Peripheral Blood-Based Biomarkers

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As cancer immunotherapy using immune checkpoint inhibitors (ICIs) is rapidly evolving in clinical practice, it is necessary to identify biomarkers that will allow the selection of cancer patients who will benefit most or least from ICIs and to longitudinally monitor patients' immune responses during treatment. Various peripheral blood-based immune biomarkers are being identified with recent advances in high-throughput multiplexed analytical technologies. The identification of these biomarkers, which can be easily detected in blood samples using non-invasive and repeatable methods, will contribute to overcoming the limitations of previously used tissue-based biomarkers. Here, we discuss the potential of circulating immune cells, soluble immune and inflammatory molecules, circulating tumor cells and DNA, exosomes, and the blood-based tumor mutational burden, as biomarkers for the prediction of immune responses and clinical benefit from ICI treatment in patients with advanced cancer.

peripheral blood biomarker cancer immunotherapy

1. Introduction

In the past decade, cancer immunotherapy using immune checkpoint inhibitors (ICIs) has demonstrated promising clinical efficacy in the treatment of various malignancies. It enabled the complete regression of advanced tumors, resulting in long-term survival in a fraction of patients [1][2][3][4]. However, this potent immunotherapeutic efficacy is not always possible due to an immunosuppressive tumor microenvironment (TME) that lacks or excludes anti-tumor effector T cells [5][6][7][8][9]. Therefore, the identification of patients who are likely or unlikely to respond to ICI treatment before or as early as possible during treatment is crucial to elicit optimal immunotherapeutic efficacy [2][10][11].

Tumor programmed death-ligand1 (PD-L1) expression was initially suggested as a plausible biomarker for predicting the response to anti-PD-1/PD-L1 therapy. A multitude of clinical studies have demonstrated the enhanced efficacy of immune checkpoint blockade in patients with higher levels of intratumoral PD-L1 [2][4][11][12]. However, tumoral PD-L1 positivity alone is insufficient for patient stratification because some PD-L1 negative patients also respond to immunotherapy and PD-L1 levels show spatiotemporal variations during cancer treatment [10][13]. Other tumor factors associated with enhanced clinical benefit from ICIs include mismatch repair protein deficiency, microsatellite instability (MSI), high tumor mutational burden (TMB), and the effector T-cell gene signature [2][4][10][12][14].

Although tumor tissue-based biomarkers partially aid in identifying patients who will benefit more from ICIs, many challenges still exist in clinical practice [4][11][12][15][16][17]. First, tumor biopsies are generally invasive, and obtaining patient tissue samples using biopsy is severely limited by tumor accessibility and the condition of the patient. Furthermore, repeated tissue biopsy could increase the likelihood of procedure-related complications and delay cancer treatment. Moreover, because of tumor heterogeneity, the local immune response within one metastatic lesion may not represent the systemic anti-cancer immunity of the patient. Lastly, although cancer immunity may constantly change during ICI treatment, longitudinal immune monitoring using repeated tissue biopsy is usually not feasible in the clinic, especially during rapid clinical deterioration. Therefore, clinicians usually make decisions based on a singe-timepoint tumor biopsy at the time of treatment initiation, rather than performing repeated tumor biopsies to monitor the updated immunological profiles of the TME.

With the advent of high-throughput multiplexed analytical technologies, peripheral blood is now suitable for deeper immune profiling. As peripheral blood sampling is readily available, minimally invasive, and repeatable, the use of blood-based immune biomarkers can compensate for the abovementioned limitations of tissue-based immune biomarkers during cancer immunotherapy [11][15][18][19][20][21][22][23]. This review aimed to summarize pivotal findings related to blood-based immune biomarkers in patients with solid cancer treated with ICIs (**Figure 1**).

