**New Strategies in Hodgkin Lymphoma: Better Risk Profiling and Novel Treatments**

Catherine Diefenbach and Christian Steidl

**Abstract**

Recent advances in Hodgkin lymphoma research are expected to prelude a promising new treatment era for patients and their treating physicians. Scientific investigations over the last few years have provided new insights into risk stratification, and, simultaneously, a plethora of novel targeted therapies are emerging for patients with relapsed and refractory disease. These novel therapies will be tested primarily in high-risk patients because 75% of the patients are cured with conventional therapies. The challenges, as Hodgkin lymphoma therapy moves forward, will be using these biologic insights to identify the patients who may benefit earlier in treatment from these novel agents, and tailoring the therapy to the tumor biology of the patient. These dual aims are intertwined; as our therapeutic arsenal increases, these biologic determinants of risk may themselves inform the design of therapies and the choice of treatments for high-risk patients.

**Background**

Classical Hodgkin lymphoma (cHL) is a B-cell lymphoid neoplasm, characterized by the presence of large mononucleated or multinucleated cells with prominent nucleoli termed Hodgkin/Reed–Sternberg (HRS) cells. Immunohistochemistry is characteristically positive for CD15 and CD30, and this pattern confirms diagnosis. The malignant HRS cells, which comprise only a small fraction (0.1%–10%) of the total cellular population, reside in a milieu of inflammatory cells, which produce soluble and membrane-bound factors that promote HRS cell growth, evasion of self-immunity, and survival (1–4). HRS cells orchestrate their microenvironment to avoid immune attack by suppressing antitumor immune surveillance (5). The HRS cells secrete cytokines such as TARC (CCL17), CCL5, and CCL22 attracting T-helper 2 (T_{H2}) and regulatory T (Treg) cells to the tumor microenvironment, and interleukin (IL)-7, which induces differentiation of naive CD4^{+}T cells toward FoxP3^{+}Treg cells (6–9). As one of the hallmarks of cHL, HRS cells constitutively express NF-κB, in part as a result of somatic mutations in pathway members and regulators, as well as other antiapoptotic proteins that inhibit both the intrinsic and extrinsic pathways of apoptosis (4, 10). HRS cell overexpression of surface molecules, such as Fas ligand, which induces apoptosis in tumor-specific CTLs, and galectin-1, which is correlated with decreased infiltration of CD8^{+} effector cells at the tumor site, maintains tolerance (11–16). Upregulation of the programmed death ligand PD-L1 on HRS cells induces energy in peritumoral T cells (17, 18). Moreover, chromosomal rearrangements of CIITA, the master regulator of MHC class II expression, have been found in approximately 15% of cHL leading to
expression of in-frame gene fusions (19). In vitro, CIITA gene fusions were shown to result in downregulated MHC class II expression and overexpression of fusion partners such as PD-L1 and PD-L2. Overall, T-cell exhaustion and deficient antitumor immunity play a key role in propagating a permissive milieu for cHL growth.

cHL is the most common lymphoid neoplasm in young patients; with a median age of 38 years at diagnosis and approximately 40% of patients under the age of 35 years at the time of diagnosis (20). Over the past 30 years, advances in treatment have led to successful clinical outcomes, with roughly 75% of patients cured with standard chemotherapy or combined modality chemoradiotherapy. Despite this success, patients with chemotherapy-resistant disease continue to have poor outcomes; there remains an estimated 1,300 deaths in the United States annually from cHL (21). Compounding this problem, many long-term cHL survivors suffer from late therapy-related toxicities; for early-stage low-risk patients, the goal is to maximize cure while minimizing toxicity. To optimize the management of cHL, we must develop biomarker strategies that allow us to better identify patients with favorable-risk disease at diagnosis, to better distinguish response from resistance early in treatment, and simultaneously to integrate novel therapies into treatment planning for patients with relapsed disease. Positron emission tomography (PET) scanning is currently the only biomarker that impacts treatment decision making in cHL; however, although PET is sensitive, it lacks specificity (22), and is further limited by cost. The Hasenclever Risk Score, although informative, does not impact management (23). In summary, besides the distinction of limited versus advanced stage, a reproducible, highly-predictive, cost-effective, and early biomarker has not to date been validated in cHL.

