High-dimensional profiling of immunotherapy-responsive immune cells in cancer

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T CELLS IN BLOOD: WITNESSES AND EXECUTERS OF IMMUNOTHERAPY IN CANCER

ABSTRACT

Immunotherapy has revolutionized the treatment of many cancers, exerting the cytotoxic power of T cells against tumor cells. Specifically, blockade of immune checkpoint markers like programmed cell death protein-1 (PD-1) or cytotoxic T-lymphocyte antigen-4 (CTLA-4) has been found to be effective for the treatment of melanoma or other cancer types with high mutation load. While a better understanding of the composition of tumor infiltrates may help tailoring immunotherapy, tumor inaccessibility often hampers proper analysis. The recent advances in single cell technologies have led to identification of several new biomarkers and immune cell subset definition in the blood which correlate with disease progression and clinical outcome. In depth immunophenotyping of blood of cancer patients may reveal relevant information regarding immunotherapeutic efficiency. Here, we discuss how peripheral immune-based biomarkers can predict the efficacy of immunotherapeutic agents. We review the current knowledge of T cell subsets and their fates can contribute to improve immunotherapy efficacy.

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INTRODUCTION

From the first study conducted in cutaneous melanoma more than two decades ago (1), it is now well-established that a correlation exists between the tumor infiltrating lymphocytes (TILs) landscape and overall survival (OS). In 1996, 285 primary cutaneous melanoma cases have been analyzed and the presence of TILs had a very strong predictive value regarding OS. Ten years later, it has been demonstrated that detailed characterization of tumor-infiltrating immune cells by flow cytometry better reflected the prognosis of colorectal patients than histopathological methods currently used to stage colorectal cancer (2, 3). This observation led to the development of "Immunoscore", becoming a reference in classification of malignant tumors (4) by implying relative few immunological markers (CD3, CD8, CD45RO) (5). This approach gave more insight in the role of the immune system in different cancer types and helped to predict treatment efficacy (6). In parallel, cancer therapies have been developed to target the immune system rather than only targeting tumor growth. The last decade, immune checkpoint blocking antibodies have been increasingly used in the clinic. Specifically, two main immunotherapeutic agents blocking two inhibiting markers called programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte antigen-4 (CTLA-4) have been developed, unleashing the cytotoxic power of T cells. PD-1 is expressed by T cells and its natural function is to inhibit the activation and function of potential pathogenic (self-reactive) cytotoxic and helper T cells but also suppressing desirable cancer-reactive T cells (7). CTLA-4 binds competitively with CD28 to CD80 or CD86 and break the activation signal of T cells (8). CTLA-4 belongs to the immunoglobulin superfamily, is mainly expressed by activated T cells and transmits an inhibitory signal to T cells. In a similar way as PD-1, such signal potentially dampens the reactivity of T cells towards cancer cells.

Blocking those molecules by immune checkpoint blockers (ICB) show significant but variable clinical outcome in cancer patients (9, 10) which coincides with unwanted immune-related adverse events (11) by breaking T cell tolerance. To avoid unnecessary exposure to potentially hazardous agents, there is an urging need for biomarkers that correlate with clinical activity and can predict immunotherapeutic efficacy in individual patients.

The recent development of deep phenotyping technologies such as mass cytometry (12), single-cell RNA sequencing (13) or TCR sequencing revealed undiscovered specific immune cell subsets. The advances of single-cell technology highlighted specific intratumoral subsets undergoing immunological changes when treated by ICB (14). Such changes can help guiding therapeutic alternatives (15) and tailoring precision treatments.

Investigation of the cellular composition of the tumor microenvironment often requires invasive surgery which strongly delays decision making for therapeutic options. Peripheral bio-markers are therefore highly needed to adjust or confirm treatment strategy in the clinic. To enhance a routinely monitoring system, we propose a review of immune parameters detectable in the blood, at different timepoints, which may predict efficiency of a potential immunotherapeutic treatment strategy. By focussing on studies involving

patients with solid tumors, we will evaluate the effects of ICB on the immune cell subsets in the blood over time and review the importance of an early based-blood investigation for a better prediction of adequate immunotherapy for individual patients.

QUANTIFICATION OF THE MAJOR LEUKOCYTE LINEAGES IS INFORMATIVE FOR ICB EFFICIENCY

One of the first basic parameters studied for efficacy prediction was the concentration of leukocytes in the blood at different timepoints before and during ipilimumab therapy (anti-CTLA-4) of melanoma. In two different studies (16, 17), a threshold of 1000 lymphocytes/ µL has been described to be associated with a better survival of melanoma patients. Ku *et al.* confirmed that the absolute lymphocyte count after 2 treatments, at week 7, correlated with validated clinical benefits later (18). Delyon *et al.* studied the eosinophil count, for which an increase of >100/ µL between the first and second infusion surprisingly correlated with an improved OS in melanoma. A possible explanation could rely on the release of cytotoxic granules from eosinophils in the tumor microenvironment (TME) (19). A more recent study (20) on large cohort of 615 melanoma patients validated these previous findings and similarly concluded that a high relative lymphocyte count is associated with a better clinical outcome.

By extending their investigation to the myeloid compartment, the same authors showed that a lower absolute monocyte counts correlates as well with a better OS. The baseline values of the parameters studied by Martens *et al.* are sufficient to predict the efficacy of ipilimumab treatment. Finally, a more recent study from 2018 (21) focused on baseline level of derived neutrophil-to-lymphocyte ratio of 720 melanoma patients in blood and showed a threshold of 3, for which survival rates is decreasing beyond this value. These results were confirmed in a larger study, involving patients treated with ipilimumab and pembrolizumab-treated (anti-PD-1) patients (22), by showing the baseline parameters of the relative eosinophil count ≥1.5% and relative lymphocyte count ≥17.5 were associated with favourable OS. These early findings were later confirmed on other cancer types like the non-small cell lung cancer (NSCLC). In NSCLC patients treated with anti-PD-1 antibodies (Nivolumab mainly) it was reportedthat a higher baseline of absolute neutrophil or monocyte count was correlated with worse clinical outcome. The same holds true for the myeloid to lymphocyte ratio and the neutrophils to lymphocyte ratio, both correlated with worse outcome in patients who underwent anti-PD-1 treatment.

Combination therapy of anti-CTLA-4 and anti-PD-1 showed significant independent variables for favourable OS including high relative levels ofeosinophils, high relative levels of basophils, low absolute numbers of monocytes, low LDH, and a low neutrophil-to-lymphocyte ratio (23).

The above results suggest that an overall count of the main blood cell lineages, without requiring complex staining or specific immune marker of the immune system during the first weeks of treatment can be indicative of a good efficiency of both anti-CTLA-4 or anti-PD-1 treatments. Briefly, a high lymphocyte and eosinophil count are correlated to a better overall survival, whereas a high basophil, neutrophil and monocyte count have the opposite effect (Figure 1). These results might be explained by the cytotoxic power of the lymphocytes or eosinophils. The role of eosinophils has not been clearly unraveled but their potential cytotoxic power might have an effect on decreasing the tumor burden (19). The other myeloid lineages basophils and neutrophils might release anti-inflammatory cytokines in the tumor micro-environment, which has an unwanted effect on promoting tumor growth over time.

CENTRAL AND EFFECTOR MEMORY T CELL COMPARTMENTS ARE MODULATED UPON IMMUNOTHERAPY

As reviewed above, the main immune blood cell lineages can predict, to a certain level, the baseline efficiency of immunotherapy. This suggests that more specific categories of immune cells belonging to these major lineages are responsible for modulating the treatment efficiency.

