

Poster Presentation Abstracts

**Kinetics of Host Transcription, EBV, and KSHV in Primary Effusion Lymphoma**  
**Rachel Bagni, M.S., NCI-Frederick/SAIC-Frederick, Inc.**

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This study aims to identify cellular genes that are affected by gamma-herpesvirus reactivation (Kaposi's Sarcoma-Associated Herpesvirus [KSHV] and Epstein-Barr Virus [EBV]) and to examine the kinetics of viral-cellular gene interactions during viral reactivation. KSHV and EBV were reactivated in the JSC-1 Primary Effusion Lymphoma (PEL) cell line using 0.3mM sodium butyrate in a 72-hour time course experiment. DNA viral loads were determined using real-time quantitative PCR assays. The Affymetrix microarray platform was used to measure cellular gene expression. Real-time quantitative RT-PCR for each KSHV and EBV gene was used to profile viral transcripts. KSHV and EBV transcription commenced in an ordered cascade of early, delayed early, and late classes and preceded increases in viral load. KSHV mRNAs clustered together rather than with their EBV homologs. KSHV genes Rta/orf50 and Mta/orf57 were transcribed 6 to 12 hours earlier than EBV genes Rta/BRLF-1, Zta/BZLF-1, and Mta/BMLF-1, suggesting that KSHV controls the cellular signaling pathways that normally reactivate EBV from latency in dually infected PEL. As EBV transcripts accumulated, KSHV mRNA levels declined. Analysis of cellular transcription profiles showed cellular replication, and cell-division genes were coordinately downregulated by virus reactivation, including a G2/M block. Several genes in the JAK/STAT pathway were induced upon viral reactivation. IL-6 and cytokine signaling typically activate the JAK/STAT pathway, and the KSHV-encoded vIL-6 was immediately induced upon reactivation in JSC-1 cells. These observations provide insight into the interactions of viral and host-cell gene transcript regulation, which may provide novel targets for antiviral and antilymphoma therapies.

**The Cellular Redox Environment as a Target in the Treatment of Lymphoma**  
**Margaret Briehl, Ph.D., University of Arizona**

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Chronic inflammation in various tissue types has been associated with the development of cancer. There is a history of chronic inflammation, for example, in many cases of MALT lymphoma. One idea, yet to be proven, is that the environment of constant oxidative stress is the carcinogen in this setting. Using a lymphoma cell model, we have found that selection for resistance to oxidative stress confers cancer phenotypes, including increased net tumor growth and resistance to chemotherapeutic agents. To explore the potential clinical relevance of this finding, we mined the diffuse large B-cell lymphoma microarray data generated by the Leukemia and Lymphoma Molecular Profiling Project (as reported by Rosenwald et al., *N Engl J Med*, 2002). Our goal was to determine whether lymphomas from patients with short survival times after diagnosis and treatment have a gene-expression profile consistent with an altered response to oxidative stress. Towards this end, we used a systems biology approach to calculate a redox score for each patient. The score reflects the expression of genes preselected based on: (1) encoding antioxidant defense proteins; (2) encoding proteins that regulate growth and survival via redox reactions; or (3) being expressed in response to oxidative stress. The analyses showed that a low redox score was significantly correlated with short patient survival time. The score provided additional predictive value to the previously identified germinal center and activated B-cell gene-expression patterns. We propose that molecular regulators of the cellular redox environment should be explored as treatment targets in lymphoid malignancies.

