developed grade II to IV AGVHD, and 15 (20%) developed grades III to IV AGVHD. In univariate analysis, advanced diseases, donor and recipient age were significant risk factors for developing AGVHD. On the other hand, omitting day 11 MTX, Class I and/or Class II HLA allele disparities, CMV seropositivity, or sex mismatch were not identified as risk factors. The mean blood level of tacrolimus during the first three weeks were significantly lower in those who developed grade II to IV or III to IV AGVHD than that of patients who did not (17.5 vs.15..8 vs.15.3 $p = 0.007$, $p = 0.015$), and the level >15ng/ml during the third week was associated with the occurrence of moderate or severe AGVHD($p = 0.023$). In multivariate analysis, only the mean concentration of tacrolimus remained as a risk factor to develop moderate to severe AGVHD. The incidence of nephrotoxicity (doubling of serum creatinine) or hyperglycemia (BS > 200mg/dl) did not significantly correlate with the mean blood level of tacrolimus until three weeks after transplant; however, the incidence of those events significantly correlated with the tacrolimus blood level of a week before the events thereafter ($p = 0.0001$). These data suggest the importance of maintaining tacrolimus blood level above 15 ng/ml during the first three weeks after transplantation to optimize the efficacy while minimize its side effects.

## 117

### THERAPEUTIC ANTIBODY MEDIATED DEPLETION OF ACTIVATED DENDRITIC CELLS AND THE PREVENTION OF GRAFT VERSUS HOST DISEASE

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Graft versus host disease (GVHD) is the most common complication of haemopoietic stem cell transplantation and contributes significantly to the morbidity and mortality of the procedure. Current GVHD prevention targets donor T lymphocytes involved in recipient tissue destruction. This strategy is associated with long term immunosuppression, loss of the graft versus leukaemia effect and graft failure. Dendritic cells (DCs) are a leucocyte population of antigen presenting cells (APC) which, when activated, stimulate the donor T lymphocyte attack in GVHD and as such, represent a possible alternative therapeutic target. To investigate the effect of DC depletion in GVHD, we used an established mouse-human chimeric model of GVHD in which human T lymphocytes are known to be effector cells. Briefly, severe combined immunodeficient (SCID) mice were injected with whole or depleted human PBMC, causing a GVHD-like syndrome that was measured by mouse survival and human cell engraftment. Preliminary data suggests that human and not murine APC are required to induce GVHD. Contrary to a previous report, human B lymphocytes do not appear to contribute to GVHD in the mouse. We found that SCID mice injected with human PBMC that had been depleted, *in vitro*, of activated DC with CMRF-44 antibody survived longer than undepleted controls ($p < 0.05$). Work is currently underway to achieve and assess *in vivo* depletion of human DC in this model.

## 118

### AN EFFICACY OF INTERFERON-α (IFN-α) AND INTERLEUKIN-2 (IL-2) IN THE TREATMENT OF HEMATOLOGICAL MALIGNANCIES RELAPSE-PERSISTENCE-PROGRESSION AFTER THE ALLOGENEIC NONMYELOABLATIVE STEM CELL TRANSPLANTATION (NSCT)

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**Introduction:** GvL reaction belongs to the key mechanisms of tumour control. Its realisation depends on following circumstances: 1) the tumour cells express antigens identified by donor T-cells, 2) the engraftment of donor T-cells, 3) the proliferation activity of tumour and 4) its burden. IFN-α and IL-2 belong to the cytokines allowing to enhance the GvL effect, unfortunately, as well as the risk of GvHD. We present an experience with the IFN-α and IL-2 administration after allogeneic NSCT in 3 patients suffering from the different haematological malignancies. **1st case:** 45-years old male with refractory peripheral T-cell lymphoma underwent NSCT (fludarabin+busulphan+ATG) from MUD. He achieved the complete donor chimerism and the CR of disease. A prophylactic immunosupression was stopped on day +100 without the development of GvHD. 10 months after NSCT the skin relapse of lymphoma was proven and the treatment with cytokines was initiated. IL-2 (3 MIU t.i.w.) has been added after 3 weeks of IFN-α (3 MIU t.i.w.) only. At least 25% resolution of skin involvement was observed 3 months after the start of cytokines administration. **2nd case:** 49-years old male with B-CLL and the transformation to diffuse large cell lymphoma was allografted from HLA-identical sibling after conditioning fludarabin and cyclophosphamide. He developed the extensive chronic GvHD that has completely resolved on corticosteroids and CSA and this immunosuppression was stopped 10 months after NSCT. 11 months after NSCT the patient developed the relapse of disease with lymph nodes bulk and bone marrow involvement. The treatment with IFN-α was started. 10 doses of 3 MIU applied in 2 months have led to the CR without the need of IL-2 addition. **3rd case:** 44-years old male with refractory AML was allografted from HLA-identical sibling after reduced intensity conditioning (fludarabin+ARA-C+idarubicin). The bone marrow aspirate evaluation revealed 30% of leukemic blasts on day +14. CSA was stopped and 5 doses of IFN-α (3 MIU) and 3 ones of IL-2 (6 MIU) were administered. The last myelogram evaluation on day +56 proved the reduction of blasts to 11%. Since the start of cytokines no GvHD has developed in these 3 patients, yet. **Conclusion:** The presentation of the 3 cases of different haematological malignancies treated with IFN-α and IL-2 but without any DLI demonstrates the efficacy of this approach that might become a possible way how to control some malignancies after NSCT.