In melanoma following ipilimumab treatment, during the two first cycles of therapy increases were observed in the central and effector memory T cell compartments (24). Similarly, anti-CTLA-4-treated responders presented a higher level of effector memory T cells (T_{EM}) and central memory T cells (T_{CM}) in metastatic melanoma setting (25). The authors could not demonstrate similar changes in patients treated with nivolumab (anti-PD-1). The baseline of $CD8^+$ T_{CM} cells by itself might also be predictive of ipilimumab efficiency (26). A higher presence of $CD8^+$ T_{CM} cells and elevated $CD8^+$ activated T-cells is associated with clinical response. A year later, confirmation study on ipilimumab-treated melanoma patients (27) showed that a higher level of $CD27^+$ $CD28^+$ $CD8^+$ T_{EM} cells in the blood correlated with a positive clinical outcome.

Following the studies with ipilimumab in melanoma, a year later it was demonstrated that nivolumab-treated cancer patients, who present high CM/Effector T cell ratios, are more likely associated to a higher inflammatory signature and therefore had longer progression-free survival 3 months after the treatment initiation (28) regardless of the tumor type. Interestingly, in their study, the authors also showed that the treatment did not trigger major changes in CM/Effector T cell ratios as suggested earlier by the previous authors (25). In other words, only the ratio CM/Effector cells before the treatment starts was predictive of the clinical outcome. The treatment itself had limited effect on the ratio.

In a nutshell, these studies show that the levels of T_{CM} and T_{EM} in blood are associated with immunotherapy clinical outcome, in some cases the latter can be modulated upon treatment, but often already determined before the treatment itself (Figure 1). This would suggest that immunotherapy can enhance a pre-existing T cell pool and therefore its efficiency can be predicted based on the T cell presence at baseline. These results indicate a primary response already exists in cancer patients and that ICB can promote anti-tumor responses and not create these *de novo*.

THE CENTRAL AND EFFECTOR MEMORY T CELLS MARKERS CD45RA AND CD45RO AND IMMUNOTHERAPY EFFICIENCY

The recent development of single cell technology allowed a systemic approach with a larger screening of immune cell subsets detected by mass cytometry or single-cell RNA sequencing. Such characterisation is helpful in the development of new targeting strategies, for example to enhance modulation of specific immune populations.

Following anti-CTLA-4 therapy in melanoma patients, an increase of $CD4^+$ T cells, especially activated $CD45RO^+$, $CD45RO^+TBET^+EOMES^+CD4^+$ and $CD45RO^+ICOS^+PD\text{-}1^+CD4^+$ effector T cells was shown (29). Tremelimumab (anti-CTLA-4) administered to 12 melanoma patients led to an increase in CD45RO on $CD4^+$ T cells after 3 doses and significantly differentiated responders from non-responders (30). Similarly, the hypothesis of an early detection of such cell subsets has been supported by another study showing that baseline levels of $CD45RO^+CD8^+$ T cells correlate with response and longer survival to ipilimumab (31).

Other studies have highlighted CD45RA as a determinant marker for immunotherapy responsiveness. A stronger intensity of CD45RA on T cells is indicative of non-responders receiving anti-CTLA-4, whereas responders display lower frequencies of $CD45RA^+$ at baseline (25). Low abundance of peripheral $CD45RA^+CD8^+$ T_{EM} cells associates with favorable clinical outcome (27). These studies enlighten the role of Th1-like $CD4^+$ T cells in tumor immunity in melanoma, especially after anti-CTLA4 treatment.

Surprisingly, a recent study focussing on evaluating the effect of Nivolumab (anti-PD-1) in 71 NSCLC patients (32) presented unexpected results that $CD8^+$ T cell compartment in responders patients displayed a higher abundance of $CD45RA^+$ T_{EM} and $CD95^+$ or $CD69^-$ T cells and lack stimulatory receptors (ICOS, CD40L, CD28, OX40, 4-1BB) only after 1 or 2 cycles. Such findings might be specifically valid for NSCLC and was not validated in other cancers. On the opposite, in 8 melanoma patients receiving anti-PD-1 treatment, an increase of $CD45RA^-CD4^+$ T_{CM} cells after treatment in long-term survivors, but not in non-responder, has been measured by mass cytometry (33). These studies might benefit from a validation cohort to corroborate their results.

It is not the first time that a crucial role for $CD4^+$ T cells has been reported. A central role of $PD\text{-}1^-CD127^{low}$ $CD4^+$ T cells has already been highlighted on a systemic level and associated with effective therapy (34). Deficient $CD4^+$ T cell help has been reported to reduce the response of CTLs and conversely a maximizing $CD4^+$ T cell help was seen to improve outcomes in cancer immunotherapy strategies (35). Altogether, these studies support the idea that immunotherapy benefits from the expansion of a Th1-like CD4 effector T cell subset, but is thus far not consistently reported across all tumor types and immunotherapeutic agents (Figure 1).

