that the continued presence of CD4 T cells after HSCT is required for the development of CD8 memory. Upon investigating the mechanism(s) of memory loss, we observed an accelerated contraction of tumor-reactive CD8 cells in the CD4-depleted mice. This accelerated loss of tumor-reactive CD8 cells correlated with increased percentages of annexin-V+ pre-apoptotic CD8 cells during the contraction phase (day 21 after HSCT). A significant proportion of the annexin-V+ CD8 cells co-expressed programmed death receptor-1 (PD-1), had an effector phenotype (CD62L-CD44+), and expressed diminished levels of the receptors for IL-7 and IL-15. These data suggest that PD-1/PD-L1 interactions may play a role in the loss of CD8 memory. Together, these results show that CD4+ cells both promote and inhibit anti-tumor responses. These observations should help us design immunotherapeutic approaches that can generate "optimal" acute anti-tumor reactivity after HSCT with development of long-term immunity.

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LONG-TERM FOLLOW-UP OF METASTATIC RENAL CANCER PATIENTS UNDERGOING REDUCED-INTENSITY ALLOGRAFTING
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Stem cell transplantation from a HLA-compatible sibling donor is an adoptive immunotherapy for cytokine-resistant, metastatic renal cancer (RCC). However, the recent introduction of several targeted therapy compounds has reduced the interest in this therapeutic strategy. We have reanalyzed our transplant series with the aim to detect long-term benefit form allografting. From February 1999 to May 2005, 25 patients with cytokine-refractory RCC received a reduced-intensity allograft from a HLA-id sibling donor. Median age was 53 years; most (24) had clear-cell histology. Median number of previous treatments was 1 (0–3). Median days from diagnosis to allograft were 822. All patients received a thiopeta, fludarabine, and cyclophosphamide conditioning regimen, and a cyclosporine-based GVHD prophylaxis. Six patients received DLI at escalating doses for progressing or non-responding disease. One-year OS was 48% (95% CI: 28–68), and 3y-OS was 20% (95% CI: 4–36). At a median observation time of 65 months, 5 patients are alive, one in CR, one in VGPR, and three with stable disease. We have analyzed the correlation of the following variables with survival: age at transplant, time from diagnosis to transplant, serum calcium corrected for albumin levels, lactate dehydrogenase (LDH), C-reactive protein (CRP), haemoglobin level before transplantation, Karnofsky performance status, number of CD34+ cells infused, number of CD3+ cells infused, and disease status at transplant. Survival of patients at favourable/intermediate-risk according to the MSKCC score that underwent allografting was better in comparison to the survival predicted by historical controls. We conclude that transplantation is able to induce long-term disease control in 20% of cytokine-refractory RCC patients. It is unknown if relapse or PD after targeted therapy will be susceptible to allograft-mediated GVT effect. The place of allografting in the treatment of metastatic RCC, alone or in combination with targeted therapies, needs reappraisal.

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LOW MORBIDITY AND MORTALITY IN A PILOT STUDY OF BUSULFAN, MELPHALAN AND TOPOTECAN AS PREPARATIVE REGIMEN FOLLOWED BY AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PEDIATRIC PATIENTS WITH HIGH RISK SOLID TUMORS (PRELIMINARY RESULTS).
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Autologous hematopoietic stem cell transplantation (HD) has been utilized as consolidative therapy for pediatric patients with some encouraging improvement in survival. We hypothesize that by intensifying therapy which poor prognosis patients currently receive, it is possible to reduce the risk of relapse after HD and the morbidity and mortality will be acceptable. Phase I pediatric studies of topotecan demonstrated activity against solid tumors. The dose-limiting toxicity of Phase I studies has been hematopoietic suppression. The use of hematopoietic stem cell support following a topotecan-containing preparative regimen will allow utilization of topotecan in combination with established chemotherapeutic agents. Patients were assigned to receive topotecan at 2 mg/m2 continuous infusion in combination with fixed doses of busulfan and melphalan (B-M-T). In this study, the primary outcome variable is to evaluate the activity of combined HDT with B-M-T. Secondary objectives is to analyze the pharmacokinetics, and outcomes.

