geal carcinoma (NPC) and Hodgkin's disease (HD). We have shown in the past that LMP1- and LMP2-specific T-cells can be activated from EBV-seropositive individuals with adenoviral vectors encoding either an inactive form of LMP1 (dLMP1) or LMP2. The aim of this study was to construct and characterize an adenoviral vector containing dLMP1 and LMP2 (Ad_dLMP1-I-LMP2) for the generation of LMP1- and LMP2-specific T cells. **Methods:** To evaluate the activity of Ad-dLMP1-I-LMP2 to reactivate LMP1- and LMP2-specific CTL, we took advantage of the tropism of Ad5F35 adenoviral vectors, which preferentially transduce monocytes after incubation of peripheral blood mononuclear cells (PBMC). Transduced monocytes expressed LMP1 and LMP2, and reactivated LMP1- and LMP2-specific T cells, that could be expanded further with autologous LCL transduced with Ad-dLMP1-I-LMP2. The presence of LMP1- and LMP2-specific CTL was determined by tetramer analysis, IFN-γ ELISPOT assays and cytotoxicity assays. **Results:** Stimulation of PBMC with Ad-dLMP1-I-LMP2 transduced monocytes from 10 HLA-A*2, EBV-seropositive donors resulted in an at least 30 to 100 fold expansion of LMP1- or LMP2-specific T-cells as judged by the presence of HLA-A*2 restricted, tetramer positive T-cells. LMP2-specific responses were detected in all donors where as LMP1-specific responses were found in 7/10 donors. The presence of LMP1- and LMP2-specific T-cells was confirmed in a subset of donors with IFN-γ ELISPOT assays. In addition, generated LMP1- and LMP2-CTL killed autologous fibroblasts expressing LMP1 and LMP2, as well as LCL. **Conclusion:** The constructed adenoviral vector, Ad-dLMP1-I-LMP2, is a promising candidate for the ex vivo generation of LMP1- and LMP2-specific CTL and may also be used as a vaccine for the in vivo boosting of adoptively transferred T cells. Targeting the subdominant LMP antigens expressed in NPC and HD may improve the efficacy of adoptive immunotherapy approaches.

LATE EFFECTS/QUALITY OF LIFE

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LONG-TERM OUTCOME AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION (HCT) FOR CML

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We studied 6548 recipients of allogeneic HCTs performed between 1978 and 1997 to determine long-term rates of overall survival, disease-free survival and relapse. As of December 2002, 2710 of the 6548 had survived for >5 yr, 1926 for >7 yr, 1044 for >10 yr, 212 for >15 yr and 7 for >20 yr. 2234 patients were alive and in continuing remission 5 or more years post-HCT. Of these, the median age at HCT was 34 yrs, 60% had received TBI as part of their conditioning; 67% had received cyclosporine and methotrexate for graft-versus-host disease (GVHD) prophylaxis. Among patients alive and in remission 5 yrs after HCT, the cumulative incidences of subsequent relapse at 15 yrs post-HCT were 17%, 15%, 12% and 7% for recipients of sibling transplants in first chronic phase (CP), recipients of sibling transplants not in first CP, recipients of alternative donor transplants in first CP and recipients of alternative donor transplants not in first CP respectively. The latest relapse occurred at 16 yr post HCT. Corresponding survival rates at 15 years in the patients were 85%, 83%, 80% and 75%. 174 of the 2234 patients surviving in remission at 5 years post HCT subsequently died; the causes of death were CML (2% of 5 year survivors), GVHD (1%), second cancer (<1%), infection (1.4%), organ failure (<1%) and other (1%). We conclude that remissions after allogeneic HCT are generally durable. However, after a high-risk period early post-HCT, there is a low but constant risk of relapse. Rescue strategies in patients with late relapse may include donor cell infusions or imatinib, though few data exist regarding the efficacy of these approaches in this patient group.

LEUKEMIA

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DETECTION OF BCR-ABL EXPRESSION IN HUMAN CML MODEL USING RADIOLABELED ANALOGUE OF ABL-PROTEIN KINASE INHIBITOR AND POSITRON-EMISSION TOMOGRAPHY (PET)

