176 ORAL CHRONIC GVHD IN CHILDREN—TREATMENT WITH TOPICAL TACROLIMUS OINTMENT
Albert, M.H., Klein, B., Schuster, F., Binder, V., Nienhoff, C., Füßer, M., Berkhards, A. Dr. von Haemmeres Children's Hospital, Pediatric Hematology/Oncology, Munich, Germany.

Oral chronic graft versus host disease (GVHD) frequently presents as lichen-type changes, hyperkeratotic plaques, pseudomembranes, or decreased oral range of motion in patients with sclerotic features of skin GVHD and is often associated with significant limitations of oral food intake and a generally decreased quality of life. Decreased oral intake is especially problematic in children with already reduced caloric uptake due to chronic gastrointestinal GVHD. Systemic tacrolimus is well established and efficacious for prophylaxis and treatment of acute and chronic GVHD and topical tacrolimus has shown activity in chronic GVHD skin lesions. We therefore initiated a pilot study to investigate the safety and efficacy of topical tacrolimus ointment in pediatric stem cell transplant recipients with debilitating oral chronic GVHD. Two patients (6 and 13 years) with β-thalassemia were included in the study at 179 and 382 days post transplant respectively. Both suffered from progressive onset, moderate to severe chronic GVHD with involvement of the oral mucosa exhibiting lichen-type changes alongside pain, erythema and small ulcerations of the oral mucosa. One patient additionally presented with progressive sclerosis of the lips limiting opening of the oral cavity. In both patients oral lesions had been refractory to systemic GVHD treatment (cyclosporine 3 and 2 mg/kg respectively). After exclusion of infectious causes for oral lesions, tacrolimus ointment 0.1% (Protopic®, Fujisawa Healthcare) was applied twice daily using a sterile gauze that was inserted into both buccal pouches and left in place for 20 minutes without rinsing the mouth afterwards. At a follow up of 24 and 14 weeks respectively, the only side effect observed was a slight burning discomfort after the first application in one patient. Tacrolimus was absorbed in both patients as exhibited by tacrolimus plasma levels up to 4.2 and 5.6 ng/ml respectively. Pain, erythematous lesions and ulcerations disappeared within 4 weeks of treatment in both. Lichenoid changes markedly improved in both and range of oral opening normalized in the patient affected. Systemic immunosuppression was tapered during treatment with topical tacrolimus in both patients. We conclude that topical application of tacrolimus ointment holds promise as a safe and efficacious treatment for oral chronic GVHD in children. These findings deserve to be affirmed in a larger prospective evaluation.

177 TREATING DONOR MICE WITH PALIFERMIN PROTECTS AGAINST THE DEVELOPMENT OF ACUTE GRAFT-VERSUS-HOST DISEASE IN A PARENTAL STRAIN HYBRID MODEL
Edison, C.A.1,2, Fischer, J.M.M.1, Madkar, R.1, Gartner, J.G.1,4 1. Department of Pathology, University of Manitoba, Winnipeg, MB, Canada; 2. Manitoba Blood and Marrow Transplant Program, CancerCare Manitoba, Winnipeg, MB, Canada; 3. BSc. (Medicine) Program, University of Manitoba, Winnipeg, MB, Canada; 4. Department of Immunology, University of Manitoba, Winnipeg, MB, Canada.

