the adoptive transfer of HER2-specific T cells resulted in regression of established GBM in the brain of mice, yet tumors recurred in a number of treated animals. This limitation in T-cell efficacy is most likely due to a) limited T-cell persistence in vivo and/or b) tumor antigen loss variants in treated tumors. Since the majority of GBMs are positive for CMVpp65 we expressed HER2.CARs in CMV-specific cytotoxic T cells (CTLs) to a) enhance their persistence in vivo following native T-cell receptor (αβTCR) engagement with CMV-antigens and b) improve their antitumor activity by simultaneously targeting CMV and HER2.

Methods: To study the feasibility of generating CMV CTLs from GBM patients, we determined the precursor frequency of CMV-specific T cells in 12 patients using IFN-g ELIspot assays. To generate CMV CTL ex vivo, peripheral blood mononuclear cells were transduced with an adenovirus vector; encoding the immunodominant CMV-pp65 antigen (Ad5f35pp65) then stimulated with autologous EBV transformed Ad5f35pp65-transduced irradiated lymphoblastic cell lines (EBV-LCL). CTLs were then transduced with a retroviral vector encoding a HER2.CAR (FRP5.CD28.ζ). We detected the expression of the CAR transgene using flowcytometry. To concomitantly identify the CMV-specific T cell population we used pentamer analysis for CMVpp65. We performed ^{51}Cr-release assays to characterize the functionality of HER2.CAR CMV.CTLs *ex vivo*.

Results: CMV-specific T cells were readily detectable in the blood of GBM patients as judged by IFN-g secretion after stimulation with CMVpp65 PepMixes™. We were able to generate CTLs that are specific for CMV through their endogenous ab TCR and specific for HER2 through the CAR. Approximately 50% of CMVpp65-specific CTLs expressed HER2.CARs on the cell surface. CMV-specific CTLs expressing the HER2-specific CAR recognized and killed the HER2-positive GBM cell line (U373) as well as autologous OKT3-blasted T cells pulsed with CMVpp65 PepMixes™ in a standard 4 hour ^{51}Cr-release assays. Neither the HER2 –ve cell lines nor autologous OKT3-blasted T cells were killed.

Conclusion: Our data demonstrates the feasibility of generating CAR expressing CMVpp65-specific CTLs that are functional against HER2 and CMV expressed in GBM.

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OUTCOMES WITH HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN COMPARISON TO CONVENTIONAL CHEMOTHERAPY IN PEDIATRIC PATIENTS WITH HIGH RISK EWING'S FAMILY TUMORS

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Pediatric patients with high risk Ewing's family tumors (hrEFT), relapsed or metastatic at diagnosis, have very poor outcome with conventional chemotherapy. A steep dose-response curve to alkylating agents suggests myeloablative chemotherapy as a potential therapeutic modality, but controversy persists, warranting additional investigation. We retrospectively reviewed hrEFT patients treated with conventional therapy (CHEMO) in comparison to high dose alkylator-based chemotherapy and autologous stem cell transplantation (ASCT) treated at a single center. Thirty-six patients were assigned into 4 groups based on disease features and therapy received: REL/CHEMO (n = 12), REL/ASCT (n = 5), MET/CHEMO (n = 14), MET/ASCT (n = 5). Kaplan-Meier estimator was used to compare event free survival (EFS) and overall survival (OS). Seven of 10 ASCT patients received busulfan, melphalan, thiotepa and 3 of 10 received melphalan, carboplatin and etoposide prior to autologous peripheral blood stem cell rescue. Both regimens included the chemoprotectant amifostine. Patients had complete response (CR), very good partial response (VGPR) or partial disease response (PR) at the time of ASCT. REL/CHEMO patients received a variety of salvage non-high dose chemotherapies. EFS at 1 and 3 years from 1st relapse were 100% and 53% in REL/ASCT group in comparison to 25% and 8% respectively in REL/CHEMO group (p = 0.0007). OS at 1 and 3 years from diagnosis were 100% and 80% for REL/ASCT group and 92% and 42% respectively in REL/CHEMO group (p = 0.013). ASCT-related toxicities were febrile neutropenia and grade 3 mucositis (n = 10), adrenal insufficiency (n = 4), bacteriemia (n = 1), and herpes zoster reactivation (n = 3). We did not observe any veno-occlusive disease of liver (VOD) or graft failure. There were no regimen-related mortalities. Four of 5 patients are surviving in the REL/ASCT group, and all are currently disease-free (one after a further relapse) with a median time from 1st relapse of 3.3 years (range 1.7-8.9). In comparison, none of the 12 children with relapsed disease treated with chemotherapy alone are surviving, with median time from relapse to death of 1 year (range 0.1-4.8). In patients with metastatic disease at diagnosis, the 1-year OS was 75% in MET/ASCT group, and 64% respectively in MET/CHEMO group (p = 0.79). Our data suggest ASCT is tolerable and should be considered as therapeutic option for relapsed patients. Randomized studies are required to support these observations.