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Introduction of imatinib marked a pivot point in history of treatment of chronic myelogenic leukemia (CML). Specific inhibition of bcr-abl kinase interrupts pathologic signal transduction pathway, terminating tumor growth. To facilitate identification of the patients eligible for imatinib therapy we developed a non-invasive in vivo imaging technique enabling detection of *abl*-kinase expression by PET. A specific *abl*-protein kinase inhibitor (PKI) DV was developed to be radiolabeled. K562—human CML cell line (overexpressing *bcr-abl*) and A431—human GBM cell line (low for *abl*) were chosen as models. Levels of *abl* expression were confirmed by Western blot. In vitro radiotracer accumulation studies were done with [^{131}I]DV. For in vitro studies subcutaneous xenograft tumor models were created in contralateral shoulders of nude rnu/rnu rats. Upon tumor size of 1 cm^3, [^{124}I]DL was injected IV to the rats for 2 sets of scans: 40 μCi for microPET and 200 μCi for clinical PET-Advance. Acquisition was performed in dynamic 10 min frames for 60 min on microPET and 90 min on PET-Advance. After image reconstruction ROIs were analyzed for radiotracer dynamics. MicroPET imaging showed accumulation of [^{124}I]DV in K562 tumor with low background signal in the rest of the animal. Maximal accumulation in early frames appeared to be in projection of liver. Imaging on clinical PET-Advance scanner demonstrated specific accumulation of [^{124}I]DV radiotracer in human CML K562 tumor model over 90 min of imaging session. No accumulation in abl-negative A431 was detected. There was concurrent monophasic clearance of the radiotracer form the circulation. Initial high concentration of the radiotracer in the liver was gradually clearing out with concomitant increase of bowel signal demonstrating hepato-biliary clearance. Non-invasive assessment of *bcr-abl* expression is feasible by PET-visualization using [^{124}I]DV, radiolabeled *abl*-PKI. Detection of *bcr-abl* expression in vivo will not only assist identifying treatment target for imatinib therapy in patients, it might assist monitoring the course of CML therapy with imatinib. This method can also be used as a screening option for selection of patients with abl-overexpression in other types of tumors.

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GM-CSF SECRETING LEUKEMIA CELL VACCINATIONS AFTER ALLOGENEIC NON-MYELOABLATIVE PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN PATIENTS WITH ADVANCED MYELODYSPLASTIC SYNDROME OR REFRACTORY ACUTE MYELOID LEUKEMIA

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Disease relapse is a frequent cause of treatment failure in patients undergoing non-myeloablative allogeneic stem cell transplantation (NST) for advanced myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). GVAX, a cancer vaccine composed of leukemia cells modified by adenoviral vector mediated gene transfer to secrete GM-CSF, has demonstrated activity in MDS and AML. We initiated a trial investigating the feasibility and safety of administering GVAX after allogeneic NST. Patients with MDS-RAEB or AML not in remission with a donor matched at HLA-A,B, and DRB1 were eligible. Leukemia blasts were collected for GVAX generation prior to conditioning. The preparative regimen consisted of fludarabine 30 mg/m^2/d IV days −6 to −3, and Busulfex 0.8 mg/kg IV q12h × 8 doses from days −6 to −3. All patients received G-CSF mobilized PBSC. GVHD prophylaxis included tacrolimus starting day −3, and methotrexate 5 mg/m^2 IV days 1, 3, 6, 11. GM-CSF (Leukine) 250 mg/m^2 SC QD was given

from day +1 until neutrophil engraftment. GVAX was initiated between day +30 to +45 if there was adequate hematologic recovery and no grade II-IV acute GVHD. GVAX was administered ID/SC qwk × 3 doses, then q2wks × 3 doses. Taper of tacrolimus began after vaccine completion. Ten patients (6 URD, 4 MRD) have been transplanted to date: 8 AML, 2 MDS/RAEB-2. Seven had circulating myeloblasts at transplant. GVAX was successfully generated for all 10 patients. Median vaccine cell dose was 1.0×10^7cells (range, 0.4-1.0 × 10^7), and median 24-hr GM-CSF secretion by vaccine cells was 7.25 ng/ml/10^6cells (range <1.0-155.9). Only 4 of 10 patients were able to start vaccination post transplant. Reasons for failure to initiate vaccination included: death before day +30 (1); acute GVHD (2); insufficient count recovery (3). Among those who received GVAX, there was no GVHD or toxicity attributable to vaccination. Focal infiltrates of lymphocytes were observed in skin biopsies of the vaccination sites. Two of four vaccinated patients are alive: 1 in CR, and 1 in relapse 5 and 6 months post transplant, respectively. Overall, 7 of 10 patients have relapsed, 6 before day +100. Although results are preliminary and the high incidence of early relapse has hindered our ability to initiate vaccination, GVAX appears to be safe for patients with MDS/AML after NST. Further cytoreduction prior to NST is necessary for disease control and improve feasibility of GVAX vaccination in this very high-risk population.

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TREATMENT OF PATIENTS (PTS) WITH CHRONIC MYELOID LEUKEMIA (CML) AND IMATINIB FAILURE AFTER DEVELOPING BCR-ABL KINASE MUTATIONS WITH ALLOGENEIC STEM CELL TRANSPLANTATION (ASCT)