Treating recipient mice with palifermin protects against the development of acute, lethal GVHD in murine models induced without a conditioning regimen. This is associated not only with a cytoprotective effect in the recipient mice, but also with a change in the cytokine profile. Whereas untreated recipients develop a strong Th1-mediated immune response, our previous work using the C57BL/6 → (C57BL/6 × DBA/2)F1-hybrid model of acute GVHD showed that palifermin-treated recipients develop a mixed Th2/Th1 profile, with a pronounced cytokine profile. Others have made similar observations. This finding suggested that palifermin might also have potent immunoregulatory effects that mitigate the development of acute GVHD. To study the immunoregulatory effects of palifermin in GVH mice independently of cytoprotective effects that result from treating the recipients with palifermin, we induced GVHD in C57BL/6 → (C57BL/6 × DBA/2)F1 mice using grafts from palifermin-treated, C57BL/6 donors. More than 80% of these recipients survived until at least day 150. When we compared the percentages of total, non-adherent donor spleen cells as well as percentages of CD4+ and donor CD8+ on days 4, 8 and 15 in recipients of grafts from palifermin-treated donors and recipients of grafts from untreated donors, the percentages were lower in the former group at some of these early time-points. By day 148, the percentages of total non-adherent donor cells, donor CD4+ and donor CD8+ cells reached 39%, 21% and 14%, respectively, indicating that the graft had not been aborted. The percentages of CD4+ and CD8+ cells were very similar in grafts of pooled spleen and lymph node cells harvested from palifermin-treated donors and from untreated donors. Interestingly, the levels of cytotoxic activity directed against YAC-1 and BW1100 target cells by splenic effector cells from palifermin-treated donor mice were 2-fold and 5-fold higher, respectively, when compared to those seen when splenocytes from untreated donors were used. These findings show that the ability of palifermin to protect against the development of acute GVHD in this model may be due, at least in part, to its immunoregulatory effects. They further suggest that palifermin may exert these effects through a mechanism that involves the activation of donor NK and/or NKT cells. Supported by a CIHR Operating Grant to JGG and CAE. Palifermin was kindly provided by Amgen, Inc.

178 ALLOANTIGEN AFFINITY AND CD4 HELP DETERMINE SEVERITY OF GRAFT-VERSUS-HOST DISEASE MEDICATED BY CD8 DONOR T CELLS
Yu, X.-Z.1,2, Albert, M.H.1, Anasetti, C.1 1. H. Lee Moffitt Cancer Center, Tampa, FL; 2. Dr. von Haemmeres Children’s Hospital, Munich, Germany.

TCR affinity dictates T cell selection in the thymus and also has a high impact on the fate of peripheral T cells. Graft-versus-host disease (GVHD) is a pathological process initiated by activation of donor T cells after adoptive transfer into an allogeneic recipient. How TCR affinity affects the potential of alloreactive T cells to induce GVHD is unclear. Using alloreactive CD4+ and CD8+ TCR transgenic (Tg) T cells, GVHD models are presented that allow for the visualization of how CD8+ alloreactive T cells behave in response to alloantigens with different TCR affinity in the absence or presence of CD4 help. In a non-myeloablative transplant model where GVHD lethality is due to marrow aplasia, alloreactive CD8+ TCR Tg T cells induced significantly more severe GVHD in the recipients that express an intermediate affinity alloantigen than those expressing a high affinity alloantigen. In a myeloablative transplant model where GVHD lethality is due to epithelium injury, CD8+ TCR Tg cells were also more pathogenic in the recipients with an intermediate affinity alloantigen than in those with a high affinity alloantigen. The presence of alloreactive CD4+ TCR Tg cells enhanced the potential of CD8+ TCR Tg cells to cause GVHD in recipients with an intermediate, but not with a high affinity alloantigen. These findings underscore that alloantigen affinity and CD4 help control the fate and pathogenicity of alloreactive CD8+ T cells in vivo.

179 GRAFT ENGINEERING USING EX VIVO METHODS TO LIMIT GVHD: FLUDARABINE TREATMENT GENERATES SUPERIOR GV-L EFFECTS IN ALLOGENIC BMT
Li, J.-M., Giver, C.R., Waller, E.K. Hematology/Oncology, Winship Cancer Institute, Emory University, Atlanta, GA.