## **Epigenetic Regulators: Potential Role in Augmenting IFNs' Antitumor Effects in Multiple Myeloma**

**Venugopalan Cheriya, Ph.D., Cleveland Clinic Foundation**

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Epigenetic silencing of interferon-stimulated genes (ISGs) by histone deacetylases (HDACs) may be responsible for the transient response of Multiple Myeloma (MM) to interferon (IFN) therapy. Inhibition of HDACs in MM cells may re-express suppressed antineoplastic ISGs to augment IFNs' antitumor effects. To test this hypothesis, pilot studies were carried out with Scriptaid (6-(1,3-Dioxo-1H,3H-benzo[de]isoquinolin-2-yl)-hexanoic Acid Hydroxyamide), an HDAC inhibitor (HDACi) with type1 IFNs. Sub-IC50 concentration of IFN $\alpha$ 1b with Scriptaid resulted in synergy in NCI H929 and RPMI 8266 cells and a strong additive effect in U266 cells. Since both IFN and HDACi manifest their antitumor effects through transcriptional regulation, we measured the effects of IFN, Scriptaid, and a combination of the two on relative expression of ISGs and antiproliferative genes in MM cells. IFNs induced several ISGs (ISG 6-16, ISG 9-27, ISG12, and XAF1) and an antiproliferative gene—TRAIL/Apo2L—both in primary MM cells and in cell lines. Co-treatment with Scriptaid downregulated ISG 6-16 and ISG 9-27 expression by approximately 40 percent in NCI H929 and RPMI 8266 cells, suggesting that these two genes may have an opposing effect or are not needed for IFNs' antiproliferative effects. A marginal increase in TRAIL/Apo2L expression was also observed with combination treatment. Combinations of IFN $\alpha$ 2b and SAHA (Suberoylanilide Hydroxamic Acid), the HDACi currently undergoing clinical trial, synergistically inhibited MM cell proliferation. Further, IFN $\alpha$ 2b and SAHA additively reduced the mitochondrial potential of the treated cells to confer their antiproliferative effects. Further demonstration of the synergy on primary MM cells from patients and in mouse xenograft models will provide a rationale for combining these two agents for MM therapy.

## **Modeling T-Cell Lymphoma in the Mouse: Understanding the Tumor-Suppressor Activities of TCF1 and Wnt5a**

**William C. Dunty, Jr., Ph.D., NCI-Frederick**

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Wnt genes encode secreted glycoproteins that regulate essential processes during embryogenesis and are required for maintenance of adult tissues. The well-characterized canonical Wnt pathway uses B-catenin and TCF/LEF transcription factors to activate target genes. Inappropriate activation of this pathway leads to tumorigenesis. Wnt5a is thought to signal through noncanonical B-catenin-independent pathways to antagonize this pathway, suggesting that Wnt5a could function as a tumor suppressor. The purpose of this investigation was to examine the tumor-suppressor activity of Wnt5a *in vivo* and to determine the mechanisms underlying this activity. Taking a genetic approach to achieve this goal, we crossed Wnt5a heterozygotes to mice null for TCF1, an established tumor suppressor in the Wnt pathway. We report that a fraction of TCF1-null mice developed T-cell lymphoma starting at 6 months of age. Loss of one copy of Wnt5a on the TCF1 $^{-/-}$  background significantly enhanced both the severity and onset of tumor formation. Biochemical analyses of these lymphomas revealed increased LEF1 and B-catenin proteins consistent with elevated canonical Wnt signaling. Transcriptional profiles of prelymphomic thymuses and thymic tumors were generated to understand mechanisms of tumor initiation and allow direct comparisons with human lymphoma profiles. Bioinformatic analysis revealed that regulators of Wnt signaling were differentially expressed during the prelymphomic time point. Runx1, an oncogene identified in mouse retrovirus-induced T-cell lymphomas, was elevated specifically in TCF1 $^{-/-}$ ;Wnt5a $^{+/-}$  tumors, suggesting that Wnt5a can repress Runx1. Our data indicate that Wnt5a functions as a haploinsufficient tumor suppressor to antagonize the Wnt/B-catenin pathway and identifies potential new target genes for tumorigenic Wnt signals.

