Insights Into Pathophysiology of Lymphomas Help Identify Therapeutic Targets

Written by Wayne Kuznar

The Presidential Symposium explored the rapidly evolving understanding of the pathogenesis of lymphoid malignancies and how this knowledge will enable the identification of therapeutic targets.

Randy Gascoyne, MD, British Columbia Cancer Agency, Vancouver, British Columbia, Canada, provided a review of next-generation sequencing, which has both enhanced understanding of the genomic basis of non-Hodgkin lymphoma (NHL) and translated into specific, targeted therapies for patients.

Next-generation sequencing technologies, such as whole genome sequencing and RNA sequencing, are being used to search for somatic mutations, chromosomal alterations (eg, translocations), and gene expression changes in NHL tumors. Next-generation sequencing has identified several recurrent mutations in indolent B-cell NHL, aggressive B-cell NHLs, and classic Hodgkin lymphoma (cHL).

Dr Gascoyne highlighted the strength of sequencing a single cell line, extending it into sequencing of clinical samples, which led to identifying a genetic alteration (translocation of CIITA) integral to primary mediastinal B-cell lymphoma (PMBCL) [Steidl C et al. *Nature.* 2011]. Translocation of CIITA is a common genetic alteration shared between cHL and PMBCL. A search for fusion partners of CIITA uncovered the ligands of programmed death 1 (PD-1)—PD-L1 and PD-L2—which, when overexpressed, can induce T-cell anergy in the microenvironment, referred to as an immunologic "double whammy," he said.

Peer-Reviewed Highlights From the

American Society of Hematology 56th Annual Meeting & Exposition

December 6–9, 2014
San Francisco, CA, USA

Genetic aberrations of the PD-L1/2 locus are present across the spectrum of B-cell lymphoid malignancies. PD-L1/2 amplification and rearrangement are significantly correlated with transcript expression [Twa DD et al. *Blood.* 2014]. Given the function of PD-Ls in repressing the antitumor response, cases harboring a translocation or high-level amplification of the PD-1 ligands are compelling candidates for nivolumab therapy, said Dr Gascoyne.

The power of next-generation sequencing can be harnessed to finding targeted agents, as in the case of EZH2. Somatic mutations altering EZH2 were first found in follicular and diffuse large B-cell lymphomas of germinal-center origin in 2010 [Morin RD et al. *Nat Gen.* 2010]. Mutations in EZH2 represent a gain-of-function mutation. EZH2 inhibition has been found to kill mutant lymphoma cells [Knutson SK et al. *Nat Chem Biol.* 2012] and therefore may be a therapeutic strategy in some NHLs.

Margaret Shipp, MD, Harvard Medical School, Boston, Massachusetts, USA, discussed emerging insights into the genetic bases for immune escape in lymphoid malignancies and promising associated treatment strategies.

Expression of the PD-1 ligands on tumor cells allows tumor cells to engage the PD-1 receptor on T cells, and activation of PD-1 signaling generates a cascade that results in a reduced activation signal and a phenotype called T-cell exhaustion [Freeman GJ et al. *J Exp Med.* 2000], which can be reversed by PD-1 blockade, she explained.

Previously, Dr Shipp's group found a recurrent alteration at chromosome 9p24.1 in primary cHL and PMBCL. Two of the genes that were most closely associated with 9p24.1 amplification were the 2 ligands of PD-1—PD-L1 and PD-L2 [Green MR et al. *Blood.* 2010]. High copy numbers of 9p24.1 correlate with increased expression of the PD-1 ligands due to gene amplification and increased expression of the JAK2 protein and associated JAK-STAT signaling [Green MR et al. *Blood.* 2010; Hao Y et al. *Clin Cancer Res.* 2014].

Epstein-Barr virus (EBV) infection is an additional mechanism of PD-L1 induction, said Dr Shipp. PD-L1 has been detected in a majority of EBV-positive post-transplant lymphoproliferative disorders (PTLDs), and cHL and EBV-positive PTLD use complementary mechanisms to upregulate PD-L1 in tumor cells [Green MR et al. *Clin Cancer Res.* 2012; Chen B et al. *Clin Cancer Res.* 2013].