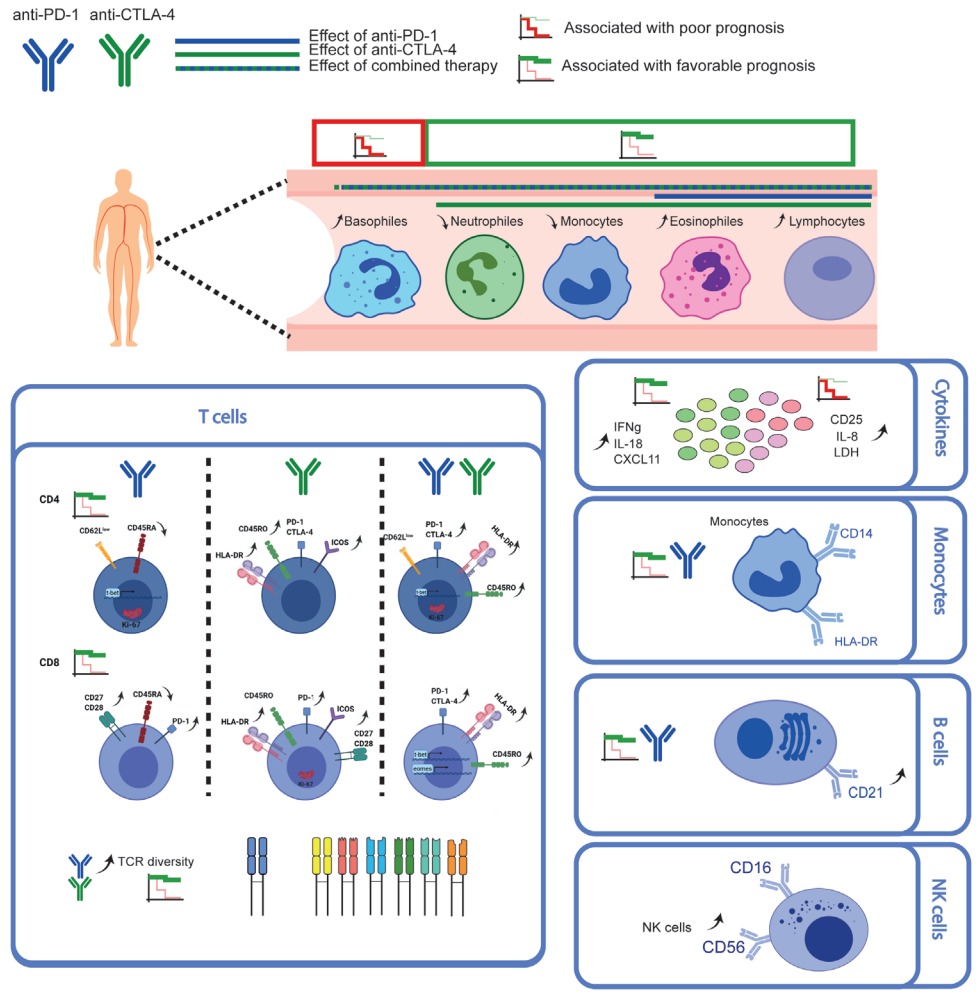

Figure 1. Graphical abstract

IPILIMUMAB SPECIFICALLY LEADS TO EXPANSION OF AN $ICOS^+$ T CELL SUBSET IN BLOOD

Further characterisation on the therapy-associated Th1-like $CD4^+$ T effector memory cell subset was performed to by detailed flow cytometry more than 10 years ago. An $ICOS^+$ Th1 subset has been discovered in 6 ipilimumab-treated-bladder cancer patients (36) and in 10 ipilimumab-treated prostate cancer patients (37), where it has been shown that therapy is correlated with an increase of $CD4^+ICOS^{hi}$ T cells in the blood together with IFN-γ expression. A year later, same evidence was brought for bladder and melanoma ipilimumab-treated patients (38). $CD4^+ICOS^{hi}$ T cells were sustained over a period of 12 weeks of therapy but already present 3 weeks after treatment. Another anti-CTLA-4 agent, tremelimumab, used on 26 hormone responsive breast cancer patients, was

reported to have the same effect (39). In this study conducted on 197 ipilimumab-treated patients, increases of activated ICOS$^+$ CD4$^+$ T cells at an early stage of the treatment, week 4 and 7, revealed a rapid immunomodulation upon the first infusion. These findings have been validated later on 36 anti-CTLA-4 treated melanoma and prostate cancer patients (40) and more recently on 26 melanoma cancer patients (41), where it was demonstrated that ipilimumab led to a higher ICOS$^+$ CD4$^+$ T cells in blood after two or three infusions. In brief, these reports show that the ICOS$^+$ T cell subset is present across multiple tumor types but present in patients specifically treated with anti-CTLA-4 agents (Figure 1).

Beyond the ICOS upregulation on CD4$^+$ T cells, similar increase has been detected on other subsets, like Gata3 on CD4$^+$. ICOS expression is not limited to CD4$^+$ T cells. Its activity has been shown on CD8$^+$ T cells at 6-month post-ipilimumab on 55 melanoma treated patients (42). The increase in ICOS expression might be linked to CD8$^+$ T cell cytotoxicity. These findings, in apparent contradiction with other studies showing no increase on ICOS CD8$^+$ T cells after therapy (24), illustrate the complexity and the dynamics of the immune response where the timing of analysis may play a crucial role. A possible explanation is a transient increase of ICOS expression which can be detected only at an early stage.

These studies showing ICOS upregulation have been confirmed in several murine models (43, 44), showing the importance of the ICOS pathway signalling in CTLA-4 therapy. The kinetic of Th1 subset shows an expansion at an early timepoint, meaning that the effect can be monitored at the start of therapy.

On the opposite, PD-1 blockade did not trigger such a clear upregulation of ICOS molecules on T cells. The populations found to be expanded after PD-1 were limited to an exhausted-like CD8$^+$ T cell phenotype. (45).

In conclusion, anti-CTLA-4 therapy but not anti-PD-1 is triggering an upregulation of ICOS on T cells, explaining a more activated phenotype against tumor cells, underlying the potential efficiency of CTLA-4 related therapies. ICOS is a stimulatory marker related to T cell immunity, and its presence in the blood reveals a tumor-reactive T cell phenotype.

ACTIVATED AND PROLIFERATIVE PHENOTYPE ON T CELLS UPON CHECKPOINT INHIBITORS

A deeper characterisation of the Th1-like CD4$^+$ cells revealed a functional feature of this subset, especially regarding their proliferative and active potential through the detection of respectively Ki-67 and HLA-DR. Ipilimumab upregulated both ICOS and Ki-67 expression on CD4$^+$ and CD8$^+$ T cells at both 3- and 6-month post therapy in melanoma patients (42). At baseline, low ICOS$^+$Ki-67$^+$EOMES$^+$CD8$^+$ T cells were associated with relapse. Some other activation markers can be expressed like HLA-DR as shown by the study conducted on 197 melanoma patients treated with ipilimumab (24). At an early stage of the treatment, week 4 and 7, patients showed a greater activated HLA-DR$^+$ CD4$^+$ and CD8$^+$ T cells with concomitant decreases in naive CD4$^+$ and CD8$^+$ T cells confirmed later in similar settings (31). Ipilimumab activates CD8$^+$ T cells by increasing HLA-DR$^+$CD25$^-$ phenotype,

implying non-specific antigen stimulation. These proliferating cells were also detected upon tremelimumab therapy in melanoma patients (30).

The ICOS$^+$ proliferative cells express inhibitory markers like CTLA-4 or PD-1 (46, 47). These cells can co-express inhibitory and activating receptors (ICOS, HLA-DR, CTLA-4, and PD-1) and are expanding after ipilimumab therapy, but no association with response could be established. These later studies would suggest that PD-1$^+$ cells expanding upon ipilimumab are not exhausted cells but can be tumor-reactive cells, as already suggested in our previous work (48).

A recent mass cytometry study on melanoma patients (49) with the aim to compare the effect of both anti–PD-1 and CTLA-4 monotherapies showed similar patterns. In both therapies, a strong enrichment of Ki-67$^+$ proliferative cells in PD-1$^+$ versus PD-1$^-$ CD8$^+$ T cells was noted. An investigation (50) on the phenotype from the blood of 29 treated NSCLC patients, revealed that therapy triggered an increase in effector-like phenotype Ki-67$^+$ PD-1^{hi+} ICOS$^+$ CD8$^+$ T cells and found out that these cells were also HLA-DR$^+$, CD38$^+$, Bcl-2^{low} and CD28$^+$, CD27$^+$ in 70% of patients, with most responses seen within 4 weeks. Conversely, 70% of patients with disease progression had either a delayed or absent PD-1$^+$ CD8$^+$ T-cell response, whereas 80% of patients with clinical benefit exhibited PD-1$^+$ CD8$^+$ T-cell responses within 4 weeks of treatment initiation. The kinetics of such subsets were further examined on patients with stage IV melanoma treated with pembrolizumab (51). The frequency of Ki-67$^+$ CD8$^+$ T cells was increased after only 3 weeks after treatment and then declined in most patients. Briefly, this study shows that Ki-67$^+$ (HLA-DR$^+$CD38$^+$) T cells in the blood are reinvigorated by anti-PD-1 therapy. Another study (52) suggests that the changes occurs even earlier than 3 weeks after treatment, by looking at the blood only one week after treatment. It was concluded from the blood of 79 NSCLC treated with anti-PD-1 agents that Ki-67$^+$ cells among PD-1$^+$CD8$^+$ T cells can predict durable clinical benefit already 7 days after the first dose. PD-1 has an immediate effect on blood cells as shown on 22 nivolumab or pembrolizumab treated patients (53), where the responder group showed higher expressions of PD-1 on CD4$^+$ T cells than the non-responder group after the first cycle of immunotherapy. The kinetics of other markers reveals a lower expression of CTLA-4, GITR, and OX40 after the second cycle of immunotherapy. Elevation of key biomarkers after the first cycle of immunotherapy, followed by a decrease in their expression after the second cycle, was associated with a better outcome from immunotherapy at an early stage of treatment of cancer. These studies underscore the importance of the timing of blood phenotyping and highlight the dynamical changes in the blood in response to therapy, often occurring within the first weeks of treatments, regardless the treatment or the tumor type (Figure 1).

