The lymphoma microenvironment comes of age in the R-CHOP era?

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The lymphoma microenvironment (LME) in diffuse large B-cell lymphoma (DLBCL) remains poorly characterized. While it has been increasingly established that DLBCL tumors vary in their degree of immune cell infiltration, how this relates to specific underlying genetic alterations, oncogenic pathways, and prognosis is less well understood. As chimeric antigen receptor (CAR) T-cell therapy and bispecific T-cell engagers (BiTE) therapy gain or are expected to gain approval for use in relapsed/refractory DLBCL, it is critical for those in the field to work towards an improved understanding of the DLBCL LME, its impact on treatment outcomes, and how it might be leveraged for successful therapeutic intervention.

In this issue of *Haematologica*, Song *et al.*[1] analyzed the LME of 57 newly-diagnosed, *de novo* DLBCL cases through multispectral immunofluorescence (mIF) and enumerated several immune cell types, including $CD4^+$ T cells, $CD8^+$ T cells, natural killer (NK) cells, and macrophages. The presence of these cell types was then correlated with R-CHOP therapy efficacy. While proportions of NK cells and macrophages in the LME were not correlated with survival outcomes, DLBCL cases harboring low T-cell proportions had significantly inferior survival than those with a high proportion of T cells. This striking survival difference was accentuated when considering proportions of $CD4^+PD\text{-}1^+$ T cells and $CD8^+PD\text{-}1^+$ T cells, suggesting a role for these putative exhausted cells in contributing to therapy resistance in DLBCL. These findings were validated with CIBERSORTx immune cell deconvolution in an independent R-CHOP-treated DLBCL cohort for which gene expression profiling was available. Importantly this overall trend for "cold" DLBCL cases is consistent with other mIF data[2] and a recent large-scale transcriptomic study from Kotlov *et al.*,[3] where DLBCL cases with a "depleted" LME were associated with significantly worse survival to rituximab-based chemoimmunotherapy.

The authors next correlated the proportions of immune cell populations in the LME with protein expression of B2M, HLA-I, and HLA-II. Interestingly, DLBCL cases with decreased expression of these proteins contained fewer T cells, consistent with previous observations in DLBCL.[4] Genetic alterations in *B2M*, as well as *CD58*, *FAS*, and *TNFRSF14*, were also collectively associated with lower proportions of T cells. Notably, these alterations are thought to contribute to distinct mechanisms of immune evasion; inactivation of *B2M* and *CD58* together enable evasion from both $CD8^+$ T cells and NK cells,[5] whereas inactivation of *FAS* and *TNFRSF14* is thought to enable evasion from T-follicular helper cell-mediated deletion during the germinal center reaction.[6] Still, it remains unclear why these "cold" DLBCL tumors acquire genetic alterations to evade immune destruction. It may be that these lymphomas were "hot" early in their development after which the acquisition of additional genetic alterations (including those studied here) was sufficient to mediate immune cell exclusion as the disease progressed.

In spite of this, while these mutations are interesting, they fail to elucidate the oncogenic pathways that define "hot" and "cold" lymphomas. For example, gain-of-function mutations in *EZH2* and a transcriptional signature of double-hit lymphoma (DHITsig) have both been associated with "cold" microenvironments in the germinal center B-cell (GCB) subtype of DLBCL.[4,7] However, neither of these two features significantly correlated with differences in the proportion of T cells in the current study, suggesting that there may be other oncogenic pathways associated with "cold" DLBCL tumors that have yet to be clarified. These new pathways, in turn, may explain their poorer response to chemoimmunotherapy.

This study makes clear that T-cell-enriched DLBCL cases are associated with improved survival outcomes following R-CHOP therapy, which the authors hypothesize could be related to chemotherapy-induced immunogenic cell death and a re-energized anti-lymphoma immune response. However, the authors' suggestion to add immunotherapies to the R-CHOP backbone in the hopes of prolonging survival for "hot" DLBCL cases or perhaps improving outcomes for "cold" DLBCL cases should be met with

caution. A recent phase Ib/II trial testing the combination of the anti-PD-L1 antibody, atezolizumab, with R-CHOP reported that the regimen did not lead to a noticeably higher complete response rate and introduced additional immune-related adverse events.[8] Results from ongoing trials testing the addition of an anti-CD20 BiTE to R-CHOP, such as those incorporating epcoritamab (clinicaltrials.gov identifier: NCT04663347) and glofitamab (clinicaltrials.gov identifier: NCT03467373), however, may show otherwise. While the work of Song and colleagues is limited by its small sample size and narrow analysis of immune cell subsets, it represents an important analysis of the prognostic value of the LME in treatment-naïve DLBCL. While it is clear that there is a dichotomy between "hot" and "cold" DLBCL cases, the extent to which T cells in the LME drive this difference in survival remains poorly defined. Future studies must focus on better understanding the molecular correlates of these phenotypes and should strive to develop therapies that realize the unmet need of this at-risk "cold" DLBCL population.

Disclosures

No conflicts of interest to disclose.

Contributions

Both authors contributed equally.

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