Results: Thirteen patients age 2–18 year old (median = 5.4) were treated on B-M-T-protocol. Seven patients were diagnosed with neuroblastoma (NBL), Wilms tumor (n = 2), PNET (n = 2), rhabdomyosarcoma (RBS) and Ewing sarcoma (each n = 1). Eight patients were in CR, five in PR. There were no deaths in the first 100 day post HDT. There was 1 admission (1day) to ICU for observation only. Eleven patients developed grade 1–2 and 2 patients grade 3 liver toxicity (transaminis and bilirubin elevation), all patients developed grade 2–3 mucositis; 5 patients had grade 2 diarrhea, one patient developed grade 3 respiratory toxicity, 7 patients had metabolic toxicities grade 1–2. All patients engrafted. Patients achieved ANC > 500 on day 8–15 (median 12), platelets > 25,000 on day 9–22 (median 16), no RBC transfusions were required after day 7–25, (median 16.2). Overall 1 1/2 year survival rate is 87% (11 out of 13 patients are alive); event-free survival is 78%. Three patients had recurrent disease; 2 patients with PNET (both patients expired) and 1 pt with NBL (alive).

Conclusions: Analysis of the preliminary data suggests that B-M-T regimen in the context of HDT was tolerated without unexpected or severe toxicity and allowed prompt transition to radiation and/or maintenance therapy. Further studies are needed to document the survival benefit of consolidation with B-M-T regimen in children with high risk solid tumors.

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ALLOGENEIC STEM CELL IMMUNOTHERAPY FOR ADVANCED METASTATIC BREAST CANCER: THE WAY FORWARD
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Despite continuous advances in the treatment of mBCR, some patients have very poor outcome. Over the last period, we have investigated allogeneic immunotherapy as a possibility for tumor control in patients with advanced mBCR. We have treated 31 pts with Allo SCT in 2 successive clinical trials. All pts (age: 43 (27–57)) underwent ASCT after the same RIC (Fludarabine (150mg/m2), Busulfan (8mg/kg) and Thymoglobin (2.5mg/kg or TLI (1 Cyg))) from a HLA-identical sibling (BM: 13%; PBSC: 87%) followed by CSA. Prior to ASCT a median of 3 lines of treatment (1–7) have been administered over a period of 57 months (6–143).15 (48%) pts underwent autologous SCT at a median time of 15 months (1–99) prior to ASCT. All pts were measurable and had a median of 2 metastatic sites (1–4) (liver:72%, bone:50%, lung:22% and brain:11%). At transplant : 17 (55%), 10 (32%) and 4 (13%) pts had progressive (PD), stable disease (SD) and partial response (PR) respectively. All patients engrafted. The cumulative incidences of grade 2–4 aGVHD and cGVHD were 42% (25–59) and 62% (45–79) respectively. Of note, none of the 31 pts died from TRM. Seven patients achieved an objective response (CR = 1; PR = 6) at a median of 60 days (30–150) for a 24 % (9–39) OR cumulative incidence. Eventually all pts but 3 progressed at a median of 310 days (120–560) post transplant. Four pts are alive at median of 23 months (21–30) post transplant for a 2-year overall survival (OS) probability of 29% (16–47%). Results are dramatically different in regards to disease status at time of transplant. While outcome was uniformly poor for pts with PD (OR = 0; 2 year OS probability: 6% (1–27)),

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patients with SD achieved a 40% (0–80) OR rate (p = 0.02) for a 50% (19–80) OS probability at 2 years (p = 0.001). Of the 4 patients in response at time of transplant, 3 increased their response (PR = 2; CR = 1) and 3 were alive after 2 years. This study shows that RIC-ASCT can be safely performed in BRCP pts without high transplant related mortality. However we show evidences that pts with highly progressive disease do not benefit from this approach. In contrast, a high rate of response associated with prolonged survival can be achieved in patients with slowly progressive disease. Better results could perhaps be achieved only in pts in the initial disease phase when treatment disease resistance has not occurred.