The genetic bases for overexpression of the PD-1 ligands in Hodgkin lymphoma led Dr Shipp and her colleagues to evaluate the efficacy of PD-1 blockade in this disease. T-cell reactivation through PD-1 blockade, using the nivolumab antibody, induced long-lasting responses in 87% of patients with relapsed/refractory Hodgkin lymphoma [Armand P et al. ASH 2014 (abst 289); Ansell SM et al. *N Engl J Med.* 2014]. An additional PD-1 blocking antibody, pembrolizumab, was also effective in heavily pretreated cHL in an early-phase clinical trial [Moskowitz CH et al. ASH 2014 (abstr 290)].

PD-1 blockade with nivolumab is also being evaluated in additional lymphoid malignancies including follicular lymphoma, diffuse large B-cell lymphoma, and T-cell lymphoma [Lesokhin AM et al. ASH 2014 (abstr 291)]. In these lymphoid malignancies, emerging insights regarding biological bases for PD-1 ligand overexpression may help identify patients who are most likely to benefit from PD-1 blockade [Monti S et al. *Blood.* 2005; Chen B et al. *Clin Cancer Res.* 2013; Chapuy B et al. ASH 2014 (abst 74)].

Ralf Küppers, PhD, University of Duisburg-Essen, Essen, Germany, provided new insights into the biology and microenvironment of Hodgkin lymphoma. The tumor cells in cHL are called Hodgkin and Reed-Sternberg (HRS) cells. The HRS tumor cells of cHL and the lymphocyte-predominant tumor cells of nodular lymphocyte–predominant Hodgkin lymphoma are both derived from germinal center B cells. HRS cells are generated from "crippled" germinal center B cells, losing nearly all of their B cell–specific gene expression program. Multiple factors contribute to lost B-cell marker expression, including downregulation of key B-cell transcription factors, aberrant expression of master regulators of non–B cells, and epigenetic silencing of B-cell genes [Schwering I et al. *Blood.* 2003; Tiacci E et al. *Blood.* 2012]. Reexpression of the B-cell program is toxic for HRS cells. The loss of the B-cell phenotype is presumably a critical pathogenetic event in Hodgkin lymphoma, as it is required for HRS precursor cells to escape from apoptosis as crippled germinal center B cells.

The genetic lesions involved in the pathogenesis of Hodgkin lymphoma are not fully known, but numerous members and regulators of the NF-κB and JAK-STAT signaling pathways are affected, suggesting an important role for these pathways in the pathogenesis of the disease. Some genetic lesions involve epigenetic regulators, and evidence has emerged that HRS cells have undergone extensive epigenetic alterations compared with normal B cells.

HRS cells represent < 1% of cells in lymphoma tissue. The Hodgkin lymphoma microenvironment is a mixed infiltrate of various immune cells, with T cells being the largest cell population. Reed-Sternberg cell interactions with T cells in the microenvironment are likely critical for Hodgkin lymphoma pathophysiology [Küppers R. *Nat Rev Cancer.* 2009].

Composite lymphomas are rare combinations of 2 histopathologically distinct lymphomas in the same patient, often a Hodgkin lymphoma and an NHL, or 2 NHLs. Composite lymphomas are frequently clonally related and carry somatically mutated immunoglobulin variable genes with both shared and distinct mutations, which offers a strong argument that both lymphomas derive from a common precursor: a mature germinal center B cell. Initial mutation studies validate a multistep transformation process for such lymphomas.

Prof Küppers showed results from long-term time-lapse microscopy Hodgkin lymphoma cell lines that demonstrate that refusion is a major route of Reed-Sternberg cell generation from Hodgkin cells [Rengstl B et al. *PNAS*. 2014]. This refusion is mostly, if not always, based on incomplete cytokinesis, but the mechanism for failure to complete cytokinesis is unknown. A striking downregulation of many genes involved in regulation of mitosis, cytokinesis, genetic stability, and DNA repair in HRS cells indicates a potential role for genomic instability in Reed-Sternberg cell generation.