been in sustained remission for 41.4 to 56.3 months (3.45 to 4.7 years). Four patients died from relapsed disease at a median of 14.9 months (range 7.73-42) post-HCT, and one with failure to achieve platelet engraftment, died from pulmonary hemorrhage at 4.5 months post-HCT.

Table 1. RIC HCT for relapsed AML - Characteristics and Results

	Number (Range)
Total number of patients	10
Age (yrs), median	5.2 (1.8 – 10.6)
Lansky performance score ≥ 90	10
Disease status prior to HCT	
CR1	1
CR2	3
CR3	3
Active disease	3
Neutrophil engraftment (ANC>500) in days, median	16.5 (10-40)
Platelet engraftment (Plts >50,000) in days, median	39 (16-42)
Acute GVHD, grades II-IV	4
Chronic GVHD (extensive)	1
Relapse	4
Time to relapse in days, median	114 (50-144)
Alive at last follow-up	5

Overall survival was 61% (95% CI 37-100%) at 1 year and 51% (95% CI 28-94%) at 3 years post-HCT. Disease free survival was 42% (95% CI 20-87%) at 1 year and 31% (95% CI 13-79%) at 3 years post-HCT. This modest success in our small cohort, indicate that RIC HCT can achieve long term and durable remissions in patients with relapsed AML after first HCT, and offer a potentially life-saving treatment option to this group of high risk patients.

SOLID TUMORS

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IMPROVING T-CELL IMMUNOTHERAPIES FOR SOLID TUMORS BY TARGETING THE TUMOR STROMA

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Background: Recent findings indicate that the tumor stroma is a critical barrier for effective T-cell immunotherapies and that eradicating the tumor stroma in addition to cancer cells has the potential to increase antitumor effects. Cancer associated fibroblasts (CAFs) are the central component of the tumor stroma and express fibroblast activation protein (FAP), which is an attractive immunotherapeutic target since it is not expressed in normal tissues. The objective of this project is to develop an adoptive immunotherapy approach with antigen-specific T cells targeting FAP expressed on reactive tumor stroma in addition to tumor antigens expressed on cancer cells.
Methods: We constructed a FAP-specific chimeric antigen receptor (CAR) using a FAP-specific single chain variable fragment that recognizes murine as well as human FAP. Mitogen activated T cells were transduced with a retroviral vector encoding a second generation FAP-specific CAR with a CD28.zeta-signaling endodomain (FAP-T cells). We then performed functional studies to characterize the generated FAP-T cells.
Results: FAP-T cells recognized and killed K562 cells genetically modified to express FAP in contrast to non-transduced T cells confirming specificity. FAP-T cells also recognized and killed a panel of FAP-positive solid tumor cell lines (head and neck cancer, osteosarcoma, breast cancer, and melanoma). To evaluate if targeting only the tumor stroma *in vivo* leads to a reduction of tumor growth we took advantage of LCLs, which are FAP negative. Luciferase-expreesing LCL were mixed with FAP-T cells, which recognize murine and human (mhFAP) or human FAP (hFAP), or non-transduced T cells prior to s.c. injection into flanks of SCID mice. While LCLs tumor readily established in mice injected with LCL/hFAP-T cells or LCL/NT-T cells; LCL tumors either did not establish or their growth was markedly retarted (10- to 100-fold) in mice injected with LCL/mhFAP-T cells as judged by serial bioluminescence imaging.
Conclusion: We have constructed a FAP-specific CAR that enables the rapid generation of FAP-T cells for targeting CAFs, the central component of the tumor stroma. Targeting CAFs with FAP-T cells resulted in marked reduction in tumor establishment and growth in vivo. Experiments are in progress to evaluate if cotargeting tumor cells and their supporting stroma results in enhanced tumor eradication.

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DOSE-DEPENDENT AND EPITOPE-SPECIFIC IN VIVO IRRADICATION OF THE HUMAN OVARIAN CARCINOMA CELLS EXPRESSING THE WILLMS TUMOR PROTEIN, WT1, IN NOD/SCID MICE, BY WT1 SPECIFIC T CELLS MONITORED BY BIOLUMINESCENT IMAGING

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WT1 is expressed in 60-80% of acute leukemias and ovarian adenocarcinomas. Its expression has been hypothesized to be critical for the growth or survival of tumorigenic stem cells. Previously, alloreactive HLAA0201$^-$ T cells recongizing a complex of WT1-peptide and HLA-A0201 were reported to prevent growth of leukemic HLA-A0201$^+$CD34$^+$Ph$^+$CML progenitor cells in NOD/SCID mice. In this study, we have assessed the capacity of WT1 CTLs lacking the alloreactivity, restricted by different HLA alleles and WT1 derived epitopes to prevent the outgrowth of two human ovarian adenocarcinoma cells (OvCar) with low (13%) and high (43%) expression of WT1 by FACS in NOD/SCID mice. For this study epitope-specific and HLA restricted WT1-CTLs were generated from PBMC of four normal donors by in vitro sensitization with autologous EBV transfomed B cells (BLCL) loaded with the pool of 141 15-mers overlapping by 11aa and spanning the entire sequence of WT1 protein. Each of the T cell lines was pre-incubated in vitro for 8 hours at different E:T ratios (0:1, 5:1, 10:1, 50:1, 100:1) with 0.05x10^6 OvCar tumor cells transduced to express a luciferase reporter gene. The cell mixtures were injected i.p. into NOD/SCID mice. Tumor growth was monitored weekly by the intensity of the bioluminescent signal. In all animals injected with the tumor cells alone the bioluminescent signal could be detected in the abdomen by day 10-15 and increased steadily through 60 days of observation (when all these mice died) while tumor engraftment was either markedly inhibited (in OvCar^{WT1low}) or completely abrogated (in OvCar^{WT1high}) by pre-incubation at 100:1 E:T ratio and correlated with higher survival of the animals (80%) over a period of 120 days. The animals injected with the tumor cells preincubated with the WT1-CTLs at 50:1 and 10:1 E:T ratio demonstrated weaker bioluminescent signal in the abdomen which increased over the course of the study while the pre-incubation of the tumor cells with WT1-CTL at 5:1 ratio did not significantly affect tumor engraftment and growth. The inhibition of tumor growth was more efficient with the WT-CTL specific for the $_{398\text{-}506}$LKTHTTRTHT epitope presented on the A0201 allele as compared to the A0301 or B0702 epitope ($_{(-125)\text{-}(-117)}$ RQRPHPGAL). These results suggest that tumorigenic OvCar cells containing high propotion of WT1$^+$ cells are succeptible to eradication in vivo by high doses of WT1-CTL specific only for some but not every WT1 epitope selected by T cells.