

Session 2: lymphoma and its microenvironment

011 INTRODUCTORY LECTURE

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Introduction: The role of the microenvironment in non-Hodgkin lymphomas is being increasingly recognized as an important contributor to both our understanding of fundamental lymphoma biology and our ability to predict outcome across the spectrum of lymphoid cancers. Insights into the role of non-neoplastic cells in lymphomagenesis derived largely from whole tissue gene expression profiling studies that revealed gene signatures expressed by non-malignant cells including several T cell subtypes, macrophages, endothelial cells and stromal elements, as important features related to survival across a number of lymphoma subtypes.

Methods: An analysis of the published literature will be used to highlight the major advances in our understanding of lymphoma biology with an emphasis on the contribution of the microenvironment. Studies that implicate a major role for non-neoplastic immune cells contributing to outcome prediction will be highlighted. Following this general introduction, the focus of the presentation will be largely based on classic Hodgkin lymphoma (cHL) and primary mediastinal large B cell lymphoma (PMBCL) as a paradigm of crosstalk between the lymphoma cells and the non-neoplastic cells in the tumoral microenvironment.

Results: Gene expression profiling of whole biopsy sections provided insights into the role of macrophages and benign B cells in the pathogenesis of cHL. HRS cells secrete a number of important cytokines and chemokines that sculpt their microenvironment and foster immune privilege. These signaling networks help explain the Th2 immunosuppressive milieu in cHL. Genetic perturbations harbored by Hodgkin Reed-Sternberg (HRS) cells and altered signaling pathways in these same cells in part explain the complex microenvironment, particularly for the over-expression of PD-L1 and galectin-1, respectively. Similar findings are also found in PMBCL, where copy number gains of chromosome 9p24 involving PD-L2, and to a lesser extent PD-L1, together with recurrent gene fusions involving the CIITA gene on chromosome 16p13, the master regulator of HLA-DR expression, provide a unifying theme helping to explain the functional composition of the microenvironment in these related lymphoma entities.

Conclusions: The non-neoplastic cells in the tumor microenvironment do not represent innocent bystander cells that are passively acquired during the evolution of the tumor, but instead represent active participants in the biology of these tumors that co-evolve with the malignant cells, making major contributions to the underlying biology and hopefully important targets for the next generation of successful therapies.

012 DEFECTS AND ABNORMALITIES IN INFILTRATING T CELLS IN PATIENTS WITH FOLLICULAR LYMPHOMA AT DIAGNOSIS

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Although clinical parameters can be used to define prognostic subgroups in patients with follicular lymphoma (FL), there is a need to identify robust biomarkers to aid prognosis and help define the underlying pathophysiology of the disease. Preceding global gene expression profiling studies demonstrated among the most important prognostic markers were the molecular features of non-malignant tumor-infiltrating immune cells present in the tumor at diagnosis (Sandeep et al. NEJM 2004). To investigate the molecular mechanisms whereby tumor infiltrating T cells (TILs) are altered in the FL microenvironment we studied highly purified, sorted infiltrating CD4 and CD8 T cells from previously cryopreserved single cell suspensions of lymph node biopsies at the time of diagnosis in treatment naive patients with FL (n=12) and compared them to those isolated from reactive tonsils (n=7) as well as peripheral blood (PB) (n=10) of age matched healthy donors. We performed global gene expression profiling using Affymetrix U133Plus2 chips of highly purified (>95% purity) CD4 and CD8 cells. For both CD4 and CD8 TILs, unsupervised analyses distinguished correctly healthy and FL T cell subsets. Using a log₂ fold change cut-off > 1 and false discovery rate of 5%, 265 genes were found differentially expressed for CD4 and 648 genes for CD8, with 109 genes overlapped for both subsets. In both CD4 and CD8, among the most downregulated genes in FL TILs were ACTN1 and IL17A, and the most upregulated genes were PMCH, ETV1 and TNFRSF9. Using Tissue Microarray we demonstrate that the intensity of expression in TILs in FL was significantly higher for PMCH (p<0.0001) and ETV1 (p<0.0001) than that of reactive tissue. PMCH is not expressed in PB T cells, but its expression is highly induced when healthy PB T cells are cultured, either in cell contact or in transwell culture, with FL cells. Surprisingly, culture of healthy T cells with normal B cells also induced its expression. Pathway

analysis indicated disruption in multiple pathways including cytokine signaling, T cell differentiation, cell proliferation and, actin-based motility/cytoskeleton formation. Using Time-Lapse imaging we demonstrate both CD4 and CD8 TILs from patients with FL (n=7) have significantly impaired motility compared to those of healthy TILs from reactive tonsil (n=4) (p<0.025). This is in keeping with our previous studies where we have shown altered T cell expression of genes regulating actin in CLL (Gorgun et al, JCI 2005) and AML (De Lieu et al, Blood 2009). Taken together, these data indicate that TILs in patients with FL are abnormal in terms of their function and gene expression profile, in keeping with our hypothesis that FL induces changes in immune cells in the tumor microenvironment. Now we are further characterizing the mechanisms of gene expression alteration in TILs of patients with FL and its functional consequences in the biology of disease. Since non-malignant infiltrating immune cells have a crucial role in the outcome of patients with FL, understanding the nature and impact of the abnormalities induced in TILs in these patients is vital before any immunotherapeutic strategies can be implemented to attempt to alter the immune microenvironment in FL.