233 INCREASED FREQUENCY AND RESPONSIVENESS OF PSA-SPECIFIC T CELLS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION FOR PROSTATE CANCER

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Therapies for localized prostate cancer include curative surgery and radiotherapy while treatment of metastatic disease is often insufficient. Therefore, we started to investigate the potential of allogeneic SCT as a treatment for non-curable prostate cancer. A patient underwent allogeneic SCT from his HLA-identical sister after a non-myeloablative conditioning regimen as treatment for his metastatic prostate adenocarcinoma. The patient was treated with donor lymphocyte infusions after SCT due to threatening rejection. Frequencies of prostate-specific T cells in the peripheral blood of the patient, sibling donor and a group of control individuals were determined by flow cytometry using tetrameric and pentameric HLA A2 complexes containing peptides derived from the prostate specific antigen (PSA). Cytotoxic activity of PSA-peptide-specific T-cells against peptide-pulsed target cells was analyzed ex vivo by 51Cr-release assays. Stable clinical and laboratory remission lasting for more than 5 years was observed after SCT. Using HLA containing pentamers with PSA-derived peptides we could detect prostate-specific CD8+ T cells in this patient in high frequencies over several months. Furthermore, higher frequencies of PSA-specific T-cells were revealed in the peripheral blood of the patient and female controls as compared with male healthy controls. Recently the PSA levels in the patient started to increase, and prostate biopsies showed prostate carcinoma in 3/8 biopsies, Gleason 4+4, totally 3.5 mm of cancer (of 100 mm totally). Due to this progression we have initiated a protocol for adoptive transfer of allogeneic PSA-specific T cells from the patient. We will, by using pentamers and fluorochrome specific beads separate the PSA specific T-cells from the patient. In order to break a possible tolerance we will keep them in high concentration of IL-2 and the respective peptides in vitro culture over night before injection back to the patient. Clinical data of this novel therapy will be presented at the meeting. Our data suggest that allogeneic SCT led to the generation of a T cell mediated prostate-specific immunity in the reported patient. The *in vitro* and *ex vivo* immunological monitoring performed indicate an adjuvant anti-tumor effect of PSA-specific T cells. This report presents a novel treatment approach involving allogeneic SCT in prostate cancer patients who do not respond to chemotherapy and/or cannot undergo prostatectomy.

234 COMPLETE LONG-TERM HEMATOPOIETIC ENGRAFTMENT BY LINEAGE-DEPLETED ALDH CD34+ UCB CELLS

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In previous work, we fractionated CD34+ umbilical cord blood (UCB) progenitors to purify cells that express high levels of aldehyde dehydrogenase (ALDH). The ALDH<sup>hi</sup> CD34<sup>+</sup> cells were enriched with progenitors that engraft NOD/SCID mice in both short-term (6–8 week) and long-term (>18 week) transplants. In contrast, ALDH<sup>low</sup> CD34<sup>+</sup> progenitors were not a significant source of NOD/SCID repopulating cells. These data strongly imply that transplantable human progenitors express ALDH; however, one shortcoming for that work remains that human hematopoietic development in NOD/SCID mice is limited to the myeloid and B-lymphoid lineages. Because the pace of T cell engraftment is a clinical critical concern, we have adopted the use of the NOD/SCID-IL2R<sup>−/−</sup> mouse xenograft transplant model. In the current study, 20 mice were transplanted with lineage-depleted ALDH<sup>hi</sup> CD34<sup>+</sup> progenitors at doses that ranged from 3,000 to 30,000 cells. In control studies, similar doses of ALDH<sup>low</sup> CD34<sup>+</sup> cells did not provide long-term engraftment. So that we might characterize the behavior of individual UCB, each transplant graft was derived from a single UCB. In addition, each UCB was assayed in multiple different mice. Twelve of 15 mice that survived until 21 weeks post-transplant demonstrated multiple-lineage human hematopoietic engraftment. Human T, B, NK and myeloid cells were detected in the bone marrow and spleen. In addition, in 9 mice human T cells were detected in the thymus. Furthermore, 7 mice had sufficient thymus tissue to measure T cell Receptor Excision Circles (TREC). By that assay, 3 of those mice demonstrated active human T cell rearrangements. As anticipated, the level of engraftment to all tissues was cell dose-dependent. One strength for this xenograft model was that engraftment could be monitored over time within the peripheral blood. Human B cells engrafted within 7 weeks post-transplant and were detected in 17 of 19 mice (~5 T cells/μL). At 16 weeks post-transplant, 15 of 18 mice maintained detectable B cells. In contrast, human T cells emerged later, beginning at 13 weeks post-transplant. However, even by 16 weeks post-transplant, T cells were only detectable in the peripheral blood of 6 of 18 mice (~5 T cells/μL). These studies provided evidence that ALDH<sup>hi</sup> CD34<sup>+</sup> cells establish both short- and long-term hematopoiesis in NOD/SCID-IL2R<sup>−/−</sup> mice. The emergence of T cells was the most stringent test for long-term engraftment.

