Inhibitory Checkpoint Receptors

Subjects: Oncology

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Inhibitory checkpoint receptors play a critical role in immune homeostasis. In health, the expression of checkpoint receptors is upregulated following the activation of antigen specific T-cells to temper the pro-inflammatory response. However, upon prolonged activation with a persisting antigen, such as chronic viral infections or in cancer, checkpoint expression is maintained, and effector T-cells enter a state of 'exhaustion'. Exhausted T-cells demonstrate a progressively reduced proliferative capacity and the loss of effector T-cell functions including the production of inflammatory cytokines and degranulation. Accordingly, there has been a rapid expansion in therapeutic targeting of these checkpoint receptors to reinvigorate the effector functions of exhausted T-cells.

immune checkpoint blockade PD-1 LAG-3 Hodgkin lymphoma non-Hodgkin lymphoma follicular lymphoma diffuse large B cell lymphoma

1. Introduction

Therapeutic immune checkpoint blockade (ICB) of Programmed Death-1 (PD-1) receptor has shown remarkable efficacy in restoring effector T-cell function in malignancy and consequent clinical trials have shown unprecedented therapeutic gains in many solid tumors including melanoma, non-small cell lung cancers (NSCLC), and renal cell carcinoma [1][2][3]. Unfortunately, trials of PD-1 blockade in lymphoma have been less successful and clinical responses have been limited to a proportion of patients with Hodgkin lymphoma and rare Non-Hodgkin Lymphoma (NHL) subtypes. The reasons for the sub-optimal efficacy of these agents in lymphoma remain unclear and are an area of active research.

Nevertheless, the promising anti-tumor activity of these agents in a narrow range of lymphoma subsets has prompted continued interest in the development of newer checkpoint inhibitors and the employment of rational combinations of ICB agents to overcome T-cell exhaustion in lymphoproliferative diseases (LPDs).

2. Checkpoint Molecules in Non-Hodgkin Lymphoma

2.1. Immune Checkpoint Molecules in Primary Mediastinal B-Cell Lymphoma

PMBCL is distinct from other B-NHL subtypes demonstrating clinical, morphological, and molecular features shared with cHL [4]. The genetic hallmarks of PMBCL are copy number alterations or translocations of the PDCDLG1 and PDCDLG2 genes (encoding PD-L1 and PD-L2, respectively) at locus 9p24.1 which are present in 60–70% of cases [5][6]. These genomic alterations occur at significantly higher frequency in PMBCL than other B-NHL

subtypes. Accordingly, this correlates with increased PD-1 ligand expression on tumor cells [7][8][9]. Translocations of the 9p locus are highly specific for PMBCL often involving PDCDLG2 (gene encoding PD-L2) and lead to expression of PD-L2 at higher levels than PD-L1, a phenomenon not seen in other B-LPDs, including cHL [10][6][8][11]. MHC class II transactivator (CIITA), is a recurrent gene fusion partner for 9p.24 translocations in PMBCL which further reduced tumor immunogenicity through decreased antigen presentation and these translocations are associated with poorer outcomes [9].

PMBCL has recently been described to have high expression of LAG-3 within the TME at similar levels to that found in cHL. However, in this study, the authors found the vast majority of T-cells in PMBCL with LAG-3 expression were on $CD8^+$ TILs [12] in contrast to cHL where $CD4^+$ TILs appeared to be the predominant LAG-3 expressing T-cell [13]. Data regarding the functional status of these TILs remain sparse and further description of the co-expression of other inhibitory molecules in this NHL subtype are needed.

2.2. Immune Checkpoint Molecules in Primary Central Nervous System and Testicular Lymphoma

Primary CNS (PCNSL) and primary testicular lymphoma (PTL) present in areas of ‘immune privilege’. Like PMBCL, more than half of PCSNL/PTL cases have genomic alterations of 9p24.1 that result in constitutive PD-1 ligand expression on tumoral cells [14]. Additional molecular drivers of the pathogenesis of PCNSL/PTL include gain-of-function MYD88 mutations (65% of cases) and loss of MHC I and II molecules (50% of PCNSL and 80% of PTL), both of which are independent of PD-1/PD-1 ligand expression [14][15].

