# GVH/GVL

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### PREVENTION OF IDIOPATHIC PNEUMONIA SYNDROME BY INTRA-BONE MARROW INJECTION OF DONOR CELLS

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Intra-bone marrow (IBM) stem cell transplant (SCT) technique has been recently developed and several clinical and experimental studies reported that IBM-SCT is associated with high rate of engraftment and low incidence of acute GVHD. Idiopathic pneumonia syndrome (IPS) is a significant cause of mortality and remains a major obstacle after allogeneic SCT. In the present study, the extent of IPS after IBM-SCT was compared with that after conventional intravenous SCT (IV- SCT) by using a lethally irradiated B6(H-2b) into F1 (H-2b/d) mouse IPS model. IBM-SCT showed significantly improved the clinical GVHD score and reduced total cells and CD3+Tcells in bronchoalveolar lavage fluid compared with IV-SCT ($p < 0.05$). Histopathological examination of the lung at 6 weeks post SCT showed significantly reduced IPS pathology in recipients of IBM-SCT($p < 0.05$). Recipient mice were imaged at different times, ranging from 1 hour to 3 days using a lethally irradiated luciferase expressing transgenic FVB/N (H-2q) into BALB/c (H-2d) mouse model. The majority of injected donor cells were trapped in the lung one hour after IV- SCT. In contrast, significant fewer cells were localized in the lung after IBM-SCT (2.07 vs. 6.30 x $10^6$ photons/sec/animal, $p < 0.01$). Although donor cells decreased in the lung 2 to 3 hours after SCT, continued increase in donor cells occurred in the lung from 6 hours to 3days after both IV and IBM- SCT. Significantly more donor cells detected in the lung from 6 hours to 3days after IV- SCT compared with that after IBM-SCT ($p < 0.001$). Taken together, IBM-SCT reduces trapping of injected donor cells in the lung and ameliorates IPS after allogeneic SCT. Targeting donor cells trafficking in the lung may be a promising strategy for preventing IPS.

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### PLASMA CYTOKINE CONCENTRATIONS ACCORDING TO CHRONIC GVHD SUBTYPE

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Finding biomarkers for the phenotype and activity of chronic GVHD (cGVHD) may improve understanding of the disease and allow tailored therapy. We examined plasma levels of cytokines in hematopoietic cell transplant (HCT) recipients, comparing levels of patients with and without cGVHD, and among 3 clinical cGVHD subgroups.

**Methods:** Using 867 assessments for 336 individual cGVHD patients from 6 centers in the Chronic GVHD Consortium, k-means hierarchical clustering identified three clinical subtypes of cGVHD based on the Lee symptom scale, a self-report cGVHD measure with 7 subscales. Plasma samples were selected from a single center (Fred Hutchinson Cancer Research Center) as archetypes for the 3 cGVHD subtypes: 1) high symptoms ('High', n = 20), 2) high symptoms but without oral or GI manifestations ('High, not mouth/GI', n = 20), 3) low symptoms ('Low', n = 20). Samples obtained from HCT recipients without cGVHD served as controls (n = 10). Six additional cGVHD samples that were not easily classified into a clinical phenotype were included in the cGVHD vs. no cGVHD analysis only. Plasma levels of IL-4, IL-5, IL-6, IL-8, IL-10, IL-17A, IL-17F, IFN-gamma, MCP-1, TNFR-II, and TGFb1, 2, 3 were measured using standardized Luminex assays. TGFb3 results were excluded for technical reasons. Plasma BAFF levels were measured by ELISA. Linear regression models compared log-transformed cytokine levels for cGVHD versus control, and between cGVHD subtypes, controlling for plate effects.

**Results:** Median TNFR-II levels were estimated at 54% higher in cGVHD patients compared to controls (ratio1.54, 95% CI 1.13-2.09, p = 0.007) but did not differ among cGVHD subtypes. Several other cytokines (TGFb1, TGFb2, IL-17F and IL-17A) had $0.01 < p < 0.05$ in specific comparisons but should be interpreted cautiously because of multiple testing. Other cytokines were not found to differ between cGVHD and controls or among 3 cGVHD subtypes.