## 119

### B7−/− DONOR T CELLS CAUSE LESS GRAFT-VERSUS-HOST DISEASE WHILE PRESERVING GRAFT-VERSUS-TUMOR ACTIVITY

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Recent studies have demonstrated the important role of T cell homing and its regulation by integrins, chemokine receptors and ligands in the pathogenesis of acute graft-versus-host disease (GVHD). Studies by others and by us have suggested that the α4β7 integrin (LPAM) plays a role in the homing of alloreactive T cells to the gut and the development of intestinal GVHD. We have previously demonstrated, using α4β7 positively and negatively sorted T cells, that GVHD morbidity and overall mortality are reduced in hosts receiving $\alpha4\beta7^-$ donor T cells. Further analysis demonstrated reduced T cell infiltration of the intestines and less intestinal GVHD in recipients of $\alpha4\beta7^-$ donor T cells, while infiltration other GVHD target organs (skin, thymus) was similar to that seen in recipients of α4β7+ donor T cells. Concerns remain however, that, in the setting of allogeneic hematopoietic stem cell transplantation (HSCT), using a monoclonal antibody blocking the α4 subunit could interfere with stem cell engraftment. We hypothesized that blocking only the β7 subunit would also produce less intestinal GVHD and less overall GVHD morbidity and mortality, while alleviating concerns regarding stem cell engraftment. We used several models with MHC class I and II disparity, comparing allografts containing donor T cells from either wild type mice or from β7−/− mice. To assess whether β7−/− T cells have an intact alloreactive response in vivo, we performed studies with CFSE-labeled donor T cells, which were transferred into an irradiated allogeneic recipient. We observed no differences in the fraction of alloreactive T cells or the kinetics of alloreactive proliferation between recipients of β7−/− vs. wild type T cells. Recipients of donor β7−/− T cells developed less GVHD morbidity (as determined by clinical GVHD scoring) and less GVHD mortality than those receiving wild type donor T cells. In a graft-versus-tumor (GVT) model with P815, we found no

increased mortality from tumor in recipients of β7−/− donor T cells, suggesting preservation of any graft-versus-tumor activity. We are currently performing further studies to look at histopathology of GVHD target organs and to analyze T cell infiltrates in GVHD target organs. In summary, β7−/− donor T cells as compared to wild type donor T cells cause less GVHD morbidity and mortality. Our data suggest that strategies that interfere with the β7 integrin have clinical potential to alleviate or prevent GVHD while preserving GVT activity.

## 120

### DETERMINANTS OF ANTILEUKEMIA EFFECTS OF ALLOGENEIC NATURAL KILLER CELLS

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In HLA-nonidentical bone marrow transplantation, we sought to determine the characteristics of donor NK cells, recipient leukemia cells, and the cytokine environment that predict the antileukemia effects of allogeneic NK cells. We found that the risk of leukemia relapse in a prospective cohort of 36 pediatric patients was best predicted by a model taking into consideration the presence of inhibitory killer-cell immunoglobulin-like receptors (KIRs) on the donor's NK cells and the absence of corresponding KIR ligand in the recipient's HLA repertoire (a receptor-ligand model). The risk of relapse was prognosticated less precisely by the Perugia donor-recipient KIR ligand-ligand mismatch model or by a natural cytotoxicity model. In contrast to the Perugia model, we found that the new receptor-ligand model was accurate when analysis was applied to patients with lymphoid malignancy. These findings corroborate our observations that the recipient's KIR repertoire, which was derived from highly purified HLA-disparate $CD34^+$ cells, always resumed a donor-specific pattern within 3 months of transplantation but did not correlate evidently with either the donor or recipient ligand repertoire. In an in vitro assay and an in vivo mouse model, human NK-cell cytotoxicity toward human leukemia cells with 11q23 chromosomal rearrangement increased with the number of receptor-ligand mismatch pairs or prestimulation with IL-12 and IL-18. These findings provide new insights into the determinants of antileukemia effects of allogeneic NK cells and therapeutic strategies.