013 RITUXIMAB INFUSION INDUCES NK ACTIVATION IN LYMPHOMA PATIENTS WITH THE HIGH AFFINITY CD16 POLYMORPHISM

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Introduction/Background: Evidence that NK cells play a major role in the clinical activity of rituximab includes the demonstration that genetic polymorphisms in CD16 (FcγRIII) correlate with therapeutic outcome to single agent rituximab. However, the impact this or other polymorphisms have on the biological response to rituximab clinically has yet to be reported. We therefore explored whether NK cell activation is induced following rituximab therapy of lymphoma patients, and whether genetic polymorphisms including in CD16 impact on that NK cell activation.

Materials and Methods: Peripheral blood samples were obtained from 21 B cell lymphoma subjects prior to therapy (baseline) and 4 hours after initiation of their first standard dose of rituximab. NK cell number and NK cell activation, as indicated by the percent of CD54^{bright} NK cells, was determined by flow cytometry for each sample. Each subject was also genotyped for polymorphisms including CD16.

Results: Of 21 subjects, 11 were homozygous FF at CD16 position 158 (low affinity polymorphism) and 10 were either heterozygous VF or homozygous VV (high affinity polymorphism). There was a 3-fold increase in the number of CD54^{bright} NK cells in samples obtained 4 hours after initiation of therapy compared to baseline in VF/VV subjects (4 hours vs baseline, p=0.029). There was no significant increase in the number of CD54^{bright} NK cells in FF subjects (4 hours vs baseline, p=0.51). The difference in the change in NK cell phenotype between VF/VV and FF subjects was statistically significant (p=0.023). The percentage of mononuclear cells that were NK cells was decreased at 4 hours compared to baseline for VF/VV subjects (p<0.0001) but not FF subjects (p=0.70). The change in NK cell number between VF/VV and FF subjects was also statistically significant (p=0.035). There was no correlation between changes in NK cell phenotype or number and other polymorphisms studies including CD32 and C1q.

Conclusion: We conclude that NK activation occurs within 4 hours of infusion of rituximab in subjects with the high affinity (VF or VV) CD16 polymorphism. This results in a change in NK cell phenotype and trafficking of NK cells out of the circulation. Changes in NK cell phenotype or trafficking are not seen consistently in subjects with low affinity CD16 (FF). This difference in NK cell activation could help explain the improved clinical outcome following treatment with single agent rituximab seen in patients with the high affinity CD16 polymorphism compared to those with the low affinity polymorphism.

014 AMD3100 DISRUPTS THE CROSS-TALK BETWEEN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS AND THEIR MICROENVIRONMENT: PRECLINICAL EVIDENCE FOR ITS COMBINATION WITH CLL TREATMENTS

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Introduction: Interactions with the microenvironment, such as bone marrow Mesenchymal Stromal Cells (MSC), protect Chronic Lymphocytic Leukemia (CLL) cells from spontaneous and drug-induced apoptosis. This protection is partially mediated by the secreted chemokine stromal cell-derived factor-1 (SDF-1 α) and its receptor CXCR4 present at CLL cells surface. Disrupting the SDF-1 α /CXCR4 axis, which plays a crucial role in CLL cell trafficking and survival, could be considered as a new treatment strategy.

Material and Methods: In the present preclinical study, we investigated the ability of AMD3100 (Plerixafor®), a CXCR4 receptor antagonist, to sensitize CLL cells to chemotherapy in a CLL/MSC co-culture model.

Results: We first showed that a concentration of 5 μ g/ml in a 2x10⁶ cells/ml of AMD3100 decreased CXCR4 expression from 88.7 \pm 3.0% to 11.33 \pm 2.3% (n=10, P=0.0078). Interestingly, treatment with AMD3100 revealed a reduction of actin polymerization in response to SDF-1 α and also a decrease of CLL pseudoemperipolesis into a stromal layer (from 11.5 \pm 2.6% to 4.5 \pm 2.2% - n=10,

P=0.0010), suggesting that AMD3100 was interfering with CLL cell trafficking. Apoptosis (determined by annexin V/7AAD/CD19 staining) was not significantly different between CLL cells treated or not with AMD3100 when cultured alone (n=10, P=0.8812). When CLL cells were cultured in presence of SDF-1 α or in a MSC stromal layer, the percentage of apoptotic cells was obviously decreased (P<0.01) compared to untreated or cultured CLL cells alone but CLL cell pre-treatment with AMD3100 (30min before the co-culture) significantly inhibited the protective effect of SDF-1 α or the MSC-based microenvironment. Furthermore, the combination of AMD3100 with various drugs (fludarabine, cladribine, corticoids, bortezomib, flavopiridol, ...) in our co-cultured model revealed a significant higher percentage of apoptotic CLL cells.

Conclusions: Altogether, these data demonstrated that interference with SDF-1 α /CXCR4 axis using AMD3100, led to inhibit CLL trafficking and the protective effect of microenvironment. The combination of AMD3100 with other drugs may thus represent a promising therapeutic approach to enhance CLL cell killing.