Lymphoma immunotherapy - Section 2

Biomarkers for immune checkpoint blockers
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Take Home Messages
- Describe genetic bases for immune evasion in classical Hodgkin lymphoma (cHL) and additional lymphoid malignancies.
- Discuss the rationale for considering PD-1 blockade as a treatment option in cHL and additional lymphoid malignancies.
- Describe factors that influence the efficacy of checkpoint blockade in cHL and additional lymphoid malignancies.

Checkpoint blockade in solid tumors
Therapeutic antibodies that block checkpoint pathways have shown promising activity in multiple tumor types. The most extensively targeted immune checkpoint pathway components are CTLA-4 (cytotoxic T lymphocyte antigen 4) and the PD-1 receptor and its ligand, PD-L1. However, only ~30% of patients with sensitive solid tumors respond to single-agent blocking CTLA-4 and PD-1 antibodies. These findings have prompted searches for biomarkers of response and resistance to checkpoint blockade. In solid tumors, candidate biomarkers include: 1) relative levels of expression of checkpoint pathway components such as PD-L1; 2) genetic bases for tumor immunogenicity including mutational burden and expression of neoantigens; 3) cellular composition and cytokine milieu of the primary tumor; and 4) the circulating immune cell signature.

Checkpoint blockade in lymphoid malignancies
Recent studies indicate that certain lymphoid malignancies have even higher response rates to specific types of checkpoint inhibition, particularly PD-1 blockade. Additionally, emerging data highlight specific biomarkers of response and resistance to PD-1 blockade in these tumors.

Genetic bases of immune evasion

Alterations of PD-1 pathway components
Classical Hodgkin lymphoma (cHL) is a lymphoid malignancy with the highest reported response rate to PD-1 blockade. CHLs exhibit near-uniform copy number alterations (CNAs) of chromosome 9p24.1 which includes the PD-L1, PD-L2 and JAK2 loci. In cHL, the magnitude of chromosome 9p24.1 copy gain is associated with copy number-dependent increased expression of the PD-1 ligands. In cHL and other B-cell lymphomas, the most accurate way to assess PD-L1 expression by malignant cells is a dual immunohistochemical assay for PD-L1 and the B-cell transcription factor, PAX5.

In newly diagnosed patients with cHL who were treated with standard induction therapy, progression-free survival (PFS) was significantly shorter for patients with the highest level 9p24.1 alterations (amplification). Additionally, the incidence of 9p24.1 amplification was increased in patients with advanced stage disease. However, in patients with relapsed/refractory cHL, higher level 9p24.1 copy number alterations and increased PD-L1 expression were associated with more favorable responses to PD-1 blockade. These findings suggest that this genetically driven immune evasion pathway makes CHLs less responsive to empiric chemotherapy but more sensitive to targeted PD-1 blockade. In cHL, additional bases of enhanced PD-1 ligand expression include chromosomal rearrangements involving either PD-L1 or PD-L2 and viral (EBV) infection.

Alterations of antigen presentation pathway components
Like other lymphoid malignancies, cHLs exhibit additional genetic bases of immune evasion that have implications for the mechanism of action of PD-1 blockade. For example, Hodgkin Reed Sternberg (HRS) cells have frequent inactivating mutations and copy loss of the PD-L1 ligands. HRS cell expression of MHC class II mediating antigen presentation and CD4+ infiltrating T cells in select solid tumors and the identification of tumor neoantigens that are primarily recognized by CD4+ T cells.

Analyses of the intact tumor microenvironment
Recent imaging techniques have allowed a more complete characterization of the intact tumor microenvironment in cHL.
Using multiplex immunofluorescence and digital image analysis, we recently characterized the T cell infiltrate in immediate proximity to HRS cells. PD-L1+ HRS cells were significantly more likely to be in physical contact with PD-1+ CD4+ T cells than PD-1+ CD8+ T cells. In addition, PD-L1+ HRS cells physically co-localized with PD-L1+ tumor-associated macrophages in microenvironmental immunoprotective niches. These studies highlight the utility of more comprehensive imaging approaches to define the specific architecture of lymphoid malignancies and their associated immune cell infiltrates. More recent approaches to characterize the tumor immune microenvironment utilize panels of over 30 metal-labeled antibodies and lasers or ion beams to liberate tags for subsequent mass spectrometry detection.

**Genetic analyses of recurrent alterations**

The paucity of Reed Sternberg cells in the cHL tumor microenvironment has limited comprehensive genetic approaches to the detection of recurrent 9p24.1 alterations and additional perturbations of antigen presentation pathway components. However, recent studies suggest that genetic signatures of cHL may be captured in cell-free DNA potentially facilitating serial monitoring and additional assessment of important parameters such as mutational load and neoantigen expression.

**Implications for other lymphoid malignancies**

Additional lymphoid malignancies, including primary mediastinal large B-cell lymphoma, primary central nervous system lymphoma and primary testicular lymphoma, were found to have similar recurrent alterations of chromosome 9p24.1 and increased expression of the PD-1 ligands. Analyses of such genetic features will likely lead to a more precise targeted strategy for checkpoint blockade in lymphoid malignancies and the development of rational combination therapies.

**References**

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In patients with relapsed/refractory classical Hodgkin lymphoma who were treated with PD-1 blockade, genetically driven PD-1 expression and MHC class II positivity on HRS cells were predictors of favorable outcome. In contrast, clinical responses to PD-1 blockade were not dependent on MHC class I expression on HRS cells.

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