## **Candidate Molecular Targets in Follicular Lymphoma: A Proteomic Approach** **Andrew Feldman, M.D., National Cancer Institute**

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Follicular lymphoma (FL) is the second most common non-Hodgkin's lymphoma and generally is incurable. There is no consensus that existing treatments offer a clinically significant survival advantage; thus, identifying new therapeutic targets is critical. Though Bcl-2 is overexpressed in most cases, it appears neither necessary nor sufficient for lymphomagenesis. To determine whether a proteomic approach could identify additional molecular characteristics of FL, lymphoid follicles were microdissected from 20 cases of FL and 15 cases of benign follicular hyperplasia. Lysates were spotted on reverse-phase protein microarrays and probed with 21 antibodies to proteins in the intrinsic apoptotic pathway, including those specific for post-translational modifications such as phosphorylation. Phospho-Akt(Ser473) and Bcl-2 were significantly increased in FL ( $P=0.001$  and  $P<0.0001$ , respectively). Phospho-Akt levels did not correlate significantly with those of Bcl-2, suggesting that Akt activation may be another mechanism important in FL. High ratios of Bcl-2/Bak and Bcl-2/Bax were associated with early death from disease, with differences in median survival times of 7.3 years ( $P=0.0085$ ) and 3.8 years ( $P=0.018$ ), respectively. There was no significant association between survival and Bcl-2, Bak, or Bax alone; thus, interactions of Bcl-2 with proapoptotic molecules may be more critical than total Bcl-2 levels. Small-molecule inhibitors that inhibit such interactions, as well as drugs that suppress Akt signaling, may be promising for treating FL. These findings underscore the importance of incorporating proteomic endpoints in larger, multicenter trials to validate candidate targets and to monitor the real-time effects of drugs on the protein pathways they inhibit.

## **Post-Treatment Biopsies in Lymphoid Malignancies** **Vishnu Reddy, M.D., University of Alabama at Birmingham Hospital**

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Post-treatment residual mass lesions are often biopsied to rule out refractory tumor versus fibrosis/granulation tissue. These include excisional, core needle, and fine-needle aspiration (FNA) biopsies. Post-treatment biopsies may play a key role in assessing therapeutic response in some emerging molecular diagnostic tests (e.g., gene arrays). All post-treatment (6 months–3 years) biopsies conducted in the past 3 years ( $N=127$ ) are compared with pretreatment biopsies on the quality of sample/routine H&E morphology, flow cytometry sample yield/viability/clonality, immunohistochemical staining, and gene rearrangement studies (PCR). Most significant interval changes noted (95%) are on H&E morphology, ranging across fibrosis, necrosis, mixed inflammatory cell infiltrate, and crush artifacts. Flow cytometry cellular yield/viability/clonality was low in the analyzed samples (60%), and often, specimens were inadequate for flow analysis. The most useful immunohistochemical stains were CD20, CD79a, CD15, CD30, LMP-1, and ALK-1. Neoplasm was found in 53 percent of the biopsies. Gene rearrangement studies were useful in 18 percent of the biopsy samples. Post-treatment biopsies pose new challenges. Decreasing biopsy size and FNA procedures further compound the problem. The most common morphologic findings noted are fibrosis, mixed inflammatory cell infiltrate, crushing, and necrosis. These further limit neoplastic cell yield/viability for ancillary tests, including flow and gene array testing. New gentle and modified tissue disaggregation techniques are needed for processing these small fibrotic biopsies. In addition, effects of mixed inflammatory cell infiltrate/fibrosis on gene array tests warrant further exploration.

## **Targeting Major Histocompatibility Class II Antigens in Non-Hodgkin's Lymphoma Using Histone Deacetylation**

**Lisa Rimsza, M.D., University of Arizona**

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Gene expression profiling has demonstrated the strong relationship between decreased major histocompatibility class II (MHC Class II) antigen expression and shorter patient survival in diffuse large B-cell lymphoma (DLBCL), presumably through loss of tumor immunosurveillance. We have shown a logarithmic increase in the risk of death as MHC Class II expression drops below the median expression level, as well as a decrease in cytotoxic CD8+ cells in those cases with lowest expression. In order to determine what available therapies might increase MHC Class II, we are currently investigating multiple possible mechanisms of decreased MHC Class II in DLBCL, including deletions, mutations, and epigenetic changes such as DNA hypermethylation and histone deacetylation at the promoter regions of both the MHC Class II and its regulatory molecules. In particular, acetylation of histone proteins creates steric hindrances that open sections of DNA to transcription. The level of acetylation at histone sites results from the balance between the opposing actions of histone acetylases and deacetylases. Histone deacetylase inhibitors (HDAC-Is) are a new class of drugs that antagonize histone deacetylases, resulting in an overall increase in histone acetylation. While these drugs can affect many genes, our data using MHC Class II (+) and (-) cell lines and three different HDAC-I drugs show that MHC Class II expression can be favorably targeted by HDAC-I therapy in DLBCL.