Given the TME and PD-1 axis have a significant role in dictating treatment outcome in PCNSL/PTL they are promising prognostic biomarker candidates. As described above, PD-L1 is over-expressed in the ‘immune privileged’ TME by several distinct mechanisms. While the total PD-L1 and tumor cells-restricted PD-L1 expression appears to have no association with clinical outcome, a favorable outcome is observed in patients with high PD-L1 expression on TAMs in both PTL and PCSNL treated with conventional therapy [16][17]. In PTL, high PD-1 expression on TILs ($CD4^+$ and $CD8^+$) correlates strongly with intra-tumoral PD-$L1^+$ TAMs and is also associated with improved outcomes [17][18]. By contrast, high PD-1^+ TILs in PCSNL conveys a poor prognosis, potentially reflecting high levels of T-cell exhaustion, which is particularly enriched in the rare EBV^{POS} subset occurring in immunocompromised patients [19][20][21][22]. Gene expression and multiplex IHC studies of PCNSL have found that co-expression of other immune checkpoint molecules (i.e., LAG-3 and TIM-3) in the TME is more strongly associated with poor outcome than PD-1 alone [18][23]. This implies that multiple markers to define states of T-cell exhaustion may be more valuable as a prognostic biomarker than PD-1 alone.

As seen in some cases of cHL, EBV is involved in lymphomagenesis through activation of the JAK/STAT pathway and transcription factor AP-1 [24]. EBV^{POS} PCNSL represents a rare but distinct subset of patients typified by unique immunobiology and poorer clinical outcomes [25]. Unlike the EBV^{NEG} counterparts, EBV^{POS} PCNSL seldom demonstrate increased rates of genomic alterations of 9p24.1 that could increase constitutional expression of PD-1 ligands [26]. Despite this, PD-L1 gene expression is several fold higher in EBV^{POS} cases which are also enriched for expression of LAG-3 and CD163 [17][27][28]. These findings are consistent with other EBV-infected LPDs

including EBV^{POS} cHL [27][29], post-transplant lymphoproliferative disease, and plasmablastic lymphoma [30][28]. Further IHC studies have demonstrated that the majority of PD-L1/PD-L2 expression in EBV^{POS} PCSNL appears to be on microenvironmental cells, most notably TAMs, which co-expressed high PD-L1 and PD-L2 [15][16][17][25][31]. This is associated with significant T-cell exhaustion of intra-tumoral T-cells that co-express PD-1 along with other checkpoint molecules, LAG-3 and TIM-3 [25]. As such, EBV^{POS} lymphoma represent an attractive entity for trials of dual-checkpoint blockade to reinvigorate the intra-tumoral immune response.

Together, these findings indicate that 'immune privilege' is conferred through a variety of mechanisms in PCSNL and PTL. EBV^{NEG} tumors are dependent on genetically mediated immune evasion including 9p24.1 gains or translocations and loss of HLA-I/II loci whereas immune evasion in EBV^{POS} PCSNL is orchestrated by up-regulation of $PD\text{-}L1^{+}$ M2 monocyte/macrophages along with LAG-3 upregulation and subsequent T-cell exhaustion.

3. Future Directions

Both PD-1 and LAG-3 represent emerging mechanisms of immune escape in LPD and are promising targets for therapeutic intervention. Pre-clinical studies suggest the synergistic role of dual blockade of these pathways may be more efficacious than either strategy alone due to improved re-activation of exhausted effector TILs as evidenced in DLBCL or by targeting separate populations in the TME as evidenced in cHL. Additionally, combinations of single or dual ICB therapy with sensitizing agents that promote immunogenic cell death (i.e., radiotherapy, immune vaccines, and oncolytic viruses) are hypothesized to improve tumor immunogenicity may broaden the cohort of patients that are responsive to immunotherapy as suggested by recent developments in FL.

As well as opportunities to enhance immunogenicity, manipulation of the PD-1 and LAG3 axis also show promise as a strategy to improve responses to adoptive T-cell therapies such as chimeric antigen receptor T-cells (CAR-T). Studies using CRISPR-Cas9 mediated gene editing demonstrate that the knockout of PD-1 and LAG3 in CAR-T cells overcome the immunosuppressive nature of the tumor environment, a key factor limiting CAR-T efficacy [32][33][34][35]. As such, the outcomes of current clinical studies of dual checkpoint blockade and associated translational studies in lymphoproliferative disease are eagerly awaited.

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