For patients with relapsed or refractory cHL, maximal cytodestruction before autologous stem cell transplant (SCT) confers the highest potential of cure, yet the current standard salvage chemotherapy regimens have a low complete response (CR) rate despite a high overall response rate (ORR; refs. 24, 25). The median time-to-progression (TTP) for patients relapsed after SCT treated with subsequent therapy is 3.8 months, and median survival is 26 months (26, 27). Expanding our therapeutic arsenal may allow more patients to benefit from SCT, and prolong survival for patients who are not transplant candidates.

On the Horizon

Risk profiling

As the understanding of the interdependence between the malignant HRS cells and their inflammatory microenvironment has increased, the search for biomarkers of treatment response that may alter clinical practice has grown. Evaluation of these biomarkers in combination rather than individually, in larger scale clinical trials, and the emergence of newer, robust multigene predictors of outcome may address some of their earlier limitations. This knowledge may have therapeutic as well as predictive benefit.

Immunohistochemistry

There has been a profusion of recent immunohistochemical studies in cHL, and an exhaustive discussion is beyond the scope of this review. Some recent and prominent examples are discussed below. The impact of the tumor microenvironment on clinical outcome has been well established in cHL and in other lymphomas (3, 28, 29). In Epstein–Barr virus (EBV)-positive cHL, lack of HLA class I and II expression on the surface of HRS cells is correlated with reduced immunogenicity and adverse outcome (30). Increased numbers of CD68⁺ macrophages in the affected lymph nodes of patients with cHL are associated with inferior progression-free survival (PFS) and disease-specific survival (31, 32). The prognostic significance of tumor-associated macrophages was further investigated in a subset of 287 patients from the E2496 Intergroup trial. Increased CD68 and CD163 expression were significantly correlated with inferior failure-free and overall survival (OS), and confirmed in a multivariate analysis (33). Also correlated with adverse PFS and/or OS are the abundance of grancyte B- and TIA-1-positive cytototoxic T cells; the expression of ALDH1A1, which functions in oxidative pathway metabolism, in macrophages and HRS cells; and increased numbers of PD-1-expressing peritumoral T cells (17, 31). Conversely and interestingly, high numbers of FOXP3-expressing regulatory cells have been associated with favorable prognosis (29), in contrast to their association with adverse prognosis in many solid tumors. This study did not classify regulatory cells beyond FOXP3⁺ expression. Future studies that expand on the phenotype of these peritumoral regulatory cells, and differentiate between inductive Tregs and natural Tregs, may help to explain this paradox. Peritumoral CD20-expressing background B cells are associated with favorable outcomes in two independent studies (31, 34), yet anti-CD20 antibody therapy with rituximab has induced objective responses in a subgroup of patients with relapsed cHL (35). The activity of rituximab may be due to a direct effect on HRS cells (that are occasionally CD20-positive) rather than depletion of supporting B cells (36, 37), or the association of peritumoral CD20⁺ cells with favorable outcome may be coincidental rather than causal. As both of these examples show, much about the biology of the peritumoral infiltrate and the role of its various cellular components in promoting or inhibiting lymphomagenesis remain to be discovered.

Peripheral blood biomarkers

Peripheral blood biomarkers have the advantages that they are accessible and reproducible. In addition, they can be easily evaluated at multiple time points during therapy, allowing for a dynamic assessment of clinical response. If validated, they have the potential to function as surrogates for both disease burden and systemic immunity, and to inform treatment decision making earlier in therapy than PET/CT. Many studies have focused on cytokines and chemokines as a window onto the tumor microenvironment. These include CCL17 (TARC; refs. 6, 38, 39), the cytokines IL-6 and IL-2 (40), galectin-1 (41), soluble CD30, and vascular cell adhesion molecule-1. Elevations in these
biomarkers have shown associations with advanced stage and adverse outcome, yet many of these studies have been retrospective analyses or small in size. Larger scale prospective trials of these biomarkers evaluated jointly are needed to more thoroughly evaluate their predictive capacity, and to examine whether through them we can create an immune signature of poor-risk cHL that guides therapeutic decision making.