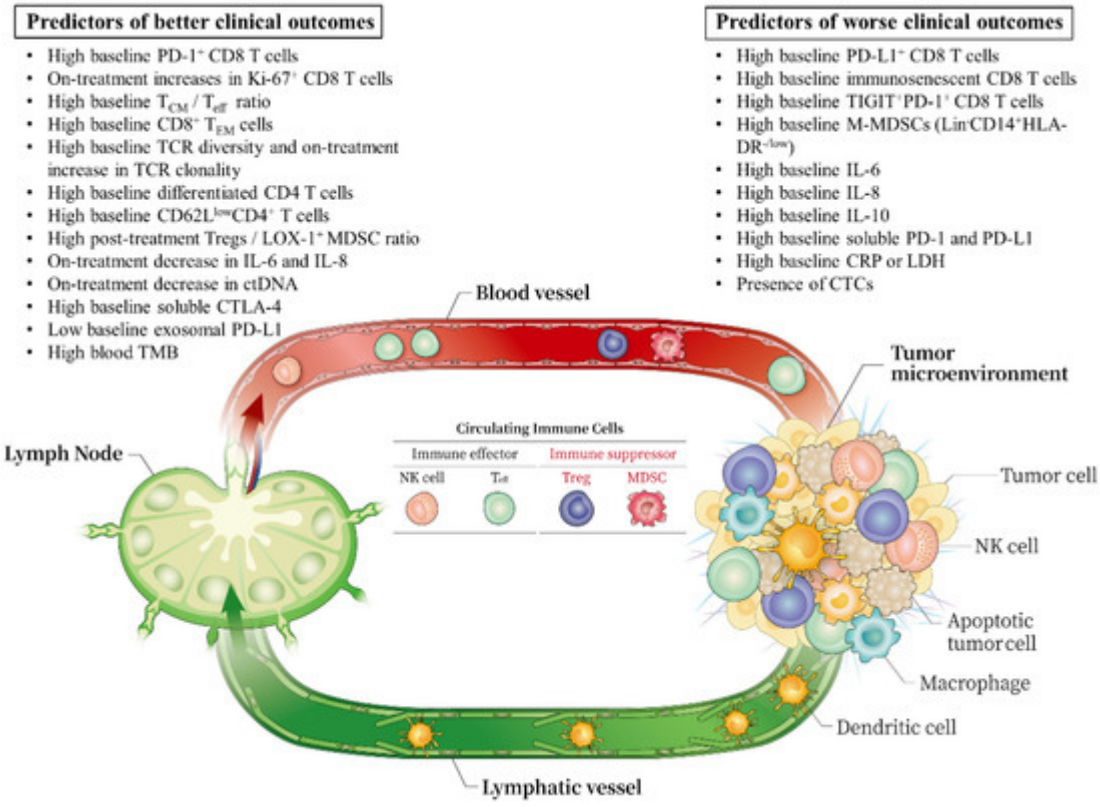

Figure 1. Summary of various circulating immune biomarkers during ICI treatment. Central memory T cells, TCM; effector memory T cells, TEM; effector T cells, Teff; T cell receptor, TCR; regulatory T cells, Tregs; myeloid-derived suppressor cells, MDSCs; polymorphonuclear-MDSCs, PMN-MDSCs; tumor mutation burden, TMB; circulating tumor cells, CTCs; circulating tumor DNA, ctDNA; monocytic-MDSCs, M-MDSCs; natural killer cell, NK cell.

2. Circulating Immune Cells

2.1. Memory T Cells

T cells undergo a natural differentiation since antigen recognition and are phenotypically subdivided based on the following surface markers: naïve T cells (T_N, CD45 RO^- $CCR7^+$), central memory T cells (T_{CM}, CD45 RO^+ $CCR7^+$), effector memory T cells (T_{EM}, CD45 RO^+ $CCR7^-$), and terminal effector T cells (T_{TE}, CD45 RO^- $CCR7^-$) [24][25]. T cells at each stage of differentiation could have a distinct role in ICI-induced restoration of anti-tumor immunity and show varied potential as predictive biomarkers of ICI treatment outcomes. It seems that a higher frequency of memory T cells compared to that of effector T cells (T_{eff}) in the peripheral blood at baseline were associated with better response and prognosis after ICI treatment [26][25]. However, this finding needs further validation in prospective studies due to the heterogeneous population studied and inconsistent sample processing.

Some researchers have reported the predictive potential of T_{CM} cells in ICI treatment response. Circulating $CD4^+$ T_{CM} cells seem to be associated with a good response and better prognosis in NSCLC and RCC patients receiving anti-PD-1 inhibitors [27]. Furthermore, Manjarrez-Orduno et al. found that patients whose tumors exhibited increased inflamed signature and PD-L1 expression showed higher levels of $CD4^+$ and $CD8^+$ T_{CM} cells compared to those of T_{eff} cells in the peripheral blood of patients with melanoma and NSCLC [26]. Intriguingly, a high T_{CM}/T_{eff} ratio at baseline was associated with longer PFS ($p < 0.05$) in a NSCLC cohort treated with nivolumab. However, there were no major changes in the T_{CM}/T_{eff} ratio in the follow-up blood samples at 3 months.