## **Abrogation of G2 Checkpoint by HSP90 Inhibitors Sensitizes p53 Mutant Cells to Chemotherapy**

**Ana Robles, Ph.D., National Cancer Institute**

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HSP90 inhibitors, the naturally occurring ansamycin antibiotic geldanamycin (GA) and its derivatives, 17-AAG and 17-DMAG, bind the ATP-binding pocket of HSP90 and inhibit essential ATPase activity, leading to destabilization and eventual degradation of HSP90 client proteins. These include a variety of signal-transducing regulators of cell growth and differentiation. The p53 protein is a tumor suppressor that is functionally inactivated in over half of all human cancers. Upon DNA damage, cells that express wild type p53 undergo G1/S and G2/M arrest followed by apoptosis, whereas cells without functional p53 bypass G1/S arrest while retaining an intact G2/M checkpoint. It has been proposed that this transient arrest in G2/M is essential for recovery from genotoxic stress and that its abrogation results in sensitization of p53-deficient tumor cells to chemotherapeutic agents and radiation. Presence of p53 mutations is associated with poor prognosis and resistance to therapy in hematological malignancies. Consistent with checkpoint activation upon DNA damage, we have found that the checkpoint kinases CHK1 and CHK2 were phosphorylated in response to doxorubicin in lymphoma and lymphoblastoid cell lines. In the absence of functional p53, cells accumulated in G2/M. Addition of 17-DMAG sequentially to doxorubicin resulted in abrogation of G2/M arrest, premature entry into mitosis, and cell death. This sensitization was accompanied by downregulation of CHK1 and/or CHK2. CHK1 has been previously characterized as an HSP90 client, thus providing an insight into the molecular pathway behind the drug schedule. Our studies indicate that this combination therapy may enhance current regimens for drug-resistant cancers.

## **Gallium Nitrate, Rituximab, and Dexamethasone for Relapsed or Refractory Lymphoma**

**Scott Smith, M.D., Ph.D., Loyola University Medical Center**

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As our aging patient population develops worsening performance status and comorbidities, it seems appropriate to develop effective and safe lymphoma treatments. One approach is to combine nonmyelosuppressive therapies. One nonmyelosuppressive agent that has efficacy in lymphoma is gallium nitrate. This agent has been shown to bind to transferrin, which is highly expressed in higher-grade histology lymphomas. Gallium nitrate's binding of transferrin impairs iron metabolism, which is a necessary component of the intracellular cytochrome systems and, ultimately, oxidative phosphorylation. The current study is a Phase II trial investigating the combination of gallium nitrate, rituximab, and dexamethasone (GaRD) for relapsed or refractory DLBCL, MCL or transformed follicular B-cell lymphomas. The gallium nitrate (200mg/m<sup>2</sup> CIV, days 1–7), rituximab (375mg/m<sup>2</sup> IVPB, day 1), and dexamethasone (40 mg po, days 1–4) are given every 21 days. Eligible patients have proven relapsed or refractory disease and a SWOG PS ≤3. Patients may have failed prior ASCT or allogeneic SCT. Accrual goal is 37 patients; 12 patients have enrolled in the study to date and have the following results: ORR 9/12 (75%); CR 2/12 (17%); PR 7/12 (58%); PD 2/12 (17%); and SD 1/12 (8%). Most of these patients (8/12 [67%]) were refractory to prior salvage regimens, including ESHAP, DHAP, and high-dose cyclophosphamide. No patients developed grade 3 or 4 toxicities, with the exception of grade 4 lymphopenia. Conclusions: Gallium nitrate, rituximab, and dexamethasone (GaRD) appear to be an effective and nontoxic salvage regimen for patients with relapsed DLBCL, MCL or transformed FL.