Similar findings were extended by targeting PD-L1 with atezolizumab in a study investigating the blood of 14 patients suffering from metastatic bladder cancer (54). By comparing the blood at baseline and after treatment, it was eventually concluded that HLA-DR$^+$Ki-67$^+$ CD8$^+$ T cells increased after atezolizumab injections. This has been validated the same year in 185 patients in other cancer types (RCC, NSCLC, melanoma,

cutaneous, mucosal and ocular) in anti-PD-L1 therapy where HLA-DR^{+}Ki-67^{+} CD8^{+} T cells increase was detected (55) after only one treatment, at early stage. Therefore, blocking PD-1 or its ligand PD-L1 might lead eventually to the same cellular effects.

To summarize, these studies highlight the upregulation of activating markers on T cells, including ICOS, HLA-DR and proliferative markers like Ki-67. The possible transient upregulation of those markers demonstrates the importance of kinetic analysis of the systemic immune response and that a correct timing is crucial for accurate immune correlation. These results emphasized the very rapid effect of PD-1 on systemic immunity. PD-1^{+} expression on T cells does not only delineate exhaustive cells but can also point to an ongoing cellular response.

THE SYNERGIC EFFECT OF PD-1 AND CTLA-4 COMBINED TREATMENT

In 2017, a study showed that higher PD-L1 expression on peripheral T cells, was correlated with worse progression-free survival on ipilimumab-treated patients (56). This leads to the conclusion that PD-L1 is still playing an inhibiting role which cannot be overcome by anti-CTLA-4 alone. A combination therapy might be the answer for a better prognostic, by modulating the immune system. As described earlier, the proliferating CD4^{+} and CD8^{+} T cells expressing HLA-DR, ICOS and/or Ki-67 have been found after combined therapy on the two main lineages CD4^{+} and CD8^{+} T cells (57) and of terminally differentiated TBET^{+}EOMES^{+}CD8^{+} T cells (49). The combination blockade leads to nonoverlapping (Ki-67, granzyme A/B, IL-8 and HLA-DR, IFNγ) expression changes with monotherapy (46) highlighting their synergistic effect (58). The number of studies studying the blood compartment in patients treated with the combination PD-1/PD-L1 together with CTLA-4 is still limited, especially due to its increased toxicity(59).

ARE REGULATORY T CELLS IMPACTED BY ICB?

Anti-CTLA-4 and anti-PD-1 therapy efficiency can be partially explained by the expansion of effector subsets. However, these immunotherapies might have an independent effect by reducing the number of T regulatory cells, thereby indirectly enhancing tumor immunity. The absence of impact of CTLA-4 blockade on regulatory T cells has already been proposed in melanoma patients (60), where no decrease in the expression of CD25^{+} CD4^{+} T cells in whole PBMC, nor a decrease in FoxP3 gene expression in the CD4^{+} or purified CD25^{+} CD4^{+} T cell populations post-treatment was shown. These results were confirmed in a larger study involving 197 ipilimumab-treated melanoma patients (24) and 12 tremelimumab-treated patients (30). Low pre-treatment frequencies of regulatory T cells were also found to be associated with a better clinical outcome (61), suggesting that T_{regs} are not a potential target of anti-CTLA-4 therapy and that this treatment does not deplete FoxP3 as recently demonstrated (62). However, contradictory results have been reported (63) where, following anti-CTLA-4 treatment, a greater increase in T_{regs} (CD25^{hi+} Foxp3^{+} CD4^{+}) was unexpectedly associated with improved progression-free survival (PFS). Finally,

findings were reported where baseline frequencies of CD25^{+}FoxP3^{+} CD4^{+} T_{regs} ≥ 1.5% were associated with favourable prognosis after initiation of ipilimumab (20). It might be that T_{regs} represent target cells of ipilimumab due to their CTLA-4 expression. Therefore, a high baseline frequency might render patients more susceptible to anti–CTLA-4 antibodies. This hypothesis is strongly supported by the observed correlation between decreasing levels of circulating T_{regs} during ipilimumab and favorable outcome (18).

In summary, the potential impact of ICB regarding T_{regs} still needs to be defined. If in some cases, no depletion of T_{regs} can be measured, it might be possible that the regulatory potential of the T cells is modulated upon anti-CTLA-4 therapy, due to its constitutive expression on T_{regs} or that the T_{regs} are not measurable in the blood but be present in other compartments, like in the tumor or the lymph nodes.

A CROSS-TALK BETWEEN IMMUNE CELL LINEAGES

PD-1 and CTLA-4 expression is not restricted to T cells and the blocking effect of ICB is not limited to the T cells. In 2014, a study on ipilimumab therapy on melanoma patients showed a decrease in circulating monocyte MDSC Lin^{-}/HLA-DR^{-}/CD33^{+}/CD11b^{+} cells was associated with improved PFS (63). The same year, it was also demonstrated that monocytic-MDSC frequencies were inversely correlated with peripheral CD8^{+} T-cell expansion following ipilimumab treatment in melanoma (64, 65). Myeloid cell frequencies could be correlated to the CD8^{+} T cell frequencies and therefore play a role in tumor immunity through their impact on CD8^{+}T cells.

An extensive study was performed later to define more precisely the myeloid subsets predicting PD-1 blockade effect. Before commencing therapy, a strong predictor of progression-free and overall survival in response to anti-PD-1 immunotherapy was the high frequency of CD14^{+}CD16^{-}HLA-DR^{hi} monocytes (66). On the opposite, it has been shown that the CD14^{+}CD16^{-} monocyte subsets before therapy were higher in the resistance group (67)

Following immunotherapy checkpoint blockade, it was noticed that in anti-PD-1 treated melanoma patients but not in anti-CTLA-4 treated patients, CD69^{+} NK cells are correlated with clinical response (25). Melanoma patients responding to immunotherapy had a higher frequency of NK cells and CD56- CD16^{hi} T cells (67) and showed higher expressions of PD-1 on NK cells than the non-responder group after the first cycle of immunotherapy (53). In peripheral blood of PD-1 treated patients, an unusual population of immune blood cells expressing CD56, HLA-DR, CD14^{mid} and CD4^{mid} increased by 9-fold from the patients with a response to therapy (68). Lastly, B cells are also subject to changes. Anti-PD-1 therapy led to decline in circulating B cells and an increase in CD21^{low} B cells (with higher PD-1 expression and higher clonality) and plasmablasts. Interestingly, patients with early B cell changes experienced higher rates of grade 3 or higher IRAEs 6 months after immunotherapy (69) (Figure 1).

The presence of these cells might be connected to effector T cells and the lymph nodes are enhancing such cross-talks. A recent study (70) showed that lymph nodes are critical for successful PD-1 therapy.

CONCLUSION AND DISCUSSION

The use of an increasing number and combinations of immunotherapeutic agents against cancer results in a large offer of treatments, which needs to be optimized by increasing its efficacy and minimizing adverse events. Blood is an easily accessible compartment where relevant information can be extracted to tailor treatment on an individual patient-basis.