## 121

### PROTEINURIA RELATED TO CHRONIC GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Proteinuria related to chronic graft-versus-host disease (GVHD) is an uncommon manifestation post allogeneic stem cell transplantation (SCT). Several papers have recently addressed this issue. We report our experience in four allogeneic SCT. Between April-2001 to November-2002, four women received full matched sibling allografts for aplastic anemia (n = 2), RAEB-I (n = 1) and CML (n = 1). Median age 28 years (range 20-40). Median time from diagnosis to transplantation was 7 months (range 6-12). Conditioning regimen for aplastic anemia was: antilymphocyte globulin + cyclophosphamide , for RAEB-I: total body irradiation + cyclophosphamide, finally for CML: Busulfan p.o.+ cyclophosphamide. GVHD prophylaxys were given with cyclosporine and methrothexate . All patient engrafted, neutrophils engraftment at median of 10 days (range 9-11), non-platelets failure was observed. Acute GVHD was observed in three cases. Proteinuria during chronic GVHD exacerbation was developed at a median time of 12 months (range 10-18) post peripheral blood SCT. None of them developed renal failure neither hypertension. Edema and hypoalbuminemia was observed in several degrees. During chronic GVHD exacerbation, one patient developed lung GVHD, another polimyositis and two showed positivity to cytomegalovirus (CMV) antigenemia with extensive GVHD manifestations. Two of them developed proteinuria at nephrotic range. Renal biopsies were carried out showing: diffuse proliferative glomerulonephritis, membranous nephropathy with IgG , C3 and Lambda immune complex deposit (F/24) and focal interstitial atrophy and fibrosis, mild membranous nephropathy with C1q immune complex deposit (F/32). Prednisone based therapy with mycophenolate mofetil (n = 3) and cyclosporine (n = 1) combined with gancyclovir for CMV antigenemia, resulted in renal function stabilization and gradual proteinuria decreases. Currently all of the patients are still alive in complete remission without transfusion and chronic GHVD improvement. Our data suggest that the kidney may be a target organ in chronic GVHD with immune complex-mediated disease. Finally we recommend that proteinuria has to be tested under chronic GVHD exacerbations.

**Table.** *Patients Characteristics*

| Sex/age | Primary Disease | Acute-GVHD | Chronic-GVHD | Initial Proteinuria (mg/24 h) | Proteinuria Post-SCT (months) |
|---|---|---|---|---|---|
| F/32 | Aplastic anemia | II | Extensive | 1248 | 18 |
| F/40 | Aplastic anemia | I | Extensive | 466 | 15 |
| F/24 | RAEB I | II | Extensive | 2294 | 10 |
| F/20 | CML Ph + ICP | none | Extensive | 427 | 10 |

## 122

### TACROLIMUS IN COMBINATION WITH STEROIDS FOR THE TREATMENT OF CHRONIC GVHD

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Background: Tacrolimus is an effective drug for the treatment of graft-versus-host disease (GvHD). Recent studies have shown that tacrolimus may be better than cyclosporine for the prevention of GvHD. Other studies have shown that it was an effective salvage therapy for chronic GvHD, even in patients previously treated with cyclosporine. We report the efficacy of tacrolimus/steroids as frontline treatment of chronic GvHD. Methods: Retrospective evaluation of 104 patients who had an allogeneic HSCT between 1/99-12/00 treated with a combination of tacrolimus and steroids for chronic GvHD. Results: Among the 104 patients (M/F = 74/30), 64 had HLA-matched sibling, 36 matched unrelated and 4 mismatched related transplants. The underlying diseases included: AML/MDS = 33, CML/MPD = 25, Lymphoma = 28, ALL/CLL = 11, Myeloma = 4, others = 3. Chronic GvHD was de novo in 33 cases, relapsing in 59 and progressive in 12 patients. The disease was limited in 20 and extensive in 84 patients. In 79% cases ≥2 organs were involved (skin = 84, liver = 43, mouth = 40, GI = 37, eyes = 24, lung = 13, hematologic = 13, musculoskeletal = 2, other = 5). GvHD was documented by histology in 56/81 cases where biopsy examination was performed at the time or following diagnosis of chronic GvHD. The overall CR/PR rate to tacrolimus/steroids was 72% (n = 75). Twenty-eight (27%) patients developed did not respond (NR) or developed progressive disease (PD). The majority of responses were seen in skin (n = 56, 79%) and oral (n = 19, 76%) chronic GvHD. Most failures were seen in patients with GVHD of the eye (n = 5, 50%), GI tract (n = 11, 41%) and liver (n = 9, 29%). In 49 cases (47%) salvage immunosuppression was required after first line treatment with tacrolimus/steroids. The majority of patients (n = 34, 69%) responded to salvage therapy. Fifty patients (48%) in this series died with an overall cGvHD-related mortality of 34%. Conclusions: Responses