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AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN UNTREATED FIRST RELAPSE (REL1) OR FOLLOWING RE-INDUCTION CHEMOTHERAPY (CT) FOR PATIENTS (PTS) WITH ACUTE MYELOGENOUS LEUKEMIA (AML)

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The timing of ASCT for AML after first relapse is controversial. The purpose of this study was to review our results in AML pts in first relapse by comparing outcomes between pts taken directly to ASCT and pts receiving re-induction chemotherapy prior to ASCT. Between January 1990 and March 2003, 37 pts with AML in REL1 or CT underwent ASCT. Survival was analyzed using the Kaplan-Meier (KM) methodology with statistical comparisons using the log-rank test. Univariate predictors of survival were examined using Cox proportional hazard models. Of the 37, 25 and 12 pts underwent ASCT at CT and REL1 (median age 46 vs 42 years), respectively. There was only 1 pt in the CT group who had refractory disease. In the CT group, 18 pts (72%) and 7 pts (28%) had stem cells harvested during CR1 and CR2, respectively. In the REL1 group, all pts had stem cells harvested in CR1. Thirty of 37 pts received cyclophosphamide and total body irradiation (Cy/TBI) for conditioning and the remainder received busulfan and cyclophosphamide (Bu/Cy). There were no statistically significant differences in the baseline characteristics between the two groups. The percentage of bone marrow blasts at first relapse was increased in the CT versus the REL1 group (median 40% vs 15%, p=0.06). KM analysis showed that there was no significant difference between the two groups in time to neutrophil and platelet engraftment. The REL1 group had a significantly shorter time to relapse than CT. The CT group had significantly higher DFS than REL1 and a trend toward better OS with no difference in TRM. The Table details estimated probabilities of these endpoints. Univariate Cox proportional hazard analysis for overall survival showed that younger age at diagnosis and transplantation, unfavorable cytogenetic abnormalities at diagnosis and relapse were significantly associated with a higher risk of death. Higher percentage of blasts in bone marrow at first relapse and shorter duration of CR1 were not significantly associated with death. In a multivariate model, only unfavorable cytogenetic abnormalities at first relapse were significantly associated with an increase risk of death. Although OS between CT and REL1 groups was not significantly different, the time to relapse and DFS were significantly longer for the CT group. Although retrospective and limited by selection bias, this study suggests that there may be an advantage for re-induction chemotherapy for AML in first relapse before ASCT.

	REL1	Post CT	p value*
Probability of Relapse (%)			**0.0075**
1 year	**83.3**	**45.8**	
5 years	**83.3**	**59.9**	
Median time to relapse (mos)	**6.1**	**13.7**	
Overall Survival (OS) (%)			**0.09**
1 year	**41.7**	**68.0**	
5 years	**8.3**	**40.8**	
Median time of OS (mos)	**8.7**	**17.9**	
Disease free survival (DFS) (%)			**0.006**
1 year	**16.7**	**52.0**	
5 years	**8.3**	**39.0**	
Median time of DFS (mos)	**6.1**	**13.7**	
Transplant related mortality (TRM) (%)			**0.50**
1 year	**0**	**4.0**	

*Log rank test comparing distribution of event times over multiple comparisons.

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HIGH AVIDITY CYCLIN E1-DERIVED PEPTIDE-SPECIFIC CTL KILL LYMPHOID LEUKEMIA CELLS AND CROSS-RECOGNIZE A HOMOLOGOUS CYCLIN E2-DERIVED PEPTIDE