All studies conducted with checkpoint blockers and monitoring changes in the different blood immune cell subsets showed that baseline parameters at the start of therapy should be taken in account to predict the outcome of the specific therapeutic agent. The presence of specific immune cell subsets at baseline has an impact on immunotherapy efficiency, explaining potential resistance of different individuals. Timing should be considered during immunomonitoring, changes might be transient and only detectable during first weeks of treatment.

The prediction potential of the blood has been realized with the venue of the new single cell technology like mass cytometry, single cell RNA-sequencing, epigenetic analysis, multianalyte serum immunoassays, metabolic analysis or glycoprotein modification analysis; and the importance of other components, like the microbiome (41) has been recently evaluated. Other immune compartments like lymphoid organs and bone marrow are also part of the systemic immune response and might play an additional role in immunotherapy efficiency.

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LIST OF ABBREVIATIONS

OS	Overall survival
TME	Tumor Microenvironment
TIL	Tumor Infiltrating lymphocyte
PD-1	Progammed cell death protein-1
CTLA-4	Cytotoxic T-lymphocyte antigen-4
ICB	Immune checkpoint blocker
LDH	Lactate dehydrogenase
TCM	T central memory cell
TEM	T effector memory cell
NSCLC	Non-small cell lung cancer

Table 1. Summary of the findings on peripheral biomarkers

Study	Year	Timing	Immunotherapy	Cancer type	Peripheral findings
(Ferrucci, Ascierto et al. 2018)	2018	Baseline, week 12 and every 12 week thereafter	Ipilimumab i.v. 3mg/kg every 3 weeks for 4 infusions	720 advanced melanoma patients	In blood at baseline: neutrophil-to-lymphocyte ratio > 3 means prognosis worsened.
(Martens, Wistuba-Hamprecht et al. 2016)	2016	Baseline	Ipilimumab: treatment with at least one dose at 3 or 10 mg/kg	Advanced Melanoma: 209 patients (identification and confirmation cohort), validation cohort (406).	Low baseline LDH, absolute monocyte counts (AMC), Lin(-)CD14(+) HLA-DR(-/low)-MDSC frequencies, and high absolute eosinophil counts (AEC), relative lymphocyte counts (RLC), and CD4(+)CD25(+)FoxP3(+)-Treg frequencies are associated with better survival
(71)	2018	Baseline and each cycle	11 patients treated with pembrolizumab 2 mg/kg every 21 days and 146 with nivolumab 3 mg/kg every 14 days	157 patients non-small cell lung cancer	Higher baseline absolute neutrophil count (ANC), AMC, ANC: ALC ratio and myeloid to lymphoid ratio correlated with worse clinical outcomes in patients who underwent anti-PD-1 treatment
(Ku, Yuan et al. 2010)	2010	Week 7 (after 2 doses of ipilimumab)	Ipilimumab 10 mg/kg every 3 weeks for 4 doses	51 refractory melanoma patients	Patients with an absolute lymphocyte count (ALC) ≥1000/μL after 2 ipilimumab treatments had a improved clinical benefit rate and median OS compared with those with an ALC <1000/μL
(Delyon, Mateus et al. 2013)	2013	Baseline, after 1st and 2nd treatment	Ipilimumab 4 doses every 3 weeks 3mg/kg	73 melanoma patients	A lymphocyte count >1000/mm^3 at the start of the second course and an increase in the eosinophil count >100/mm^3 between the first and second infusions were correlated with an improved OS
Rosner, Kwong et al. 2018	2018	Baseline	Nivolumab + Ipilimumab	209 unresectable stage III ou IV melanoma	High relative eosinophils, high relative basophils, low absolute monocytes, low LDH, and a low neutrophil-to-lymphocyte ratio correlated with improved OS
(Simeone, Gentilcore et al. 2014)	2014	baseline and Weeks 4, 7, 10 and 12	Patients received ipilimumab 3 mg/kg every 3 weeks for four doses	95 melanoma patients	Survival was significantly associated with decreasing levels of lactate dehydrogenase, C-reactive protein and FoxP3/regulatory T cells, and increasing absolute lymphocyte count, between baseline and the end of dosing (Week 12)
(Weide, Martens et al. 2016)	2016	Baseline	pembrolizumab	616 patients	Relative eosinophil count (REC) ≥1.5%, relative lymphocyte count (RLC) ≥17.5%, ≤2.5-fold elevation of LDH, and the absence of metastasis other than soft-tissue/lung were associated with favorable OS
(Kelderman, Heemskerk et al. 2014)	2014	Baseline, week 6	4 cycles of 3 mg/kg ipilimumab every 3 weeks	230 cutaneous melanoma patients	Long-term benefit of ipilimumab treatment was unlikely for patients with baseline serum LDH greater than twice the upper limit of normal
(Liakou, Kamat et al. 2008)	2008	Baseline, week 3 (before 2nd dose) and week 7	Ipilimumab at 3 mg/kg, every 3 weeks, for total of 2 doses	bladder cancer (6) Healthy donor (10)	Th1 CD4$^+$ ICOS$^+$ T cells are increased after CTLA-4 immunotherapy in blood and produce IFNγ and recognize the tumor antigen NY-ESO-1
(Fan, Quezada et al. 2014)	2014	N/A	CTLA-4, ICOSL, CTLA-4 + ICOSL, CTLA-4 at D3/6/9/12	B16 model mouse (melanoma and prostate cancer)	Concomitant CTLA-4 blockade and ICOS engagement by tumor cell vaccines engineered to express ICOS ligand enhanced antitumor immune responses in melanoma and prostate cancer in mice.

Table 1. (continued)