235 HUMAN EMBRYONIC STEM CELLS-DERIVED HEMANGIOBLAST EXPRESS HLA-ANTIGENS

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The initial differentiation of vascular endothelium and hematopoietic cells during embryogenesis occurs in the mesodermal layer of the yolk sac, yielding structures called blood islands. We hypothesized that under specific conditions this process can be recapitulated in embryoid body (EB)-derived cell cultures and that such cells will be able to form colonies of functional cells with dual hematopoietic/endothelial potential. We used two-step differentiation technology to obtain the bipotential blast cells from human embryonic stem cells (hES). This involved short differentiation in our EB system followed by differentiation in semisolid culture media supplemented with mixture of cytokines. We have shown that the occurrence of hemangioblast (blast colony-forming cells) – BL-CFC during EB differentiation (day 0–6) is transient and peaks on day 3. The emergence of this event was associated with expression of T (early mesoderm gene), and was suppressed by the development of endoderm layer. Similarly, the highest BL-CFC count was associated with dramatic increase in early hematopoietic/endothelial genes: CD34, CD31 and KDR. The similar pattern of expression of Patch1 and Gli1 genes suggested association with hedgehog (Hh) pathway. BL-CFC colonies were composed of 30–50 blast cells on day 6. These cells had homogenous morphology in Wright-Giemsa stain with a big nucleus containing disorganized chromatin and a narrow rim of cytoplasm filled with large-size granules. As shown by FACS staining, they were heterogenous and expressed markers of early hematopoietic/endothelial potential. We used two-step differentiation technology to obtain the bipotential blast cells from human embryonic stem cells (hES). This involved short differentiation in our EB system followed by differentiation in semisolid culture media supplemented with mixture of cytokines. We have shown that the occurrence of hemangioblast (blast colony-forming cells) – BL-CFC during EB differentiation (day 0–6) is transient and peaks on day 3. The emergence of this event was associated with expression of T (early mesoderm gene), and was suppressed by the development of endoderm layer. Similarly, the highest BL-CFC count was associated with dramatic increase in early hematopoietic/endothelial genes: CD34, CD31 and KDR. The similar pattern of expression of Patch1 and Gli1 genes suggested association with hedgehog (Hh) pathway. BL-CFC colonies were composed of 30–50 blast cells on day 6. These cells had homogenous morphology in Wright-Giemsa stain with a big nucleus containing disorganized chromatin and a narrow rim of cytoplasm filled with large-size granules. As shown by FACS staining, they were heterogenous and expressed markers of early hematopoietic and endothelial cells (CD31, CD34, VE-cadherin, Flt-1) and mature differentiated cells (CD45, CD33, CD146). Some of them expressed fetal and embryonic globin genes as shown RT-PCR. Moreover, they could be characterized by higher expression of HLA class I molecules when compared to hES and EB cells.