Gene expression profiling and tumor-associated macrophages

Gene expression profiling (GEP) has been challenging in cHL due to the paucity of HRS cells in normal tumor samples. Laser capture microdissection techniques have enabled a detailed analysis of the malignant HRS separated from the cells of the tumor microenvironment. Steidl and colleagues recently examined microdissected HRS cells from 29 patients with cHL. Using integrative analysis, they identified target genes in primary HRS cells with expression levels that significantly correlated with genomic copy number changes, and found a macrophage-like signature including colony-stimulating factor-1 receptor (CSF1R) that was significantly correlated with treatment failure in an independent set of 132 patients. In multivariate analysis, a combined score of CSF1R expression and high numbers of CD68+ macrophages was an independent predictor of short PFS (42). Other GEP studies suggest that expression of a B-cell signature is associated with favorable outcome, and that plasmacytoid dendritic cell, cytotoxic T-cell, or angiogenic signatures are associated with poor outcomes (31, 34). Using NanoString digital expression profiling, a gene expression-based predictor applicable to routinely available formalin-fixed, paraffin-embedded tissue biopsies, a gene signature has recently been described that identifies high-risk patients from a cohort of patients with advanced-stage cHL (43). Importantly, this multigene predictor was validated on an independent patient cohort treated at the British Columbia Cancer Agency, showing its power as a potential clinical tool that can be integrated into a diagnostic workflow.

Novel therapeutic strategies

Novel therapeutic approaches continue to be necessary for the patients who relapse or have refractory disease. Several of the biomarker candidates described above such as CSF1R are themselves the targets of novel agents, and many more may inform future treatment strategies. Many agents under active investigation show modest single-agent activity; going forward, the challenge will be how to combine them with each other and with standard chemotherapy to augment the efficacy of both. Figure 1 depicts these agents...
in the context of their targets. Table 1 provides references and clinical trial numbers for all agents discussed below.

**Therapies targeting receptors expressed on HRS cells**

CD30, a 120-kDa type I transmembrane glycoprotein, belonging to the TNF superfamily, is highly expressed on the HRS cells of cHL. CD30 signaling is associated with pleotropic downstream effects including cellular survival, differentiation, and lymphocyte activation. Early clinical trials targeted CD30-expressing HRS cells using monoclonal antibodies. Two unconjugated antibodies, SGN-30 (cAC10), a chimerized immunoglobulin G1 monoclonal antibody, and MDX-060, a fully human monoclonal antibody with enhanced antibody-dependent cell-mediated cytotoxicity, were evaluated both as single agents and in combination with conventional chemotherapy; however, few objective responses were seen in patients with cHL (44–46).

Conjugating antibodies to a therapeutic drug allows specific targeting of potent toxins or chemotherapeutic agents to differentially expressed antibody targets. Upon binding to the cell surface target, antibody–drug conjugates (ADC) are internalized primarily via clathrin-mediated endocytosis, and subsequent lysosomal proteolysis, allowing the therapeutic agent to be delivered into the target cell, while ideally sparing normal cells, which lack the antigen specificity. The chimeric antibody cAC10 (SGN-30) was subsequently modified by the addition of a valine–citrulline peptide linker to monomethyl auristatin E, a synthetic analogue of the naturally occurring antimitic agent dolastatin 10, to form the ADC brentuximab vedotin (SGN-35). Brentuximab vedotin showed striking antitumor activity in two phase I clinical trials (47, 48). In the PIVOTAL phase II clinical trial of 102 heavily pretreated patients with relapsed cHL, the ORR was 75%, with a CR rate of 34% (25). On this basis, brentuximab vedotin received accelerated U.S. Food and Drug Administration approval for the treatment of relapsed/refractory cHL. Ongoing clinical trials are investigating brentuximab vedotin in first-line therapy in combination with standard chemotherapy, and in relapsed disease both as a single agent or in combination with conventional chemotherapy. The success of CD30 targeting is proof-of-concept that targeting the HRS tumor cells themselves can result in clinical response. However, the relatively short 5.6-month median PFS duration for the patients on this study calls into question the durability of this response in the absence of concomitant targeting of the tumor microenvironment.