Study	Year	Timing	Immunotherapy	Cancer type	Peripheral findings
(Ng Tang, Shen et al. 2013)	2013	Baseline, week 3 before 2nd dose, and week 7	Ipilimumab at 3 mg/kg, every 3 weeks, for total of 2 doses	10 controls, 36 anti-CTLA-4 patients, 10 gp100 DNA vaccine (melanoma and prostate)	ICOS$^+$ CD4 T cells increase after ipilimumab treatment
(Metzger, Long et al. 2016)	2016	2 weeks after tumor implantation	Treated on days 9, 13, and 16	CT26-bearing mice	Anti-OX40 efficacy was impaired with ICOS-L blockade. OX40 stimulation alone was sufficient to drive an increase in the frequency of ICOS expression among all T-cell subsets
(Chaput, Lepage et al. 2017)	2017	Baseline and before each treatment	Ipilimumab 3 or 10 mg/kg every 3 weeks then every 12 weeks	26 patients with MM treated with ipilimumab	Baseline gut microbiota enriched with Faecalibacterium associated with beneficial clinical response to ipilimumab. Ipilimumab led to a higher ICOS CD4+ T cells in blood and higher CD25 in serum.
(Vonderheide, LoRusso et al. 2010)	2010	Baseline, 4 and 8 weeks	tremelimumab (3-10 mg/kg) every 28 days or every 90 days plus exemestane 25 mg daily	26 hormone responsive breast cancer	Treatment was associated in most patients with increased peripheral CD4$^+$ and CD8$^+$ T cells expressing inducible costimulator (ICOS) and a marked increase in the ratio of ICOS$^+$ T cells to FoxP3$^+$ regulatory T cells
(Wang, Yu et al. 2012)	2012	Baseline, after 2 and 4 doses (3 and 6 months)	resected stage IIIC/IV melanoma received ipilimumab with or without a peptide vaccine.	55 melanoma patients	Ipilimumab up-regulated Ki-67 and ICOS on CD4$^+$ and CD8$^+$ T cells at both 3- and 6-month post ipilimumab. At baseline, low Ki-67+EOMES+CD8+ T cells were associated with relapse
(Carthon, Wolchok et al. 2010)	2010	Baseline, after 1/2 dose and after 2/4 doses	Ipilimumab at 3 or 10 mg/kg/ dose	12 urothelial carcinoma bladder cancer patients and 14 melanoma patients	In melanoma and bladder cancer patients: increase in CD4$^+$ICOS^{hi} T cells. CD4$^+$ICOS^{hi} T cells, sustained over a period of 12 weeks of therapy, correlates with increased of clinical benefits Melanoma patients: no significant differences in ICOS expression on T cells from frozen samples as compared with fresh samples.
(Weber, Hamid et al. 2012)	2012	Baseline, week 4, 7 and 12	Ipilimumab 3 mg/kg (n=40) or 10 mg/kg (n=42) every 3 weeks for 4 doses (induction) and vaccines.	197 melanoma patients	At week 7, most patients showed greater humoral responses relative to baseline titers and increases in the percent of activated HLA-DR$^+$ CD4$^+$ and CD8$^+$ T cells with concomitant decreases in naive CD4$^+$ and CD8$^+$ T cells Increases from week 4 observed in central memory, effector memory, and activated ICOS$^+$ CD4$^+$ T cells, but not in ICOS$^+$ CD8$^+$ T cells or in FoxP3$^+$ CD4$^+$ regulatory T cells.
(Chen, Liakou et al. 2009)	2009	Baseline and after treatment	2 doses 3 mg/kg or 10 mg/kg of ipilimumab with a 3-week interval	7 treated patients	Increase in the frequency of CD4$^+$ICOS^{hi} T cells in the blood following treatment
(Wei, Anang et al. 2019)	2019	From 6 to 184 days In average: anti-CTLA-4 (39), anti-PD-1 (25), combo (52), donor (3)	anti-PD-1 and anti-CTLA-4	PBMC from melanoma patients: ipilimumab therapy (13), anti-PD-1 monotherapy (22), combo (13), donor (3)	Following anti–PD-1 or CTLA-4 monotherapy: strong enrichment of Ki-67$^+$ proliferative cells in PD-1$^+$ versus PD-1$^-$ CD8$^+$ T cells anti-CTLA-4: increase of CD4$^+$ T cells, activated CD45RO$^+$ and CD45RO$^+$ TBET$^+$ EOMES$^+$ CD4$^+$ effector T cell, CD45RO$^+$ ICOS$^+$ PD-1$^+$ CD4$^+$ effector T cell Combination therapy: increase in overall frequency of CD8$^+$ T cells and increase of frequency of a terminally differentiated TBET$^+$ EOMES$^+$ CD8$^+$ T cells
(Callahan, Horak et al. 2013)	2013	Not specified	Combination nivolumab and ipilimumab	35 patients advanced melanoma	T-cell subsets, including increases in the percentage of CD4 and CD8 expressing HLA-DR, ICOS and/or Ki-67 were seen with combination therapy

Table 1. (continued)

Study	Year	Timing	Immunotherapy	Cancer type	Peripheral findings
(Yi, Ready et al. 2017)	2017	Baseline and end of treatment	Ipilimumab, neoadjuvant chemotherapy, paclitaxel	24 NSCLC	Increased of ICOS, HLA-DR, CTLA-4, and PD-1 on T cell after ipilimumab, but no association with response.
(Kamphorst, Pillai et al. 2017)	2017	Baseline and every treatment cycle.	anti–PD-1 or anti–PD-L1 blocking antibodies	29 NSCLC patients	Following therapy: increase in effector-like phenotype Ki-67$^+$ PD-1^{hi+} CD8$^+$ T cells (also HLA-DR$^+$, CD38$^+$, Bcl-2^{lo} and CD28$^+$, CD27$^+$, ICOS$^+$) following therapy in ~70% of patients, most responses seen within 4 weeks. 70% of patients with disease progression had either a delayed or absent PD-1$^+$ CD8$^+$ T-cell response, whereas 80% of patients with clinical benefit exhibited early PD-1$^+$ CD8$^+$ T-cell responses
(Huang, Postow et al. 2017)	2017	Baseline and before each treatment	pembrolizumab (2mg/kg) infusion every 3 weeks for 12 weeks.	Patients with stage IV melanoma	Frequency of Ki-67$^+$ CD8$^+$ T cells was increased at 3 weeks after pembrolizumab treatment and then declined in most patients. Ki-67$^+$ (HLA-DR$^+$CD38$^+$) T cells in the blood are reinvigorated by anti-PD-1 therapy and contain T-cell clones that are also present in the tumour
(Das, Verma et al. 2015)	2015	Before and after (not specified)	anti-PD-1, anti-CTLA-4, combination	Cancer patients: anti–PD-1 (n = 24), anti–CTLA-4 (n = 9) or combination therapy (n = 12).	CTLA-4 blockade: induces a proliferative signature in T_{EM} (Ki-67, CTLA-4 and ICOS, IFNγ) PD-1 blockade: changes in genes implicated in cytolysis (granzyme A/B, FCRL3 and KLRF1, IFNγ) Combination blockade: leads to nonoverlapping (Ki-67, granzyme A/B, IL-8 and HLA-DR, IFNγ) changes
(Kagamu, Kitano et al. 2019)	2019	Baseline, between 12 and 92 weeks.	Nivolumab 3 mg/kg body weight every two weeks	non-small lung cancer discovery cohort (40) and validation cohort (86)	Blood: Higher CD62L^{low} CD4$^+$ (T-bet$^+$, CD27$^-$, FOXP3$^-$ and CXCR3$^+$: Th1) % T cells prior to PD-1 blockade (correlated with long-term survival in NSCLC patients). CD25$^+$FOXP3$^+$ CD4$^+$ % T cells were significantly higher in non-responders.
(61)	2013	Baseline and every 4 weeks	Ipilimimuab and GVAX vaccine	28 prostate cancer patients	Significantly prolonged overall survival (OS) was observed for patients with high pre-treatment frequencies of CD4+CTLA-4+, CD4+PD-1+ or low pre-treatment frequencies of differentiated CD4+ or regulatory T cells.
(Manjarrez-Orduno, Menard et al. 2018)	2018	Baseline, 8 and 12 weeks	Nivolumab	22 patients	Patients with, at baseline, high CM/Eff T cell ratios (associated with higher inflammatory signature), had longer progression-free survival (PFS) 3 months after the initiation of nivolumab but treatment did not show major changes in CM/Eff T cell ratios
(Takeuchi, Tanemura et al. 2018)	2018	Before and after (timelines not specified)	Nivolumab (3 m/kg) every 2 weeks .Pembrolizumab (2 m/kg) every 3 weeks	8 malignant melanoma patients (4 as exploratory and 4 as validation).	Anti-PD-1 mAb treatment: increase of CD4T CM (CD27+FAS-CD45RA-CCR7+) after treatment in long-term survivors, but not in non-responders.
(Subrahmanyam, Dong et al. 2018)	2018	Baseline	anti-CTLA-4 and anti-PD-1 therapies according to FDA protocol for metastatic melanoma	67 melanoma patients	Anti-CTLA-4: In non-responders: stronger intensity of CD45RA (in CD4$^+$ and CD8$^+$ T cells) In responders: lower frequencies of CD45RA+ (in CD4+ and CD8+ T cells) higher T_{CM} and T_{EM} cells In anti-PD-1: CD69+ NK cells correlated with clinical response