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Using a similar strategy that was used to identify PR1 as a leukemia-associated antigen (LAA), we identified two homologous HLA-A2-restricted peptides from cyclin E1 (CCNE1M) and cyclin E2 (CCNE2L) that could be used to elicit peptide-specific CTL from healthy donors. The two peptides differ by a single amino acid at position 7 and have equal binding affinity for HLA-A2, and each elicited peptide-specific CTL with equal efficiency. Because each CCNE1M- and CCNE2L-CTL clone, derived from limiting dilution, cross-recognized the other homologous peptide, we hypothesized that each clone would efficiently kill leukemia that over-expressed either or both CCNE1 and CCNE2 proteins. Sorted high avidity CTL showed higher specific lysis of peptide-pulsed T2 than did low avidity CTL (38.8% vs 31.9% specific lysis, respectively, at E:T 10:1, p = 0.02). The fluorescence decay of tetramer dissociation (ln (peptide/HLA-A2 tetramer)) over time was linear for each clone, showing that avidity was proportional to TCR affinity and tetramer dissociation t1/2 was determined based on first order kinetics. CCNE1M-CTL had higher affinity for CCNE1M/HLA-A2 (CCNE1/A2, t1/2=84.5 min; CCNE2/A2, t1/2=25.3 min) and preferentially killed CCNE1M-pulsed T2 cells (CCNE1, 56.9% vs CCNE2, 38%, respectively, at E:T 10:1). CCNE2L-CTL also had higher TCR affinity for CCNE1M/HLA-A2 (CCNE1/A2, t1/2=29.5 min; CCNE2/A2, t1/2=10.7 min), but showed only slightly higher specific lysis of CCNE1M-pulsed T2 cells (CCNE1, 49.3% vs CCNE2, 44.2% specific lysis, respectively, at E:T 10:1). Each clone specifically lysed HLA-$A2^+$ T-ALL leukemia cells in proportion to both CCNE1 and CCNE2 protein overexpression (CCNE1M-CTL, R^2=0.89; CCNE2L-CTL, R^2=0.88) in an HLA-A2-restricted manner. Both the high and low affinity clones showed equal lysis of T-ALL cells that expressed large amounts of each protein (CCNE1M-CTL, 24.3% vs CCNE2L-CTL , 23.8%, at E:T 10:1). However, high affinity CCNE1M-CTL killed T-ALL cells significantly better than low affinity CCNE2L-CTL (16.8% vs 6.6% lysis, respectively, at E:T 10:1) when the T-ALL expressed a 2.5-fold lower amount of both CCNE1 and CCNE2 proteins. We conclude that the CCNE1M and CCNE2L homologous self-peptides are lymphoid LAA. Furthermore, while the higher TCR affinity of CCNE1M-CTL suggests that the CCNE1M peptide is the more dominant epitope, ultimate target susceptibility is enhanced due to degeneracy of the resulting CTL clones against homologous peptide epitopes.

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NON-MYELOABLATIVE CONDITIONING FOR ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH ACUTE MYELOID LEUKEMIA. THE IMPACT OF THE REMISSION STATUS

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Background: Multicentre randomized trials have shown that allogeneic hematopoietic stem cell transplantation (HSCT) is the most effective strategy for preventing relapse in patients in first complete remission (CR) of acute myeloblastic leukemia (AML), offering up to 50% disease-free survival. However in adult patients with good risk AML, allogeneic HSCT is usually indicated in second complete remission or first untreated relapse. We analyzed the outcome of 16 AML patients who received a reduced intensity conditioning regimen for allogeneic HSCT in first or second remission. **Patients and Methods:** Sixteen AML patients (1 M1, 8 M2, 3 M3, 2 M4 and 2 M5), 9 in first CR (FCR) and 7 in second CR (SCR) were included. All patients received Busulfan 4 mg/kg/d/2 days, Fludarabine 30 mg/m^2/d/3 days and cyclophosphamide 350 mg/m^2/d/3 days as conditioning regimen. The median age was 32 years (range 3–56) in both groups. The source of hematopoietic stem cells (HSC) was peripheral blood and donors were HLA

