screened 12 OS and EWS cell lines for HER2 expression by FACS analysis and analyzed the ability of HER2-T cells to 1) proliferate, 2) produce immunostimulatory cytokines, and 3) kill HER2+ tumor cells in cytoxicity assays upon exposure to HER2+ OS and EWS cell lines. The *in vivo* function was tested in a murine intraperitoneal xenograft model, which allows for serial imaging by bioluminescence with representative OS (LM7) and EWS (TC71) cell lines. Imaging results were confirmed by pathological examination. **Results:** 10 of 12 cell lines were HER2+ by FACS analysis. HER2-T cells recognized and killed all HER2+ OS and EWS cell lines in cytotoxicity assays, whereas HER2-negative tumor cells were not killed. Coculture of HER2-T cells with HER2+ tumor cell lines resulted in T-cell proliferation, and secretion of IFN-γ and IL-2 in a HER2-dependent manner. *In vivo*, HER2-T cells eradicated established intraperitoneal xenografts in 80% of animals harboring LM7 and 70% of those harboring TC71 tumors, resulting in long-term tumor free survival of treated animals. In contrast, delivery of non-transduced T cells did not change the tumor growth pattern. **Conclusions:** This study shows that HER2 is a target antigen for adoptive immunotherapy of OS and EWS. HER2-redirected T cells not only recognized and killed HER2+ tumor cells *ex vivo*, but also eradicated experimental xenografts *in vivo*. Hence, adoptive transfer of HER2-redirected T cells may represent a promising immunotherapeutic approach for OS and EWS.

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OPTIMIZING IMMUNOTHERAPY IMMEDIATELY AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) TO EFFECTIVELY TREAT ESTABLISHED NEUROBLASTOMA

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Based on our previous success at inducing "protective" immunity to neuroblastoma immediately after syngeneic HSCT using a combination of adoptive immunotherapy and tumor vaccination, we hypothesized that a similar immunotherapeutic approach would be able to effectively treat mice with established tumors. Mice bearing established AGN2a (neuroblastoma) tumors were given lethal total body irradiation followed by HSCT consisting of bone marrow supplemented with 6×10^6 T cells. On days 2, 7, and 14 after HSCT, mice were vaccinated with irradiated AGN2a cells that had been genetically modified to express CD54, CD80, CD86, and CD137L. Bioluminescent imaging and caliper measurements were used to monitor tumor growth. Our best results – delayed tumor growth in all mice and 70% tumor-free survival – were seen when mice were given HSCT, T cells derived from tumor-vaccinated syngeneic donors (pre-sensitized T cells), and vaccination. Vaccination was an important component of the immunotherapy in these mice, as only 36% of mice given pre-sensitized T cells only (no vaccine) survived long-term. Several of the mice given pre-sensitized T cells without vaccination had late tumor recurrences, which were not observed in vaccinated mice. This may reflect the requirement of a vaccine-based approach for the induction or maintenance of long-term immune memory. Mice given HSCT, adoptive T cell transfer from naïve mice, and vaccination also showed delayed tumor growth, but tumor-free survival was lower (33%). Overall survival correlated with the frequency of IFN-γ-producing, tumor-reactive CD8+ cells in lymphoid tissues of treated mice. Moreover, we demonstrate that mice with regressing tumors had higher frequencies of tumor-reactive T cells in their lymphoid tissues than mice with progressing tumors. When specific immune cell subsets were depleted from the adoptively transferred T cells two important observations were made. First, CD4+ T cell depletion was found to initially benefit tumor-bearing hosts, but the generation of anti-tumor T cell memory was inhibited. Second, more specific depletion of CD4 + 25+ cells from the adoptively transferred T cells can result in better tumor immunity without decreasing anti-tumor T cell memory. In summary, our results suggest that a multi-faceted immunotherapeutic strategy including HSCT, T cell adoptive transfer and early post-transplant tumor vaccination can effectively treat mice with established neuroblastoma.

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HIGH-DOSE CHEMOTHERAPY WITH AUTOLOGOUS STEM-CELL SUPPORT VERSUS STANDARD-DOSE CHEMOTHERAPY FOR HIGH-RISK BREAST CANCER: META-ANALYSIS OF INDIVIDUAL PATIENT DATA FROM 15 RANDOMIZED ADJUVANT TRIALS

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Background: The role of adjuvant high-dose chemotherapy (HDC) with autologous hematopoietic stem-cell transplantation for primary breast cancer at high risk of recurrence (at least 4 involved axillary lymph nodes) remains ill-defined. Data from individual trials have limited power to show overall or subgroup benefit for this indication. **Methods:** Individual patient data from 15 known randomized trials (HDC vs standard-dose chemotherapy [SDC]) were merged into a single database. Disease-free survival (DFS) was defined as time from surgery to recurrence or death; breast cancer-specific survival (BCSS) was time from surgery to death from breast cancer or treatment-related toxicity; and overall survival (OS) was time from surgery to death. Cox proportional hazards regression was used to compare the effect of HDC vs SDC on DFS, BCSS, and OS adjusted for age, trial, hormone receptor (HmR) status (positive if either estrogen or progesterone receptor was positive), and menopausal (MP) status. Subgroup analyses were by age, HmR and MP status. **Results:** Median follow-up for 6,210 patients (3,118 HDC, 3,092 SDC) was 6 years (range, 0–15.3); median patient age was 46 years (range, 20–67). HmR status was positive in 46.8% of patients, negative in 23.7%, and unknown in 29.5%; 68.9% were premenopausal, 29.4% were postmenopausal, and 1.7% was unknown.