STEM CELL BIOLOGY

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SYSTEMIC ADMINISTRATION OF PLEIOTROPHIN INDUCES HEMATOPOIETIC STEM CELL (HSC) REGENERATION IN VIVO

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The elucidation of bone marrow microenvironmental signals which promote HSC regeneration in vivo would have important implications for the treatment of patients undergoing radiation therapy, chemotherapy and stem cell transplantation. We recently reported that pleiotrophin, a soluble heparin-binding growth factor, induced a 10-fold expansion of long-term repopulating HSCs in culture (Himburg et al. Blood, Nov 2008; 112: 78). Based on this observation, we hypothesized that PTN might also be a regenerative growth factor for HSCs. Here we tested the effect of systemic administration of PTN to mice to determine if PTN could promote HSC regeneration in vivo following total body irradiation (TBI). C57Bl6 mice were irradiated with 700 cGy followed by

intraperitoneal administration of 2 μg PTN or saline × 7 days, followed by analysis of BM stem and progenitor cell content. Saline-treated mice demonstrated a significant reduction in total BM cells, BM c-kit+sca-1+lin- (KSL) cells, colony forming cells (CFCs) and long term culture-initiating cells (LTC-ICs) compared to non-irradiated mice. Irradiated, PTN-treated mice demonstrated a 2.3-fold increase in total BM cells ($p = 0.03$), a 5.6-fold increase in BM KSL stem/progenitor cells ($p = 0.04$), a 2.9-fold increase in BM CFCs ($p = 0.004$) and an 11-fold increase in LTC-ICs ($p = 0.03$) compared to irradiated, saline-treated mice. Moreover, competitive repopulating assays demonstrated that BM from irradiated, PTN-treated mice contained 5-fold increased competitive repopulating units (CRUs) compared to irradiated, saline-treated mice ($p = 0.04$). Taken together, these data demonstrate that the administration of PTN induces BM HSC and progenitor cell regeneration *in vivo* following injury. To determine whether PTN acted directly on BM HSCs to induce their proliferation and expansion in vivo, mice ingested BrDU × 7 days and compared BrDU incorporation in BM KSL cells in saline-treated versus PTN-treated mice. PTN-treated mice demonstrated a significant increase in BrDU+ BM KSL cells compared to saline-treated controls ($p = 0.04$). PTN is a novel, secreted growth factor for BM HSCs and induces their regeneration *in vivo* while preserving their repopulating capacity. PTN has therapeutic potential as a growth factor to accelerate hematopoietic reconstitution in vivo in patients undergoing myelosuppressive radiotherapy or chemotherapy.

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THE ROLE OF SALL4 IN EMBRYONIC STEM CELL PLURIPOTENCY

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Murine embryonic stem cells (mESCs) can grow indefinitely *in vitro*, and yet retain the potential to differentiate into all three primitive germ layers. They hold significant promise within the field of regenerative medicine, particularly through the production of patient specific induced pluripotent cells (iPS), which mimic the properties of embryonic stem cells. The ability of mESCs to maintain their pluripotency is regulated by a number of transcription factors, including Sall4, the causative gene in Duane-Radial Ray syndrome (DRSS), consisting of radial, cardiac, and eye abnormalities. Sall4 is critical for maintaining mESCs in a pluripotent state, and interestingly exists as two isoforms (Sall4a, the long isoform and Sall4b, the short isoform); Sall4b has been implicated as an oncogene in acute myelogenous leukemia (AML). Using genome wide location anaylsis, we have been able to show that both isoforms of Sall4 exist in mESCs and bind to overlapping but not identical target genes. In fact, Sall4b (the short isoform) binds to most pluripotency targets, whereas Sall4a binds predominantly to differentiation genes. Using RNAi technology to generate mESCs containing only one of the two isoforms we demonstrate that Sall4b but not Sall4a is required for maintaining mESCs in a pluripotent state. Lastly, proteomics reveals that Sall4a and Sall4b may have different protein:protein interaction partners which affects their ability to regulate pluripotency targets. Taken together, these data indicate that differences in splice variants at a single locus can play key roles in regulating the pluripotent states. Further studies need to be performed to understand how these differences may translate into the unique roles these splice isoforms play in human diseases.