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ASCT is curative for many pts with CML, and may be effective after imatinib failure. Resistance to imatinib is most often associated with point mutations in the Bcr-Abl kinase domain. The outcome of pts with Bcr-Abl kinase mutations after ASCT is not known. We assessed the outcome of ASCT in 9 pts with CML (chronic phase [CP] = 3, accelerated phase [AP] = 3, blast phase [BP] n = 3) harboring 8 different protein kinase mutations. P-loop mutations were detected in 4 (44%) pts; T315I mutation was detected in 2 pts (one AP and one CP). Seven male and 2 female pts, median age of 44 years (range, 26-63 years), received their ASCT between June 2003 and July 2005. At the time of ASCT, one pt was in major molecular remission (MMR) (BP, Q252H), one was in major cytogenetic response (CP, T315I), and 2 were in complete hematologic response (2 BP, Y253H and E281A). Preparative regimen was busulfan + cyclophosphamide in 7 and fludarabine + cyclophosphamide in 2 pts. Donor was fully matched related in 4 (44%) and unrelated in 5 (56%) pts. Source of stem cells was peripheral blood and bone marrow in 7 and 2 pts, respectively. Graft-versus-host disease (GVHD) prophylaxis consisted of tacrolimus and mini methotrexate. All patients engrafted; there was no treatment-related mortality. Chimerism studies at day 30 and 100 post ASCT were available in 7 pts and were 100% of donor type. Eight pts achieved a complete molecular remission (CMR); one pt with a T315I mutation achieved a MMR. Two (22%) pts (Q252H [BP] and T315I [AP]) relapsed after a median of 7 months; one of them (T315I) died of disease progression. All the remaining 7 pts were in CMR for a median of 13 months (range, 3-20+ months). We conclude that ASCT remains an important salvage option for pts who develop resistance to imatinib through Bcr-Abl mutations. Early introduction of such strategy may result in better outcome.

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IN VIVO BIOLUMINESCENCE IMAGING OF ACUTE PROMYELOCYTIC LEUKEMIA CELL TRAFFICKING AND MOBILIZATION BY AMD3100

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Novel approaches have been developed to mobilize hematopoietic stem cells (HSC) for patients undergoing autologous and allo transplantation. These strategies may provide insights into improved HSC collection and enhanced egress of leukemic cells and thus sensitivity to anti-leukemia therapy. CXCR4/SDF-1 axis regulates the trafficking of normal HSC to and from the bone marrow (BM). AMD3100 (AMD) specifically and reversibly blocks SDF-1 binding to CXCR4, and is a promising mobilizing agent currently in clinical development. We utilized a mouse model of acute promyelocytic leukemia in which the PML-RAR_α transgene was knocked into a single allele of the murine cathepsin G locus. We transduced banked leukemia cells with a dual function reporter gene that encodes a click beetle red (CBR) luciferase, a bioluminescence imaging (BLI) optical reporter gene, and EGFP for ex vivo cell sorting (CBR/EGFP). We isolated $EGFP^+$ cells using a MoFlo cell sorter, and passaging them in secondary syngeneic recipients that developed rapidly fatal acute leukemia. Upon intravenous (iv) injection of 10^6 APL cells into syngeneic recipients, APL rapidly migrated to the BM, with increased BLI signal in the femurs, spine, ribs, and skull, at 4 days after injection, followed by spleen infiltration and by death due to leukostasis by 14-16 days. To our knowledge, this represents the only mouse leukemia model in which leukemia cells home preferentially to the BM in a manner that is similar to what is seen in human AML. AMD (5 mg/kg) at the time of APL infusion or bid on days 0-7, had no impact on the engraftment of either normal HSC or the PML. We observed rapid mobilization of the APL cells when AMD was administered 11 days after APL injection. 40% of mice that received AMD on day +11 died 2 to 4 hours after AMD injection as a result of the rapid and massive mobilization of blasts. Interestingly, CXCR4 expression in mobilized tumor cells decreased from 33 ± 3% before AMD administration to 19 ± 6%, and 7.8 ± 0.6% after 2 and 12 hours (FACS; $P < .001$). AMD + AraC (200 mg/kg) on day +11 prolonged the overall survival of mice, compared with mice treated only with AraC. In summary, we developed a mouse model to study the APL cell trafficking, and we have shown leukemia cell mobilize from the BM into PB after AMD administration. In these preliminary results we observed that AMD may sensitize APL cells to AraC. We propose that CXCR4/SDF-1 is a key regulator for leukemia migration and homing to the BM.

LYMPHOMA/MULTIPLE MYELOMA

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POSITIVE POSITRON EMISSION TOMOGRAPHY (PET) PRE-AUTOLOGOUS STEM CELL TRANSPLANT (ASCT) IN NON-HODGKIN LYMPHOMA (NHL) DOES NOT PRECLUDE SUCCESSFUL OUTCOME

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PET has become an important imaging modality for lymphoma, and has been reported to be of prognostic significance prior to ASCT for NHL. **Methods:** To assess the prognostic value of PET prior to ASCT in NHL, PET pre and post ASCT was prospectively obtained in all NHL patients. From May 2003 to December 2004, 100 patients underwent ASCT for NHL. PET was considered positive if it had abnormal FDG uptake; CT was positive if it had areas of lymphadenopathy as defined by the international response criteria. **Results:** Median age was 58 (range 17-74); 69% were male. Patients had received a median of 2 prior chemotherapy regimens (range 1-9). At relapse, prior to ASCT, the median IPI was 2 (range 1-5); 31% of patients were stage I or II; 69% were stage III or IV. Histology included DLBCL 50, transformed NHL 15, mantle cell NHL 11, low-grade NHL 8, T-cell NHL 6, primary CNS NHL 5, and high grade NHL 5. At ASCT, 43 patients were in CR, 49 in PR, 3 in untreated relapse, and 5 had resistant disease. The conditioning regimen was BEAM in 89; Zevalin/BEAM in 10. Pre-ASCT PET was not obtained in 8