After adjusting for age, trial and MP, HDC was found to prolong DFS (hazard ratio [HR] 0.87; 95% confidence interval [CI] 0.81–0.94; P = 0.0002) but not BCSS (HR 0.93; 95% CI 0.85–1.02; P = 0.12) or OS (HR 0.95; 95% CI 0.87–1.03; P = 0.18). After adjusting for HmR in the subset for which that information was available, HDC was found to prolong DFS (HR 0.83; 95% CI 0.77–0.90; P < 0.0001) and had modest but significant benefits on BCSS (HR 0.88; CI 0.79–0.97; P = 0.014) and OS (HR 0.89; 95% CI 0.81–0.98; P = 0.016) compared to SDC. For BCSS and OS, none of age, MP or HmR had a significant interaction with treatment, yet there was a significant age by treatment interaction for DFS, in which HDC was better for younger rather than older patients. **Conclusions:** HDC as used in these 15 randomized studies prolongs DFS in adjuvant breast cancer. HDC has at most a modest benefit on BCSS and OS compared to SDC. Whether HDC has benefit in the context of contemporary taxane-based regimens and targeted therapies is unknown and may be resolved by future clinical trials.

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KIR RECEPTOR-LIGAND INCOMPATIBILITY PREDICTS SUSCEPTIBILITY OF OSTEOSARCOMA TO NK-MEDIATED LYSIS

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Long-term survival for pediatric patients with high-risk solid tumors remains poor despite multi-modality treatment, and new approaches are needed. Although the success of KIR (Killer Immunoglobulin-Like Receptor) incompatible, haploidentical stem cell transplantation has been suggested in hematological malignancies in adults and children, it has not been thoroughly examined for pediatric solid tumors. In this study, we evaluated the potential for KIR-incompatible lysis of osteosarcoma cells, in vitro. We hypothesized that the killing of osteosarcoma cell targets could be predicted by the degree of KIR receptor/ligand mismatch. To test this hypothesis, healthy donor NK cells were isolated and KIR phenotype determined by flow cytometry. Consistent with previous studies, donor NK cells exhibited a high prevalence of all three

relevant inhibitory KIRs (KIR2DL1, KIR2DL2/KIR2DL3, KIR3DL1). Conversely, examination of three established osteosarcoma cell lines (HOS, SaOS, and U2OS) demonstrated significant variability in cell surface KIR ligand expression (HLA-C groups 1 and 2, HLA-Bw4) by flow cytometry and qRTPCR. Following a 12-hour incubation of donor NK cells with IL-2, lysis of osteosarcoma targets was measured in an annexin V flow cytometric assay. Greater KIR receptor-ligand incompatibility was predictive of higher killing of osteosarcoma cells. These findings were consistent using NK effectors from different donors. Additionally, we observed that at high passage number (>20), SaOS cells demonstrated down-regulation of KIR ligand expression. These changes correlated with increased lysis by the same donor NK cells. Our findings suggest: 1) Variable expression of KIR ligands in osteosarcoma allows potential susceptibility to KIR-incompatible, NK cell-mediated lysis; 2) The killing of osteosarcoma cells by NK cells can be predicted by the degree of receptor/ligand mismatch; and 3) During expansion, osteosarcoma cells may down-regulate expression of KIR ligands, resulting in increased susceptibility to lysis by KIR-incompatible NK cells. Further studies are needed to explore the utility of KIR-incompatible, haploidentical stem cell transplantation for patients with recurrent or metastatic osteosarcoma.

Patient Features

Age (yrs)	Diagnosis	Ultra high-risk features	Number of prior treatment regimens	Disease status at on-study/SCT	Day 28–42 CD4 count/mm3	Time to relapse/PD (days)	Survival post-SCT (mos)
20	RMS	MR	5	PD/PR	253	100	34.0
19	RMS	MR	3	PD/SD	442	42	4.3
10	EWS	ER, MR	2	PD/SD	324	28	3.6
14	EWS	ER, B/BM	3	PD/SD	335	100	5.8
18	RMS	B/BM	2	PD/SD	443	100	14.5
21	EWS	PD, B/BM	1	PD/SD	226	160	20.7
18	RMS	MR	2	PD/SD	176	60	7.3
29	EWS	B/BM	2	PD/SD	294	100	24.8
12	RMS	MR	3	NED/NED	205	100	35.2*
15	RMS	B/BM	2	PD/SD	241	70	13.3
8	EWS	B/BM	1	NED/NED	152	NED	8.9[£]
19	EWS	PD	1	PD/PD	481	42	32.2*
25	EWS	B/BM	2	PD/PD	148	100	14.9
25	DSRCT	Metastatis§	2	NED/NED	903	150	28.6*
18	EWS	B/BM	3	PD/PD	1003	38	4.4
15	EWS	B/BM	1	NED/NED	171	NED	19.0*,[£]
21	EWS	B/BM	1	PD/PR	346	100	11.5*
14	DSRCT	Metastatic§	2	NED/NED	463	NED	7.6*,[£]
Median 17.8					Median 309		Median 13.9