matched siblings in all instances. The median number of $CD34^+$ cells infused was 5.2×10^6/kg in both groups. All patients received cyclosporine and methotrexate as GvHD prophylaxis. Eleven patients (68%) were transplanted as an outpatient. **Results:** The range of follow-up from date of transplantation was 4–60 months. All patients showed myeloid engraftment (neutrophils $>0.5 \times 10^9$/l) after a median of 15 days, and platelet recovery $>20 \times 10^9$/l was achieved after a median of 13 days. Acute GVHD was observed in 7 (43.7%) patients and chronic GVHD in 8 (50%) patients, without significant differences in both groups. The relapse incidence and mortality for the 9 patients who were transplanted in FCR was 22%, and for patients transplanted in SCR was 85%. Death occurred because of relapse in all cases. **Conclusions:** Our data show that reduced-intensity conditioning and allogeneic HSCT can induce stable remission in AML patients transplanted in FCR. More studies are needed to determine the role of non-myeloablative conditioning and transplantation in patients with refractory or relapsing AML.

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HEMATOPOIETIC CELL TRANSPLANTATION FROM UNRELATED AND RELATED DONORS AFTER A FLUDARABINE-ALKYLATOR-BASED REGIMEN IS A REASONABLE TREATMENT OPTION FOR OLDER PATIENTS (>60 YEARS) WITH ACTIVE HEMATOLOGIC MALIGNANCIES

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The upper age limit for allogeneic hematopoietic cell transplantation (aHCT) used to be in the range of 50 to 55 years, well below the age with the highest incidence of myeloid malignancies. Here we present retrospective data on 69 consecutive patients (pts) older than 60 years of age (median 64; range 60–74) transplanted from a matched sibling (n=20) or unrelated (n=49) donor after conditioning with a uniform, dose-adapted, myeloablative conditioning regimen consisting of fludarabine 150 mg/m^2, carmustine 300 mg/m^2 and melphalan 110 mg/m^2 (FBM). GvHD prophylaxis consisted of cyclosporine A and mycophenolate mofetil with additional rabbit anti-T lymphocyte globulin (ATG) in the unrelated setting. Apart from 5 pts. receiving marrow all other pts received filgastrim mobilized peripheral blood grafts (PBHCT). Pts presented with CLL (3), follicular NHL (2), mantle cell NHL (2), multiple myeloma (1), myeloproliferative syndrome (3), MDS-RA (3) and AML or MDS-RAEB (n=55). Apart from 3 pts in CR1 and 3 in CR2, all other had active disease, either refractory or untreated. Acute GvHD >II° occurred in 10 related (50%) and 16 unrelated (45%) transplanted pts. In the related transplantations, 6 pts died due to TRM (30%) and 4 due to relapse (20%). Causes of death were infections (4 pts), acute GvHD (1 pt) and infection in the context of chronic GvHD (1 pt). In the unrelated transplantations, there were 8 deaths due to TRM (16%) and 6 due to relapse (12%). Causes of death after unrelated aHCT were graft failure (1 pt), ARDS (1 pt), infections (4 pts), acute GvHD (1 pt) and myocardial infarction (d +230 after aHCT, 1 pt). After a median follow up of approx. 1 year (range 3–1778 days), the estimated probability of overall survival was 46% and 61% for related and unrelated transplantations, respectively. This difference was not statistically significant. We conclude, that aHCT using the dose-adapted FBM protocol is a reasonable treatment option for pts older than 60 years of age with high risk lympho-hematologic malignancies. In contrast to previous reports from transplant registries, we found that aHCT from unrelated donors incorporating in vivo ATG results in outcomes comparable to HCT from related donors. We suggest that the lack of HLA identical relatives in older pts should not discourage the transplant choice.