**Conclusions:** TNFR-II was higher in cGVHD than patients without cGVHD. These preliminary results did not confirm other putative cGVHD biomarkers reported by others.

| | | High vs. High, not mouth/GI | High, not mouth/ GI vs. Low | High vs. Low | cGVHD vs. Control |
|---|---|---|---|---|---|
| Cytokine | n | ratio* (p-value) | ratio* (p-value) | ratio* (p-value) | ratio* (p-value) |
| IL_4 | 76 | 0.84 (—) | 1.14 (—) | 0.96 (—) | 1.01 (—) |
| IL_5 | 76 | 0.81 (—) | 1.06 (—) | 0.86 (—) | 1.03 (—) |
| IL_6** | 47 | 0.82 (—) | 0.83 (—) | 0.68 (—) | 1.01 (—) |
| IL_8 | 76 | 0.91 (—) | 0.90 (—) | 0.82 (—) | 1.28 (—) |
| IL_10 | 76 | 0.90 (—) | 0.99 (—) | 0.89 (—) | 1.30 (—) |
| IL_17A | 76 | 0.81 (—) | 1.12 (—) | 0.92 (—) | 1.56 (0.04) |
| IL_17F | 76 | 0.92 (—) | 0.90 (—) | 0.83 (0.04) | 1.05 (—) |
| IFNg | 76 | 0.88 (—) | 0.98 (—) | 0.86 (—) | 1.07 (—) |
| MCP_1 | 76 | 0.89 (—) | 1.02 (—) | 0.90 (—) | 1.19 (—) |
| sTNFR_II | 76 | 1.15 (—) | 1.03 (—) | 1.18 (—) | 1.54 (0.007) |
| TGFb1 | 76 | 0.62 (0.10) | 2.00 (0.02) | 1.23 (—) | 1.08 (—) |
| TGFb2 | 75 | 0.56 (0.08) | 2.21 (0.02) | 1.25 (—) | 1.35 (—) |
| BAFF | 76 | 1.11 (—) | 0.86 (—) | 0.96 (—) | 1.12 (—) |

*Ratio of median estimated concentration for first group over median for second group. (Group comparisons are ratios due to the log transformation of cytokine concentrations in regression models.)
**Concentrations on one plate were below the assay limit of detection.
– p > 0.10.

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### ADOPTIVELY TRANSFERRED NK CELLS HOME TO TUMOR SITES AND ACCUMULATE WITH TUMOR GROWTH BUT DO NOT LEAD TO TUMOR REGRESSION IN A MURINE B-CELL LYMPHOMA MODEL

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**Introduction:** Natural killer (NK) cells can reject tumor in in vitro cytotoxicity experiments and in murine models where depletion of endogenous NK cells leads to accelerated tumor growth. Given that allogeneic NK cells can be safely transferred into mice and humans, it is now important to investigate whether these NK cells can home to tumor.

**Methods:** A20 B-cell lymphoma cells (1 x 10^6) were injected subcutaneously into BalbC recipients at the same time as total body irradiation and a T-cell depleted bone marrow transplant from MHC-mismatched wild-type FVB donors. Once the tumor became palpable (10-14 days), mice were injected intravenously with 0.5 x 10^6 fresh NK cells derived from luciferase-transgenic (Luc+) FVB donors. Some animals received intraperitoneal rhIL-2. Tumor growth was monitored by regular caliper measurements and NK cell trafficking was evaluated by bioluminescent imaging (BLI). Animals were sacrificed when the tumor began to ulcerate, and the tumor mass as well as control tissues were harvested for immune cell phenotyping and functional studies.

**Results:** NK cells infused into control non-tumor bearing animals homed to lymph nodes, spleen and liver with a maximal BLI signal at the end of the second week as previously shown (Olson et al, J Immunol 2009) By contrast, in tumor bearing-animals the Luc+ NK cells homed to lymphoid organs in the first week, followed