**Table 1. Selected novel therapies in relapsed/refractory Hodgkin lymphoma and their targets**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Main target</th>
<th>Clinical trial number</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Receptor-targeted therapies</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>SGN-30</td>
<td>CD 30+ HRS cells</td>
<td>NCT00051597</td>
<td>(44–46)</td>
</tr>
<tr>
<td>MDX-060</td>
<td>CD 30+ HRS cells</td>
<td>NCT00284804</td>
<td>(44–46)</td>
</tr>
<tr>
<td>Brentuximab vedotin</td>
<td>CD 30+ HRS cells</td>
<td>NCT00947856</td>
<td>(25, 47, 48)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NCT01100502, NCT01060904</td>
<td></td>
</tr>
<tr>
<td>AFM 13</td>
<td>CD 16/30+ HRS cells</td>
<td>NCT01221571</td>
<td>(51)</td>
</tr>
<tr>
<td>HCD122</td>
<td>CD40+ HRS cells; T&lt;sub&gt;eff&lt;/sub&gt;/Treg signaling</td>
<td>NCT00670592</td>
<td></td>
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<tr>
<td><strong>Downstream signaling pathway</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Bortezomib</td>
<td>NF-κB and TNFR signaling, inhibition of IκB degradation</td>
<td>NCT00439361</td>
<td>(53)</td>
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<tr>
<td>SB1518</td>
<td>JAK-2</td>
<td>NCT01263899</td>
<td>(55)</td>
</tr>
<tr>
<td>MLN4924</td>
<td>NF-κB via inhibition of NEDD8</td>
<td>NCT00722488</td>
<td>(56, 57)</td>
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<td>Everolimus</td>
<td>PI3K signaling, mTOR, TNFR signaling</td>
<td>NCT00967044, NCT00918333, NCT01075321</td>
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<td><strong>Microenvironment-targeting</strong></td>
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<td></td>
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<tr>
<td>Panobinostat</td>
<td>Histone modification</td>
<td>NCT00742027</td>
<td>(59)</td>
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<tr>
<td>Vorinostat</td>
<td>Histone modification, STAT signaling (pSTAT6)</td>
<td>NCT00132028</td>
<td>(58)</td>
</tr>
<tr>
<td>Lenalidomide</td>
<td>Immunomodulation, antiangiogenesis</td>
<td>NCT00540007</td>
<td>(60)</td>
</tr>
<tr>
<td>Rituximab</td>
<td>CD20+ peritumoral B lymphocytes</td>
<td>NCT00654732</td>
<td>(35)</td>
</tr>
<tr>
<td>Autologous CAR.CD30</td>
<td>EBV+ CD30+ HRS cells; CD30+ HRS cells</td>
<td>NCT00881387</td>
<td></td>
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<td>EBV-specific CTLs</td>
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<td>NCT01192464</td>
<td>(62, 63)</td>
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<td>PLX3397</td>
<td>CSF1R + macrophages; CSF1R + HRS cells</td>
<td>NCT01217229</td>
<td>(61)</td>
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<tr>
<td>BMS-936558</td>
<td>PD-1-expressing peritumoral lymphocytes</td>
<td>NCT01592370</td>
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tetravalent human antibody construct targeting CD30/CD16 on the HRS cell surface (51); and HCD122, an antibody against CD40 targeting both CD40+ HRS cells and T\textsubscript{H}2/Treg signaling. To date, in early-phase clinical trials, galiximab has shown limited activity, and AFM13 primarily stable disease.

**Agents targeting downstream kinase signaling pathways in HRS cells**

A second class of drugs targets constitutively activated downstream signaling pathways in cHL such as NF-kB, Janus-activated kinase (JAK)-STAT, and the phosphatidylinositol 3-kinase (PI3K; AKT/mTOR) pathway.

Bortezomib, a reversible proteasome inhibitor that downregulates NF-kB signaling and enhances apoptosis through downregulation of the antiapoptotic molecules XIAP and c-FLIP, has a putative role as a chemotherapy-sensitizing agent (52), yet has shown little single-agent clinical efficacy (53). JAK2 inhibitors, which inhibit constitutive STAT phosphorylation in cHL cell lines and downregulate expression of the immunosuppressive antigens PD-1 and PD-L1 (54), have shown primarily stable disease (55). A study targeting NF-kB by MLN4924, a small-molecule inhibitor of neddylation 8, is currently ongoing.