Other studies have revealed the potential role of T_{EM} cells in predicting the clinical benefits of ICIs. In patients with melanoma treated with PD-1 inhibitors, a higher frequency of circulating $CD8^+$ T_{EM} cells and lower frequency of $CD4^+$ T_{EM} cells and naïve $CD8^+$ T cells at baseline were observed in responders [28]. Consistent results were also reported in melanoma patients treated with the anti-CTLA-4 antibody, ipilimumab. Wistuba-Hamprecht et al. demonstrated that high frequencies of CD8 effector-memory type 1 T cells defined as CD45 RA^- $CCR7^-$ $CD27^+$ $CD28^+$ in the peripheral blood within 4 weeks of treatment initiation were associated with higher clinical response and longer OS [29]. However, late stage-differentiated effector memory CD8 cells (CD45 RA^+ $CDR7^-CD27^-CD28^-$) were inversely related to OS. Furthermore, other researchers have simultaneously investigated the functional status and phenotype. Kim et al. reported that a lower frequency of $CD8^+$ T_{EM} cells and a higher frequency of severely exhausted T cells ($TIGIT^+$ cells among PD-1^+ $CD8^+$ T cells) at baseline were associated with HPD and shorter OS in NSCLC patients treated with PD-(L)1 inhibitors [30].

Overall, memory T cells in the peripheral blood at a certain differentiation stage could be considered when predicting responses to ICIs.

2.2. TCR Clonality and Diversity of PD-1^+ $CD8^+$ T Cells

Some researchers have focused on the role of TCR clonality in circulating T cells as a predictive determinant of ICI response. In NSCLC, dominant TCR expansion was observed not only within the tumor tissue but also in circulating T cells, and early and sustained TCR clonal expansions in the blood were present in ICI responders [31]

[32] Recently, Han et al. reported that pretreatment PD-1$^+$ CD8$^+$ TCR diversity in the peripheral blood was higher in patients with disease control than in those with disease progression, and high PD-1$^+$ CD8$^+$ TCR diversity (>3.14) was associated with better response, longer PFS, and OS in NSCLC patients treated with PD-(L)1 inhibitors [33]. Increasing PD-1$^+$ CD8$^+$ TCR clonality at 4–6 weeks after ICI treatment initiation was associated with a higher disease control rate, longer PFS, and OS. Moreover, TCR diversity was an independent prognostic factor for both PFS and OS. The higher TCR diversity of PD-1$^+$ CD8 T cells might reflect a higher probability of neoantigen recognition. After ICI exposure, the dominant clonal expansion of tumor-specific T cells indicated a good clinical response. Furthermore, it differentiated pseudo-PD from true PD. Thus, monitoring the TCR repertoire could help predict the ICI benefit. Expansion of TCR clones in the peripheral blood after ICI treatment may not completely reflect the true diversity of the TCR repertoire within the tumor and may not be sufficient to suppress tumor growth. A recent study by El Meskini et al. elucidated this by using genetically engineered mouse melanoma models. They demonstrated that the tumor response to ICI therapy requires not only clonal expansion of the TCR repertoire but also tumor access to adequate TCRs [34]. Therefore, the post-treatment expansion of TCR clones in both the blood and tumor seems critical for the therapeutic response to ICIs. The dynamic changes in TCR clonality and diversity during ICI treatment could be validated in future prospective studies.

2.3. CD4 T Cells

CD4 T cells can promote anti-tumor immunity by supporting the priming, migration, and survival of CD8 T cells [35]. Moreover, functional CD4$^+$ T cells are necessary to restore the cytotoxicity of CD8 T cells following anti-PD-(L)1 treatment. In NSCLC patients treated with PD(L)1 inhibitors, a high proportion (>40%) of highly differentiated CD4 T cells (CD27$^-$CD28 $^{low/negative}$) in peripheral blood at baseline could predict objective RR with 100% specificity and 70% sensitivity [36]. Furthermore, this was correlated with longer PFS in multivariate analysis. These CD4 T cells mainly expressed memory features with higher Ki67$^+$ expression, but lower co-expression of PD-1 and LAG-3. Moreover, a low percentage of CD25$^+$ FOXP3$^+$ CD4$^+$ Tregs was associated with a higher RR and longer PFS and OS.

A recent report showed that CD62 L^{low} CD4 T cells were associated with the clinical response to nivolumab in NSCLC [37]. The circulating level of CD62 L^{low} CD4 T cells at baseline was higher in the nivolumab responders compared to that in non-responders ($p < 0.001$). These cells showed the classical characteristics of Th1 cells and were positively correlated with the percentage of effector CD8 T cells and expression of PD-1 on CD8 T cells. Conversely, the percentage of CD25$^+$ FOXP3$^+$ CD4$^+$ Tregs was significantly lower in nivolumab responders compared to that in non-responders. Intriguingly, durable responders maintained high percentages of CD62 L^{low} CD4 T cells within the peripheral blood.

Taken together, the intrinsic functionality of CD4 T cell immunity could be a key factor for restoring anti-tumor immunity and predicting outcomes in patients treated with ICIs.