Table 1. (continued)

Study	Year	Timing	Immunotherapy	Cancer type	Peripheral findings
(Kunert, Basak et al. 2019)	2019	Baseline, 2 weeks (after 1st dose), 4 weeks (2nd dose)	NSCLC patients treated with 3 mg/kg of nivolumab (intravenously every 2 weeks)	71 NSCLC patients Healthy control samples were obtained from 15 donors	In partial response patients: $CD8^+$ T cells at baseline and during treatment is similar to healthy controls, but 2 times higher than in patients with PD or SD. CD8 in PR have more % of TEMRA (CD45RA) and $CD95^+$ or $CD69^-$ and lack stimulatory receptors (ICOS, CD40L, CD28 OX40, 4-1BB).
(Wistuba-Hamprecht, Martens et al. 2017)	2017	Baseline, 2^{nd} dose (D 19–23), 3^{rd} dose (D 40–44) and thereafter (>D45)	104 patients received 4 doses of ipilimumab, whereas in the remaining patients, the treatment was stopped after 1 to 3 doses.	137 late-stage melanoma patients.	Higher levels of CD8 T_{EM} $CD27^+$ $CD28^+$ cells in blood are predictive for good outcome Low abundance of peripheral CD8 T_{EMRA} cells associate with good outcome.
(Herbst, Soria et al. 2014)	2014	Day 1, D2, D8 after cycle 1, first day after each cycle	PD-L1 MPDL3280A, clinical activity was seen from 1 to 20 mg kg, treatment every 3 weeks for 16 cycles	RCC (56), NSCLC (n=53), Melanoma (11), Cutaneous (n=33), Mucosal (n=5), Ocular (4), Others (23)	Increase in IL-18, CXCL11 and CD8+HLA-DR+Ki-67+ T cells and IFN-γ during the first cycle of treatment IL-6 level decreases cycle 2, day 1. Changes not correlated to response or progression.
(Pirozyan, McGuire et al. 2020)	2020	Baseline (36), week 6 (39), 1 year (18).	Prembolizumab or Nivolumab	42 from melanoma patients prior to beginning anti-PD-1 treatment 39 from 6 weeks post-treatment and 18 from the 1-year time point	Responders had a higher frequency of NK and CD56-expressing $CD16^{hi}$ T cells. Patients in the primary resistance group had a higher frequency of classical monocytes.
(Spitzer, Carmi et al. 2017)	2017	3 and 8 days after immunotherapy	anti-PD-1, IFNg + antiCD40 + CD1 alloIg, IFNg + antiCD40 + B6 alloIg	Mice	A $CD4^+$ T cell subset from the periphery is sufficient to mediate anti-tumor immunity. Specific clusters PD-1-$CD127^{low}$ $PD\text{-}1^{low}$ $CD4^+$ T cells were significantly elevated in responders compared to non-responders at both time points
(Comin-Anduix, Lee et al. 2008)	2008	Baseline and at each dose	At least 2 doses of tremelimumab (up to 24) 10 mg/kg monthly	12 patients advanced melanoma. Cohort of HLA-A*0201-positive patients with MART1-positive melanoma	Tremelimumab does not increase the number or function of antigen-specific $CD8^+$ T cells in peripheral blood nor decrease FoxP3 transcripts But increase in CD45RO on $CD4^+$ T cells after 3 doses and increase in HLA-DR on $CD8^+$ T cells after 2 doses significantly differentiated responders from non-responders.
(Tietze, Angelova et al. 2017)	2017	Baseline, and 1-2 days and at each cycle	treated with ipilimumab (n = 21) and pembrolizumab (n = 9)	30 melanoma patients	Baseline levels of $CD45RO^+CD8^+$ T cells correlate with response and longer survival to ipilimumab. ipilimumab: activates $CD8^+$ T cells by increasing $HLA\text{-}DR^+CD25^-$ phenotype, implying antigen non-specific stimulation.
(Jacquelot, Roberti et al. 2017)	2017	Baseline	ipilimumab	190 patients metastatic melanoma	Higher the PD-L1 expression on peripheral T cells the worst regarding progression-free survival. Detectable CD137 on circulating $CD8^+$ T cells was associated with the disease-free status of resected stage III melanoma patients after adjuvant ipilimumab + nivolumab (but not nivolumab alone)

Table 1. (continued)

Study	Year	Timing	Immunotherapy	Cancer type	Peripheral findings
(Hiniker, Reddy et al. 2016)	2016	Baseline, 4 weeks after the last dose of ipilimumab	Ipilimumab every 3 weeks and local radiotherapy	9 melanoma patients	Higher baseline CD8 T_{CM} cells, transient on-treatment increases in MIP-1α and β, and sustained increases in IP-10 and MIG associated with CR/PR. Analysis of immune response data suggested a relationship between elevated CD8-activated T-cells and response
(Du, Hu et al. 2018)	2018	Baseline, D21 and D42	nivolumab (3 mg/kg) or pembrolizumab (2 mg/kg) alone or with chemotherapy in each of cycles.	22 different cancer patients	Responder group showed higher expressions of PD-1 on CD4+ and NK cells than the non-responder group after the first cycle of immunotherapy, and lower expression of CTLA-4, GITR, and OX40 after the second cycle of immunotherapy.
(Cha, Klinger et al. 2014)	2014	Baseline and after 1 month of treatment	Ipilimumab: up to 4 doses (1.5 to 10 mg/kg) every 4 weeks and GM-CSF daily (250 mg/m2) the first 2 weeks of cycles. Tremelimumab: 15mg/kg every 3 month	25 metastatic castration-resistant prostate cancer patients treated with ipilimumab and GM-CSF, 21 metastatic melanoma patients treated with tremelimumab, 9 controls.	Treatment increase TCR diversity. Number of clonotypes that increased with treatment was not associated with clinical outcome. Improved overall survival was associated with maintenance of high-frequency clones at baseline.
(Robert, Tsoi et al. 2014)	2014	Baseline and 30 to 60 days	tremelimumab. Administered at 15 mg/kg every 3 months	21 patients metastatic melanoma	CTLA-4 blockade diversifies the peripheral T-cell pool
(Rizvi, Hellmann et al. 2015)	2015	Baseline, D21, D44, D63, D256, D297	pembrolizumab, treated at 10mg/kg every 2-3 weeks	1 patient had stage IV non-small cell lung cancer (NSCLC)	Anti–PD-1-induced neoantigen-specific T cell reactivity in blood
(Baitsch, Baumgaertner et al. 2011)	2011	Mean of 96 days after vaccination	Monthly s.c. 100 ug Melan-A/MART-1 peptide and CpG (500 ug) in IFA (300–600 ul Montanide)	A*0201+ patients with stage III/IV metastatic melanoma 4 A2+ healthy donors	In peripheral blood from patients vaccinated with CpG upon vaccination, differences in T effector cells specific for persistent herpesviruses (EBV and CMV)
(Kvistborg, Philips et al. 2014)	2014	Baseline, post treatment (from 7 to 54 days)	ipilimumab	40 patients with advanced HLA-A*0201 melanoma	Pre- and posttreatment comparison: anti–CTLA-4 Treatment induces early stage (median 2 weeks) an increase in the number of detectable melanoma-specific CD8 T cell.
(Yuan, Adamow et al. 2011)	2011	Baseline; Week 7, 2, 24	Patients received ipilimumab at 0.3 mg/kg (n = 1), 3 mg/kg (n = 4), or 10 mg/kg (n = 139) every 3 wk for four treatments	144 advanced metastatic melanoma patients	NY-ESO-1–seropositive patients had a greater likelihood of experiencing clinical benefit 24 weeks after ipilimumab treatment than NY-ESO-1–seronegative patients. NY-ESO-1–seropositive patients with associated $CD8^+$ T cells experienced more frequent clinical benefit than those with undetectable $CD8^+$ T-cell response and survival advantage.
(Powles, Eder et al. 2014)	2014	Baseline and after treatment	15 mg kg–1 every 3 weeks of atezolizumab	14 patients metastatic bladder	IL-18 and IFN-γ levels transiently increased and $CD8^+HLA\text{-}DR^+Ki\text{-}67^+$ cells increased
(Ribas, Shin et al. 2016)	2016	Baseline and 74 days (mean)	Pembrolizumab: 0 mg/kg every 2 weeks, or 2 mg/kg or 10 mg/kg every 3 weeks	53 patients metastatic melanoma	In peripheral blood, an unusual population of blood cells expressing CD56, HLA-DR, $CD14^{mid}$ and $CD4^{mid}$ population of cells increased by 9-fold from the patients with a response to therapy.