Background: The development of new strategies to inhibit graft versus host disease (GVHD) while preserving graft-versus-leukemia (GvL) activity of donor lymphocytes remains an important challenge in the field of allogeneic bone marrow transplantation (BMT). Several methods to treat donor lymphocytes ex vivo prior to transplantation have been developed, including transfusion of T cell depleted BM pairs including marrow from mismatched (H2b/k, H2b), MHC haplo-mismatched (H2b→H2b/k, H2k→H2b/k), and MtHa mismatched (H2k1→H12k2) strain combinations. T-cell depleted BM
(TCD-BM) was transplanted in combination with untreated, fludara- 
binetreated, 7.5Gy γ-irradiated, or PUVA-treated splenocytes. CD3 
activity was assessed by administering a lethal number of H2k T 
lymphoma cells (LRBM) or H2b myeloid lymphoma cells (CI498). 
Post-transplant survival of recipient groups was determined, and 
GVHD and GVLo effects were assessed by clinical and pathological 
score. Hematopoietic chimera and donor T cell expansion were 
analyzed by flow cytometric analysis of peripheral blood samples at 
days 30 and 60 post-BMT. In addition, the short-term in vitro survival 
of memory and naive donor T cell subsets was monitored after fludarabine, PUVA, or γ-irradiation. Results: In vitro survival of all 
donor T cell subsets 2 days after γ-irradiation or PUVA was minimal, 
while fludarabine-treated T cells demonstrated preferential survival of 
memory T-cells. Allogeneic splenocytes treated with fludarabine, 
7.5Gy γ-irradiation, or PUVA had significantly diminished GVHD 
activity compared to untreated donor splenocytes, and facilitated en-
graftment of low-dose TCD-BM. Fludarabine-treated splenocytes 
(and PUVA-treated, to a lesser extent) retained GVLo activity and 
contributed more to donor T cell engraftment compared to γ-irradi-
donor splenocytes. The results are consistent with other studies 
suggesting that donor memory T-cells contribute to GVHD activity but 
do not produce GVHD. Conclusions: Among ex vivo methods tested 
that inhibited GVHD activity of allogeneic lymphocytes, ex vivo treat-
ment with fludarabine is superior to γ-irradiation or PUVA, resulting 
in better separation of GVHD and GVLo activities in murine models of 
allogeneic BMT.

180 REVEALING KINETICS OF CYTOKINE INDUCED KILLER CELL TRAFFICK-
ING AND SURVIVAL IN VIVO 
Nicholls, R., Baker, J., Beilhack, A., Wieldand, C.R., Negrin, R.S. The 
Division of Bone and Marrow Transplantation, Department of 
Medicine, Stanford University School of Medicine, Stanford, CA. 
Cytokine induced killer cells (CIK), which are generated from 
splenocytes in mice and PBMC in human by the timed addition of 
IFN-γ, anti-CD3 MAbs and IL-2, express both T cell and NK cell 
markers. CIK cells kill tumors through NKG2D mediated cytoly-
toxity and have in vivo activity in several murine models. CIK 
cells have been utilized in the clinic after both auto and allo 
transplant to treat or possibly reduce the risk of disease recurrence. 
Our goal was to explore CIK kinetics in vivo in the absence of 
exogenous cytokines. To this end, we transplanted luciferase-la-
abeled murine CIK cells to compare the trafficking patterns in 
different transplant settings (syngeneic vs allogeneic, and mye-
loablative vs nonmyeloablative) and identified the survival time of 
CIK cells in each BMT setting by in vivo bioluminescence imaging 
(BLI). BLI studies showed that CIK cells were proliferated rapidly 
in secondary lymphoid organs such as the spleen, cecal and 
mesenteric lymph nodes. This observation was similar to the pat-
tern of fresh splenocyte administration. In contrast, severe acute 
GVHD was not observed even in CIK dose escalation studies. CIK 
cell derived signals were detected more than 80 days after BMT. 
Moreover to clarify which lymphocyte subpopulations mainly pro-
liferate in vivo, we also transplanted CIK cells generated from GFP 
positive splenocytes and sequentially analyzed tissue distribution. 
We confirmed that GFP+CD8+NKG2D+ cells expanded in vivo 
in allogeneic BMT models. In contrast, syngeneic CIK cells did not 
home to any specific organs and proliferated much less com-
pared to those of allogeneic CIK cells, but GFP positive cells were 
detected for at least 21 days after transplantation. We demon-
strated that the kinetics of CIK cell survival was different among 
each BMT setting and CIK cells could survive without the addition 
of exogenous cytokines in vivo for prolonged periods, especially in 
allogeneic BMT settings.