Inhibition of mTOR has a myriad of in vitro effects, including enhancement of apoptosis, cell-cycle arrest, and autophagy. Targeting the PI3K/AKT/mTOR pathway in cHL seems to be a promising strategy. In a phase II trial in relapsed cHL of the MTOR inhibitor everolimus, the ORR was 35%, with 27% stable disease, and a median TTP of 7.2 months (56). In a phase I/II study in combination with the histone deacetylase inhibitor (HDACi) panobinostat, the ORR for 13 patients with cHL was 46% (57). A trial of the immunomodulatory agent lenalidomide in combination with everolimus is ongoing.

**Agents modulating the tumor microenvironment**

A third class of drugs targets tumor–microenvironment interactions. These include HDACi, monoclonal antibodies targeting peritumoral B cells such as rituximab, immunomodulatory agents such as lenalidomide, PLX3397, a highly selective inhibitor of CSF1R (also known as Fms), and active immunotherapy, using methods such as adoptive transfer of tumor-specific CTLs. Just entering clinical trials are checkpoint inhibitors targeting immunosuppressive molecules on the surface of peritumoral CD4+ T cells, such as anti-CTLA-4 and PD-1 inhibitors.

HDACi modulate the innate response through protean effects, including downregulation of PD-1 expression on CD4 and CD8 T cells, and engagement of the TNF superfamily ligand to stimulate antigen-specific memory T cells. Treatment with HDACi decreases serum TARC secretion in vitro (38). Multiple HDACi have been investigated against cHL, including vorinostat, a selective inhibitor of HDAC 1, 2, 3, and 11 (58), and panobinostat, a pan-DAC inhibitor (59). As single agents, these HDACi have shown primarily stable disease. Lenalidomide, an immunomodulatory and antiangiogenic agent, has similarly showed predominantly stable disease (60). PLX3397 has shown limited activity in a heavily pretreated patient cohort (61). Rituximab is under investigation combined with gemcitabine, and as an upfront therapy in combination with standard ABVD chemotherapy. Direct immune-based approaches represent a novel and promising strategy for targeting cHL. Bolland and colleagues describe an adoptive immunotherapy approach using ex vivo expansion of viral EBV antigen-specific CTLs that has produced striking results, albeit in a small study: 5 of 6 patients with relapsed EBV+ cHL had a response and 4 of 5 patients had a CR that was sustained for more than 9 months (62, 63). Other trials of adoptive immunotherapy, including genetically engineered T lymphocytes expressing a chimeric CD30 antigen receptor are ongoing. Immune checkpoint approaches that stimulate intratumoral immune cells both alone and in conjunction with chemotherapy are currently in development or in progress, with anti-CD27 antibodies (CDX1127) and anti–CTLA-4 antibodies (ipilimumab). Clinical trials of the checkpoint inhibitors PD-1 and PD-L1 include both patients with cHL and non-Hodgkin lymphoma in their current design.

**Conclusion**

As cHL investigation moves forward into the 21st century, the interdependence between the bench and the bedside has never been closer. Biologic insights will hopefully enlighten the challenge of how to easily and cost effectively risk stratify patients early in treatment. With current chemotherapy, up to 75% of patients are cured. However, the following questions remain: Can we identify a subset of these patients with such favorable outcome that they need less chemotherapy? Alternately, for patients who are at high risk, can we generate signatures of their disease that not only indicate a high likelihood of resistance to standard chemotherapy, but also suggest which novel agents or combination of agents will be most effective? As these novel therapies are integrated into the clinical armamentarium, how do we optimize synergistic combinations with standard chemotherapy, and when in treatment should these new platforms be integrated? If these remaining challenges are solved, we will be significantly closer to more individualized treatments and improved clinical outcomes for patients suffering from this disease.

**Authors’ Contributions**

Conception and design: C. Diefenbach

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Diefenbach

Writing, review, and/or revision of the manuscript: C. Diefenbach, C. Steidl

**Grant Support**

C. Steidl is supported by a Career Investigator Award from the Michael Smith foundation for Health Research. C. Diefenbach is supported in part by the NYU Clinical and Translational Science Institute NIH/NCATS UL1 TR00038.

Received December 24, 2012; revised February 15, 2013; accepted February 25, 2013; published OnlineFirst February 27, 2013.
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22. Published OnlineFirst February 27, 2013; DOI: 10.1158/1078-0432.CCR-12-3064
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Clin Cancer Res 2013;19:2797-2803. Published OnlineFirst February 27, 2013.

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