2.4. Immunosuppressive Cells: Myeloid-Derived Suppressive Cells (MDSCs) and Tregs

MDSCs are a heterogeneous group of myeloid cells that fail to differentiate into granulocytes, macrophages, or dendritic cells. They show immunosuppressive activity by inhibiting T and NK cells, stimulating Tregs, and playing an important role in various malignancies [5][38]. MDSCs are classified into two phenotypes: neutrophil-like MDSCs, called granulocytic-MDSCs (G-MDSCs), also known as polymorphonuclear-MDSCs (PMN-MDSCs), and monocyte-like MDSCs (M-MDSCs). G-MDSCs are commonly identified as Lin^{-} $CD11b^{+}$ $CD14^{-}$ $CD15^{+}$ HLA-DR^{-} or Lin^{-} $CD11b^{+}$ $CD14^{-}$ $CD66b^{+}$, and M-MDSCs are defined as Lin^{-} $CD11b^{+}$ $CD14^{+}$ $CD15^{-}$ HLA-$DR^{-/low}$. Along with MDSCs, Tregs ($CD4^{+}$ $CD25^{+}$ $FoxP3^{+}$) play another key immunosuppressive role in various underlying mechanisms [5][39][40]. They are a subset of CD4 T cells that maintain immune homeostasis by hindering the activities of CD4 and CD8 effector cells, NK cells, and antigen-presenting cells [5][40]. Therefore, these immunosuppressive cells have been investigated as potential prognostic or predictive biomarkers in cancer patients receiving ICIs.

A study examined circulating M-MDSCs (Lin^{-} $CD14^{+}$ $CD11b^{+}$ HLA-$DR^{low/-}$) in melanoma patients receiving ipilimumab [41]. M-MDSCs at baseline were more frequent in melanoma patients than in healthy volunteers. With a cut-off of 14.9% of M-MDSCs, patients with fewer M-MDSCs at baseline or at week 6 survived longer than patients with more abundant M-MDSCs. There was a statistically significant inverse correlation between percentage changes in the $CD8^{+}$ T cell number and M-MDSC frequency at week 6.

Martens et al. analyzed M-MDSCs and Tregs in a large cohort of patients with advanced melanoma treated with ipilimumab [42]. This study revealed that M-MDSCs ($Lin^{-}CD14^{+}$ HLA-$DR^{-/low}$) at baseline were negatively correlated with OS, while Tregs were positively correlated with OS. When these two parameters, M-MDSCs ≥ 5.1% and Tregs < 1.5%, were integrated with other clinical parameters into a prognostic model, OS and ipilimumab responses could be better predicted.

Furthermore, another study in patients with advanced melanoma treated with ipilimumab as a neoadjuvant, investigated the early dynamic changes in circulating MDSCs and Tregs at week 6. It was found that early on-treatment decrease in M-MDSCs ($Lin1^{-}$HLA-DR^{-} $CD33^{+}$ $CD11b^{+}$) and an increase in Tregs at week 6 were associated with longer PFS [43].

As MDSCs share common features with neutrophils, it is challenging to clearly distinguish MDSCs from neutrophils. Recently, lectin-type oxidized LDL receptor-1 (LOX-1) has been suggested as an MDSC-specific marker in patients with cancer [44]. A recent study examined LOX-1-expressing PMN-MDSCs and Tregs in NSCLC patients treated with nivolumab [45]. In the exploratory cohort, the baseline level of LOX-1^{+} PMN-MDSCs in the peripheral blood did not differ between responders and non-responders, while the level of Tregs was higher in responders compared to that in non-responders. Interestingly, LOX-1^{+} PMN-MDSCs were significantly decreased in responders after nivolumab treatment, but the levels of Tregs were unaltered. There was an inverse correlation between LOX-1^{+} PMN-MDSCs and Tregs, and a ratio of Tregs to LOX-1^{+} PMN-MDSCs (TMR) ≥ 0.39 could distinguish the nivolumab responders from the non-responders. A high post-treatment TMR was also significantly correlated with longer PFS. Therefore, relative changes in Tregs and PMN-MDSC during PD-1 inhibition could help predict a good response to and better prognosis from ICI treatment.

Taken together, MDSCs were consistently associated with poor ICI responses and clinical outcomes. However, regarding Tregs, the results are controversial; while some studies revealed a positive correlation, another study reported a negative correlation, and other studies reported no such relationship between Tregs and ICI outcomes [46][47][28][37][42][45]. Therefore, the clinical implication of Tregs in cancer patients treated with ICIs requires further investigation.

2.5. Natural Killer (NK) Cells

NK cells induce adaptive immune responses without prior antigen sensitization during ICI treatment [48]. Mazzaschi et al. showed that the NSCLC patient group that benefited from nivolumab treatment showed higher $CD56^+$ NK cells with a more cytotoxic phenotype (perforin, granzyme B, CD3ζ) than the non-responding group [46]. High baseline levels of circulating NK cells (>202/μL) were associated with prolonged OS in this study. A recent study reported similar results in NSCLC [49], which require further validation due to the small sample size.

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