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DNA TRANSFER FROM DONOR TO HOST CELLS AS A MECHANISM FOR EPITHELIAL CHIMERISM AND GENOMIC ALTERATIONS AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

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Research in the field of allogeneic hematopoietic cell transplantation (allo-HCT) revealed hidden consequences of the co-existence of two genetically distinct populations in the transplant recipient. First, epithelial cells with donor-derived genotype emerge and second, epithelial tissues of the host acquire genomic alterations. We asked whether horizontal transfer of donor-DNA to host epithelium is operating in (and linking) both phenomena. 176 buccal samples were obtained from 71 allo-transplanted patients and analyzed with microsatellite markers for the presence of donor-DNA and microsatellite instability. The presence of graft-derived hematopoietic cells in the samples was excluded by immunocytochemistry. The results were associated with clinical data. Genomic instability induction and DNA transfer in epithelial cells by allogeneic lymphocytes was assessed *in vitro*. We found a high contribution of donor-DNA (mean 26.6%) in buccal samples in 61 out of 68 evaluable patients. In addition, 32% of the samples were positive for microsatellite instability. The extent of donor-DNA was significantly correlated with the occurrence of genomic alterations ($p < 0.05$). The age of the patient and a female-to-male transplantation were significantly correlated with microsatellite instability. There was a trend for increasing risk of microsatellite instability for patients who experienced severe graft-versus-host disease. During follow up secondary malignancy was diagnosed in 5 patients (14%) who exhibited microsatellite instability but only in 1 (3%) with no instability. In an *in vitro* model, we demonstrated that apoptotic lymphocytes may not only induce genomic alterations but also transfer DNA in co-cultured epithelial cells, resulting in the creation of hybrid chromosomes. Our results indicate that continuous charge of the transplant recipient with apoptotic donor-DNA and its illegitimate integration into host epithelium may come in light as epithelial cells with donor-derived genome or instability events and may provide a new model for elucidating protean clinical manifestations after allo-HCT, such as secondary malignancy.

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ALDH^{BR} HEMATOPOIETIC PROGENITORS PROMOTE SHORT-TERM ENGRAFTMENT IN EXPERIMENTAL MODELS FOR CORD BLOOD TRANSPLANTATION

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We previously developed methods to purify human hematopoietic progenitors based on their high expression of aldehyde dehydrogenase (ALDH^{br} cells), which enrich both short- and long-term SCID-repopulating cells. One clinical trial now suggests that augmenting a conventional cord blood transplant (CBT) with ALDH^{br} cells accelerates both neutrophil and platelet engraftment. We now describe two experimental models for this clinical regimen, performed in different strains of NOD/SCID mice. Each study included three cohorts: (1) mice transplanted with 4,000 purified ALDH^{br} cells; (2) mice transplanted with total CB at a dose that contained 4,000 ALDH^{br} cells; and, (3) mice that first received the same dose of unmanipulated CB and, after 4 hours, the ALDH^{br} cells.

In NOD/SCID-IL2Rγ^{null} (NSγ) mice, human ALDH^{br} cells exhibited strong chimerism including bone marrow CD33$^+$CD15$^+$ neutrophils and circulating CD41$^+$ platelets, by 4 weeks post-transplant. Unmanipulated CB also engrafted the bone marrow of NSγ mice, but >95% of the cells were CD3$^+$ T cells. Myeloid engraftment was low to undetectable. When CB grafts were augmented with ALDH^{br} cells, bone marrow chimerism increased >2-fold relative to mice that received CB alone ($P = 0.006$). However, like mice that received CB alone, the human cells within the bone marrow were mainly T cells; myeloid engraftment remained low to undetectable.