Key: RMS - rhabdomyosarcoma; EWS - Ewing sarcoma; DSRCT - desmoplastic small round cell tumor; MR - multiply recurrent; ER - early relapse within 1 year of diagnosis; B/BM - bone or bone marrow metastasis at diagnosis; PD - progressive disease; SD - stable disease; PR - partial response; NED - no evidence of disease; mos - months; yrs - years

§Any metastatic site at diagnosis for DSRCT.

*Alive.

[£]No evidence of disease.

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PROLONGED SURVIVAL FOR ULTRA HIGH-RISK PEDIATRIC SARCOMA PATIENTS FOLLOWING REDUCED-INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION (ALLOSCT)

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Background: Pediatric sarcomas with ultra high-risk features have overall survival rates of <20% and autologous transplantation has not substantially improved outcome. AlloSCT can cure pediatric hematologic malignancies, in part due to a graft versus tumor (GVT) effect. The use of myeloablative alloSCT in sarcoma has historically been limited by excessive toxicity. We piloted a reduced intensity alloSCT (RISCT) regimen in pediatric sarcoma patients. **Methods:** 18 patients with ultra-high risk features (Table) were transplanted. Induction chemotherapy was administered for disease control and targeted CD4 count reduction. Pre-transplant conditioning consisted of cyclophosphamide 1,200 mg/m^2/day × 4, fludarabine 30 mg/m^2/day × 4 and melphalan 100 mg/m^2 × 1. Grafts were G-CSF mobilized unmodified peripheral blood stem cells from 5–6/6 HLA-matched siblings. An initial cohort (n = 13) received cyclosporine for GVHD prophylaxis and a second (n = 5) received tacrolimus and sirolimus. **Results:** The regimen was well tolerated with no transplant related mortality. All patients had rapid engraftment and achieved full donor chimerism by post-SCT day (D+) 28. Immune recovery was surprisingly brisk (median CD4+ count 309/mm^3 D + 28–42). 15/18 recipients developed aGVHD: 13 grade 1–2 and 2 grade 3. 13/13 patients surviving > 100 days receiving cyclosporine as a single agent for GVHD prophylaxis developed cGVHD whereas only 2/5 in the tacrolimus/sirolimus cohort have developed cGHVD. Median survival from initial diagnosis is 35 months and from SCT is 13.9 months. Currently 6 patients are alive, 2 with no evidence of disease. Despite early relapses, many patients experienced prolonged survival and apparent improved responsiveness to chemotherapy, suggesting a change in the natural history of these aggressive tumors following alloSCT. **Conclusions:** Ultra high-risk sarcoma patients tolerated RISCT well and experienced brisk engraftment with kinetics similar to myeloablative regimens. Alteration of GVHD prophylaxis appears to have decreased GVHD rates and severity. Median survival compares favorably with outcomes in similarly ultra high-risk patients who did not undergo SCT. Given the rapid immune recovery and favorable survival, this regimen appears to be a promising platform for post-transplant immune-based therapy in pediatric sarcoma patients.

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LONG-TERM CLINICAL OUTCOME OF HIGH DOSE CHEMOTHERAPY WITH AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION (HDCT/ASCT) FOR BREAST CANCER PATIENTS

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Background: Previous studies have shown that HDCT/ASCT offer no survival advantage to standard treatment modality for breast cancer in a short follow-up group. We have followed breast cancer patients with HDCT/ASCT in a long-term follow up (median follow up 114.5 months, ranges 79–142). **Method:** Forty-seven consecutive patients who received HDCT/ASCT for treating breast cancer at Ajou University Hospital between Dec. 1995 and Mar. 2001 were reviewed retrospectively. Overall survival (OS), progression free survival (PFS) and other clinical data were calculated according to the disease status at transplant. Student's t-test was done for statistics. **Result:** The median age was 43 years (ranges 27–59). Out of 47 patients, 11 were high risk for relapse, 9 were sensitive relapse, 22 were refractory relapse and 5 were primary metastatic disease before transplant. In high risk group, 6 patients were stage IIIA, 5 were II with intensive lymph node infiltrations. Patients with primary metastatic cancer or refractory relapse showed poor outcome (20% and 4.6% at 100 months, respectively). High risk group maintained survival of 63.6% after 120 months whereas sensitive relapse group showed 33.3% at 80 months. In high risk group, TNM stage did not affect on OS or PFS, but ER/PR positive group showed survival advantage over double negative group (p = 0.0019), whereas C-erbB2 status did not affect the survival. **Conclusion:** Long-term follow up of clinical data suggests that HDCT/ASCT offers as an alternative treatment modality for breast cancer especially in case of high risk for relapse group with ER/PR positive. **Key Words:** Breast Cancer, Myeloablative therapy, Hematopoietic Stem Cell Transplantation.