Table 1. (continued)

Study	Year	Timing	Immunotherapy	Cancer type	Peripheral findings
(Dronca, Markovic et al. 2015)	2015	Baseline and at radiographic tumor evaluation	Pembrolizumab 2 mg/kg every 3 weeks	40 (29 with baseline sample available and 14 serial) melanoma patients	In responders the levels of Bim in PD-1^{+} CD8^{+} T cells decreased after the first 3 months of treatment, and they increased/did not change in all non-responders
(Das, Bar et al. 2018)	2018	Baseline and after the first cycle of therapy	anti-PD-1; n=8, anti-CTLA-4; n=8 and anti-PD-1 and anti-CTLA-4 or comb therapy; n=23	35 melanoma patients	CCB therapy led to decline in circulating B cells and an increase in CD21^{low} B cells (with higher PD-1 expression and higher clonality) and plasmablasts. Patients with early B cell changes experienced higher rates of grade 3 or higher IRAEs 6 months after CCB.
(Maker, Attia et al. 2005)	2005	Baseline and 3 weeks after each tmt	every 3 weeks with an anti-CTLA-4 Ab with or without peptide immunization	10 stage IV melanoma or renal cell cancer patients	No decrease in the expression of CD4+CD25+ cells in whole PBMC, nor a decrease in Foxp3 gene expression in the CD4+ or CD4+CD25+ purified cell populations posttreatment. The percentage of CD4^{+}, CD8^{+}, CD4^{+}CD25^{+} and CD4^{+}CD25^{-} T cells in PBMC expressing the activation marker HLA-DR increased following anti-CTLA-4 Ab administration
(Kim, Cho et al. 2019)	2019	Baseline and Day 7 after first dose	Pembrolizumab or Nivolumab	79 NSCLC	Ki-67^{+} cells among PD-1^{+}CD8^{+} T cells 7 days after the first dose significantly predicted durable clinical benefit
(Tarhini, Edington et al. 2014)	2014	Baseline and 6 weeks	Neoadjuvant ipilimumab 10 mg/kg intravenously administered 3 weeks apart	27 melanoma patients	Following ICB, greater decrease in circulating monocyte MDSC Lin^{-} HLA-DR^{-} CD33^{+} CD11b^{+} cells and decrease of T_{regs} CD4^{+}CD25^{hi+}Foxp3^{+} was associated with improved PFS. Baseline evidence of fully activated type I CD4^{+} and CD8^{+} antigen-specific T cell immunity against cancer-testis (NY-ESO-1) and melanocytic lineage (MART-1, gp100) antigens potentiated after ipilimumab.
(Krieg, Nowicka et al. 2018)	2018	Baseline and 12 weeks after anti-PD-1 immunotherapy	Nivolumab 3 mg/kg every 2 weeks or pembrolizumab 2 mg/kg every 3 weeks for 12 weeks	Discovery cohort (20 patients) and validation cohort (31 patients) with melanoma.	Before commencing therapy, a strong predictor of progression-free and overall survival in response to anti-PD-1 immunotherapy was the frequency of CD14^{+}CD16^{-}HLA-DR^{hi} monocytes
(Kitano, Postow et al. 2014)	2014	Baseline, week 6 after therapy (after 2 doses of ipilimumab)	4 doses of ipilimumab at 10 mg/kg or 3 mg/kg i.v. every 3 weeks	Discovery cohort (28 patients) and validation cohort (40 patients) with melanoma.	monocytic-MDSC frequencies were inversely correlated with peripheral CD8+ T-cell expansion following ipilimumab: Having less than 14.9% m-MDSC pre-treatment was associated with improved OS among the 68 patients
(Meyer, Cagnon et al. 2014)	2014	Baseline, D3/D7/D20 and after 3–30 months after treatment	Ipilimumab (15), Vemurafenib (10), Ipilimumab followed by vemurafenib (1), untreated (23)	49 patients	Lower percentages of Lin^{-} CD14^{+} HLA-DR^{-} MDSC in patients responding to ipilimumab treatment compared to non-responders
(72)	2020	Baseline, 6 weeks after therapy	Pembrolizumab (6) 3 Nivolumab (3)	9 patients non-small cell lung cancer	Percentages of NK cell populations in the immune cells of PBMCs were prominently elevated in the immunotherapy responder group when compared with non-responders
(Alvarez Secord, Bell Burdett et al. 2020)	2020	Baseline	Carboplatin and Paclitaxel with or Without Bevacizumab	751 patients: epithelial ovarian cancer	IL-6 predictive of a therapeutic advantage with bevacizumab for PFS and OS. Patients with high median IL-6 levels treated with bevacizumab had longer PFS and OS compared with placebo. IL-6 may be predictive of therapeutic benefit from bevacizumab when combined with carboplatin and paclitaxel.

Table 1. (continued)

Study	Year	Timing	Immunotherapy	Cancer type	Peripheral findings
(Sanmamed, Perez-Gracia et al. 2017)	2017	Baseline, 2–4 weeks after the first dose; and at response evaluation.	nivolumab or pembrolizumab or combination nivolumab (1, 2, 3 or 10 mg/kg) or pembrolizumab (2 or 10 mg/kg) intravenously every 2 or 3 weeks	Discovery: 29 melanoma patients. Validation cohort: 19 NSCLC patients and 15 melanoma patients	Serum IL-8 levels decreased between baseline and best response and increased upon progression. Early changes in serum IL-8 levels (2–4 weeks after treatment initiation) were strongly associated with response.
(Hannani, Vétizou et al. 2015)	2015	Baseline, after 1st and 2nd tmt	ipilimumab 3 mg/kg	262 MM patients	High baseline serum levels of sCD25 were associated with compromised ipilimumab effects in MM patients.
(Sabatino, Kim-Schulze et al. 2009)	2009	Baseline	High-dose interleukin-2 (IL-2)	59 patients	Serum VEGF and fibronectin are easily measured pre-treatment biomarkers that could serve to exclude patients unlikely to respond to IL-2 therapy.
(Yuan, Zhou et al. 2014)	2014	Baseline and at completion (week 12)	176 patients treated with ipilimumab at 3 (n = 98) or 10 mg/kg (n = 68).	176 MM patients	Baseline sVEGF ≥ 43 pg/mL was associated with decreased OS no correlation between VEGF changes and clinical outcome

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