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DYSREGULATION OF THE γδ T CELL REPERTOIRE IN PATIENTS WITH ACUTE LEUKEMIA: IMPLICATIONS FOR CELLULAR IMMUNOTHERAPY

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Introduction: Despite their relatively small numbers, γδ T cells have significant innate anti-tumor activity. This is principally due to their ability to respond, without prior exposure or priming, to a broad array of stress-associated antigens commonly displayed on malignant cells. Our laboratory has shown increased allogeneic γδ T cells to have a protective effect against relapse in patients transplanted for leukemia, and that γδ T cells are not associated with increased risk of graft versus host disease in these patients. In order to gain insight into the nature of a potential antileukemic effect of γδ T cells, we sought to determine if evidence existed for an autologous γδ T cell immune response in pre-treatment leukemia patients. **Methods:** We first examined the γδ T cell receptor (TCR) phenotype and complementarity determining region 3 (CDR3) cDNA sequence from 17 leukemia patients and 3 healthy controls to determine the γδ TCR repertoire and clonality. Following this, we cultured γδ T cells from healthy first degree relatives with the corresponding patients' primary leukemic blasts. We then examined γδ T cell proliferation and TCR repertoire at weekly intervals and cytotoxicity to patient blasts after 3–4 weeks of culture and compared these results with co-cultures of γδ T cells from healthy volunteers and normal allogenic mononuclear cells (MNC). **Results:** All patients and healthy volunteers had normal absolute γδ T cell counts. In 15/17 patients, $V\delta1^+$ γδ T cells were predominant, in contrast to $V\delta2^+$ predominance in healthy volunteers. Vδ1 CDR3-region cDNA sequence analysis revealed that leukemia patients exclusively used the Jδ1 constant region, with healthy controls showing both Jδ1 and Jδ2 use. Two patients with APL showed significant CDR3 conservation as did one B-ALL and one T-ALL patient. When fresh allogeneic γδ T cells were cultured with primary patient blasts, $V\delta2^+$ T cells were quickly deleted and $V\delta1^+$ T cells proliferated and in 4 instances were able to lyse primary blasts. Insignificant γδ T cell proliferation was observed in cultures with normal MNC. **Conclusions:** These findings point to a complex dysregulation in the γδ T cell compartment in leukemia patients including deletion of the Vδ2 subtype *in vitro* and *in vivo* and a $V\delta1^+$ T cell response to leukemia *in vivo*. These findings suggest consideration of allogeneic γδ T cell therapy as part of a comprehensive immunotherapeutic strategy for acute leukemia.

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GEMTUZUMAB-OZOGAMICIN (GO; MYLOTARG®) AS PART OF CONSOLIDATION THERAPY FOR AML BEFORE AUTOGRAFT: LOW INCIDENCE OF HEPATIC VENO-OCCLUSIVE DISEASE

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Background: Gemtuzumab-Ozogamicin (GO) has been used successfully in induction therapy for *de novo* acute myeloid leukemia (AML). Several reports note a high incidence of hepatic veno-occlusive disease (VOD) when given within 115 days of transplant, particularly in allogeneic transplant recipients. Data are limited in the autologous transplant setting. **Methods:** In an ongoing ECOG (E1900) study for *de novo* AML in patients less than 61 years of age, GO was given as consolidation therapy to 23 patients median (range) age 51 (21–59) years who achieved complete remission after induction with cytarabine 100 mg/m^2/day CI over 7 days plus daunorubicin 45–90 mg/m^2/day for 3 days. Two separate consolidations then were given using cytarabine 3 g/m^2 × 6 doses. Upon recovery from the second cycle, patients were given one dose of GO 6 mg/m^2. Upon recovery of blood counts after GO, patients underwent an autotransplant conditioning regimen of IV busulfan 0.8 mg/kg × 16 doses and cyclophosphamide 60 mg/kg × 2 doses with autologous blood stem cell support. **Results:** One patient developed VOD after the administration of GO but fully recovered without further sequelae. The remaining 22 patients have proceeded to transplant. All have engrafted after their procedure with no patient experiencing VOD within 100 days post-transplant. **Conclusion:** GO is a safe consolidation approach in younger AML patients with less than 5% developing VOD with its use when