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ORAL ABSTRACTS

621.LYMPHOMAS: TRANSLATIONAL-MOLECULAR AND GENETIC

Single-Cell RNA Sequencing Reveals the Interplay between Circulating CD4 + T Cells, B Cells and Cancer-Associated Monocytes in Classic Hodgkin Lymphoma Treated with PD-1 Blockade

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Classic Hodgkin lymphoma (cHL) is a largely MHC class I-negative tumor with recurrent 9p24.1/ *PD-L1/ PD-L2* copy gains and the highest reported response rates to PD-1 blockade. In cHL, the efficacy of PD-1 blockade is closely associated with Hodgkin Reed-Sternberg (HRS) cell expression of MHC class II, highlighting the potential role of CD4 + T-cell effectors and additional non-MHC class I-mediated mechanisms of tumor cell killing. We utilized scRNA and scT-cell receptor (TCR) sequencing to characterize the peripheral immune response to PD-1 blockade and define non-CD8 + T-cell dependent mechanisms of immune evasion in cHL.

Peripheral blood mononuclear cells were obtained from 20 patients with relapsed/refractory (R/R) cHL who were treated with PD-1 blockade on the CheckMate 205 clinical trial (at cycle 1 day 1 [C1D1] and cycle 4 day 1 [C4D1]), 11 patients with newly diagnosed (ND), previously untreated cHL and 13 healthy donors.

We first analyzed circulating CD4 + T cells given their likely importance in the response to PD-1 blockade in cHL. We found that patients with R/R cHL had markedly lower baseline TCR diversity than healthy donors across the entire CD4 + naïve/central memory (CM) T-cell space. Additionally, patients with the most favorable responses to PD-1 blockade had significantly increased CD4 + naïve/CM TCR diversity at baseline (C1D1) and more abundant naïve/CM subsets on treatment (C4D1). These response-related differences likely reflect a continued capacity to mount CD4 + T-cell responses to neoantigens. We also identified several circulating CD4 + T-cell populations that were significantly more abundant in patients with cHL than in healthy donors, including follicular helper-like T cells, IFN-stimulated cytotoxic T cells and actively proliferating CTLA4 + LAG3 + T cells.

Compared to healthy donors, all evaluated patients with cHL had significantly reduced numbers of classically defined circulating NK cells and an expanded population of IFN-stimulated NK cells with likely reduced cytotoxic potential. In addition to marked deregulation of the NK cell compartment, all patients with cHL had decreased numbers of circulating B cells at all stages of differentiation. We used the scRNA sequencing data to reconstruct individual B-cell receptor (BCR) sequences with the TRUST4 algorithm and found that the numbers of total and unique BCRs, as well as chao1 diversity, were significantly decreased in patients with ND cHL in comparison to healthy donors. Additionally, patients with the most favorable

responses to PD-1 blockade had significantly higher baseline BCR diversity and circulating B-cell numbers. Altogether, these data underscore the likely importance of B cell-mediated immune responses in cHL, either directly or via Fc-binding of innate effectors.

Finally, the most abundant circulating CD3$^-$ population in patients with cHL was a newly identified monocyte subset with increased expression of multiple immunosuppressive and tumor-promoting cytokines/chemokines, as well as *PD-L1* and *SIRPa*. These inflamed IL1β^+ monocytes were virtually absent from the blood of healthy donors. Using RNAscope, we also detected macrophages with similar features in the intact cHL tumor microenvironment, in the immediate proximity of malignant HRS cells. To assess the broader significance of the inflamed IL1β^+ monocytes, we interrogated a single-cell compendium of monocytes and macrophages from multiple solid tumors and identified a population of monocytes/ macrophages with a similar transcriptional signature. These results suggest that similarly programmed inflammatory monocytes/macrophages are detectable in multiple cancers.

We next assessed the clinical significance of circulating inflamed IL1β^+ monocytes in patients with R/R cHL who were treated with PD-1 blockade. Patients who did not respond to PD-1 blockade expressed significantly higher levels of the inflamed IL1β^+ monocyte transcriptional signature which led to the development of a predictive assay. We then identified a comparable circulating monocyte population with the same transcriptional signature associated with unresponsiveness to PD-1 blockade in an additional solid tumor, renal cell carcinoma, which underscored the broad significance of these findings.

Taken together, our findings highlight the likely complementary role of CD4$^+$ T cells, B cells and cancer-associated monocytes in the response to PD-1 blockade in cHL.

Disclosures Tang: *Immunitas Therapeutics:* Current Employment. **Luu:** *PathAI:* Current Employment. **Cader:** *AstraZeneca:* Current Employment. **Ouyang:** *Bristol Myers Squibb:* Current Employment. **Lawton:** *BostonGene:* Ended employment in the past 24 months. **Armand:** *ATB Therapeutics:* Consultancy; *Xencor:* Consultancy; *IGM:* Research Funding; *AstraZeneca:* Consultancy, Research Funding; *Kite - a Gilead company:* Research Funding; *Tessa Therapeutics:* Consultancy; *Genentech/Roche:* Consultancy, Research Funding; *Merck:* Consultancy, Honoraria, Research Funding; *Foresight Diagnostics:* Consultancy; *Regeneron:* Consultancy; *Bristol Myers Squibb:* Consultancy, Research Funding; *MSD:* Consultancy, Research Funding; *Affimed Therapeutics:* Research Funding; *ADC Therapeutics:* Consultancy; *GenMab:* Consultancy; *Enterome:* Consultancy; *Adaptive Biotechnologies:* Research Funding. **Rodig:** *Immunitas Therapeutics:* Membership on an entity's Board of Directors or advisory committees; *KITE/Gilead:* Research Funding; *Bristol Myers Squibb:* Research Funding. **Liu:** *GV20 Therapeutics, LLC:* Current Employment, Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees. **Shipp:** *Bayer:* Research Funding; *Abbvie:* Research Funding; *Bristol-Myers Squibb:* Membership on an entity's Board of Directors or advisory committees, Research Funding; *AstraZeneca:* Membership on an entity's Board of Directors or advisory committees, Research Funding.

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