181 IMPACT OF ALLOGENEIC SIBLING DONOR-DERIVED PRE-TRANSP-
PLANTATION CD16/+ CD3+ CELLS 
Kim, H.-J.1, Choi, Y.1, Jeong, H.-Y.1, Min, W.-S.2, Kim, S.-Y.1, 
Eom, K.-S.1, Lee, S.1, Min, C.-K.1, Cho, S.-G.1, Lee, J.-W.1, 
Kim, C.-C.1, Kim, T.-G.2 1. Catholic HSCT Center, St Mary’s Hos-
pital, Catholic Univ of Korea College of Medicine, Seoul, Republic 
of Korea; 2. Dept of Microbiology, Catholic Univ of Korea College 
of Medicine, Seoul, Republic of Korea. 
The associations between the numbers of donor-derived pre-
transplantation CD16/+ CD3+ cells and clinical outcome were 
investigated. Blood samples were obtained from 41 adult HLA-
matched sibling donors on the day of transplantation. The median 
percentage of CD16/+ cells recovered from the total MNCs of 
the donors was 8.5% (range, 0.9–27%). In addition, the median 
percentage of CD16/+ CD3+ cells from these populations was 
3.2% (range, 0.1–10.6%). Patients who received high levels of 
donor CD16/+ CD3+ cells showed more favorable outcomes. 
The numbers of donor CD16/+ CD3+ cells were associated 
with the development of acute GVHD (P = .028) and chronic 
GVHD (P = .0318), suggest a trend towards an inverse correlation 
between the numbers of CD16/+ CD3+ cells and the incidence of 
GVHD. The numbers of donor CD16/+ CD3+ cells in association 
with the disease-free survival were not statistically signif-
ificant (P = .0943). However, the higher numbers of donor 
CD16/+ CD3+ cells infused showed significantly lower rates of 
transplant-related complications (TRC) (P = .02). These results 
suggest that the levels of CD16/+ CD3+ cells can be an eval-
"able parameter to consider when performing allogeneic he-
matopoietic stem cell transplantation in terms of limiting the 
chances of TRC, including GVHD or relapse.

182 SELECTIVE DOWNREGULATION OF ALLOREACTIVITY IN A HUMAN 
MODEL OF EXTRACORPOREAL PHOTOTHERAPY TREATMENT 
OF GRAFT-VERSUS-HOST DISEASE 
Marshall, S.R., Wang, X.N., Dickinson, A.M. Haematological Sciences, 
University of Newcastle upon Tyne, Newcastle upon Tyne, Tyne 
& Wear, United Kingdom. 
Graft-versus-host disease (GVHD) remains the most serious 
complication following haemopoietic stem cell transplantation, 
with an incidence of 40-60% and can be fatal in up to 50% of cases. 
Extracorporeal phototherapy (ECP) is a novel treatment of both 
acute and chronic GVHD involving psoralen and UVA (PUVA) 
treatment of peripheral blood cells with high reported success even 
in those resistant to conventional immunosuppressive treatments. 
ECP appears to induce selective immune suppression without 
increased rates of infection or disease relapse, but its mechanism of 
action remains poorly understood. In our development of a skin 
explant model for GVHD has been shown to be highly pre-
dictive of clinical GVHD and has been used to investigate the 
pathophysiology of the disease. The model involves sensitizing 
donor lymphocytes with recipient lymphocytes in vitro in a pri-
ary mixed lymphocyte reaction and then evaluating the second-
ary response on recipient skin biopsies by grading the graft versus 
host reactivity (grades I-IV) histopathologically using the Lerner 
grading system for GVHD. ECP only treats a small proportion of 
circulating mononuclear cells at each visit, but is able to down-
regulate GVHD through effects on untreated cells. Similarly in the 
skin explant model, Graft-versus-host reactivity (grades I-IV) histopathologically using the Lerner 
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