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REVIEW

Inhibitory Immune Checkpoint Molecules and Exhaustion of T cells in COVID-19

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Summary

COVID-19 (Coronavirus Disease) is an infectious disease caused by the coronavirus SARS-CoV-2 (Severe acute respiratory syndrome Coronavirus 2), which belongs to the genus *Betacoronavirus.* It was first identified in patients with severe respiratory disease in December 2019 in Wuhan, China. It mainly affects the respiratory system, and in severe cases causes serious lung infection or pneumonia, which can lead to the death of the patient. Clinical studies show that SARS-CoV-2 infection in critical cases causes acute tissue damage due to a pathological immune response. The immune response to a new coronavirus is complex and involves many processes of specific and non-specific immunity. Analysis of available studies has shown various changes, especially in the area of specific cellular immunity, including lymphopenia, decreased T cells ($CD3^+$, $CD4^+$ and $CD8^+$), changes in the T cell compartment associated with symptom progression, deterioration of the condition and development of lung damage. We provide a detailed review of the analyses of immune checkpoint molecules PD-1, TIM-3, LAG-3 CTLA-4, TIGIT, BTLA, CD223, IDO-1 and VISTA on exhausted T cells in patients with asymptomatic to symptomatic stages of COVID-19 infection. Furthermore, this review may help to better understand the pathological T cell immune response and improve the design of therapeutic strategies for patients with SARS-CoV-2 infection.

Key words

Inhibitory checkpoint molecules • T cell exhaustion • COVID-19 • SARS-CoV-2

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Introduction

At the end of 2019, a new respiratory coronavirus SARS-CoV-2, which causes COVID-19 disease, was discovered in Wuhan, China (Chan *et al.* 2020).

Clinical studies have shown that SARS-CoV-2 causes complex changes in cellular immunity, characterized by lymphopenia, neutrophilia, eosinopenia and a distorted distribution of T cell subpopulations and high plasma concentrations of pro-inflammatory cytokines. Massive cytokine release syndrome, also known as cytokine storm, is observed in polymorbid, elderly cases or in patients with a certain genetic predisposition. A cytokine storm can lead to the progression of the disease to a severe stage with damage to organs, mainly the lungs (Zhang *et al.* 2020, Shi *et al*. 2020). Genomic interindividual variability could at least partially explain difference in clinical manifestation of SARS-CoV-2 infection (Vašků *et al.* 2020).

It is known that cytotoxic T cells ($CD8^+$) activate other cells of the immune system or are directly involved in pathogen destruction and thus prevent the development of infection (Taylor *et al.* 1986). Detailed studies of the phenotype and function of $CD8^+$ are necessary in understanding their potential role in the pathophysiology of the disease and the development of therapeutic approaches (Ackermann *et al.* 2019). Following the induction of effector T cells with high cytotoxic and proliferative activity, it is essential to prevent excessive host immune responses. T cell activity is regulated by the co-expression of stimulatory and inhibitory receptors (Legat *et al.* 2013). The inhibitory receptors and their role in the exhaustion of T cells have been studied in detail, mainly in cancer, chronic viral infection such as hepatitis B (HBV), hepatitis C (HCV) and human immunodeficiency virus (HIV) and active viral infection, including Hantavirus and Ebola (Ackermann *et al.* 2019, McLane *et al.* 2019, Kong *et al.* 2020). The term "T cell exhaustion" is used mostly for effector T cells with a reduced ability to secrete cytokines and an increased expression of inhibitory receptors. These cells are considered hypofunctional or even dysfunctional effector T cells. The difference between normal and exhaustion T cells is in the response to long-term antigen exposure (Blank *et al.* 2019). However, the exact role and kinetics of co-inhibitory molecules in the early stages of infection have not yet been clarified. Furthermore, it is still unknown how and when T cell exhaustion and the loss of effector function occurs. Similarly, it is unclear whether cell exhaustion plays a role in the pathophysiology of acute infections (Legat *et al.* 2013, Diao *et al.* 2019).

T cell exhaustion, which often occurs during chronic infection and cancer is also found in patients with COVID-19. Previous experimental studies of T cell functionality in patients with COVID-19 have shown an upregulation of inhibitory receptors such as programmed cell death protein (PD-1; CD279), T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3; CD366), lymphocyte-activation gene 3 (LAG-3; CD223) and different expression of other immune checkpoint receptors (Table 1). Therefore, it is possible that T cell exhaustion may play a role in the pathophysiology of COVID-19 infection (Diao *et al.* 2019). However, the upregulation of inhibitory receptors on T cells in acute infections may not necessarily relate to their exhaustion. It can be considered a complex immune activation and a balance of excessive immune system reactivity (Legat *et al.* 2013). Expression of these co-inhibitory receptors has been shown to play a dual role with potentially harmful but also beneficial effects (Abel *et al.* 2018). Overexpression of inhibitory receptors may prevent a hyperactive host immune response. However, this inhibition of T cells may also suppress hyperinflammation by down-regulating T cell effector functions (Butler *et al.* 2011). Accordingly, in patients with COVID-19, it is crucial to determine the level of co-expression and the extent of the various co-inhibitory receptors (Jubel *et al.* 2020).

This review offers a systematic summary of the characteristics of T cell inhibitory checkpoint molecules in association with T cell exhaustion in patients with COVID-19. In addition, it may help to understand the pathophysiology of the disease and develop immunotherapeutic strategies for COVID-19 treatment. We also aimed to summarize the most important selected immune laboratory parameters with respect to the complications and severity of the disease (Fig. 1) (Bonifacius *et al.* 2021).

Immune response to COVID-19

The immune response to infection caused by the new coronavirus is complex dynamic and has not been clearly elucidated. It is biphasic and involves innate and adaptive immunity (Jesenak *et al.* 2020). After initial responses provided by physical barriers, mucosal and innate immunity, T and B cell immunity is gradually

developed with specific antiviral neutralizing antibodies, which result in rapid elimination of the virus (Rizzo *et al.* 2020). In severe cases, the course of the infection is more complicated. After the initial phase, which usually has the character of a classic respiratory viral infection, the infection gradually progresses. Secondary bacterial infections form and worsen over the course of the disease. In pneumonia, respiratory insufficiency occurs with the development of ARDS. The severity of lung damage correlates with extensive pulmonary infiltration of neutrophils and macrophages and higher number of these cells in the peripheral blood. Neutrophils are known to be a major source of chemokines and cytokines. It is the cytokine storm which can lead to the development of ARDS. The infection gradually disseminates throughout the body, resulting in sepsis, tissue destruction and multiorgan failure (Wu *et al.* 2019).

Significant deviations from the reference values have been observed after evaluation of several laboratory parameters in patients with mild and severe courses. Many clinical studies clearly indicate impairment of specific cellular immunity, mainly lymphopenia, differences in number of T cells ($CD3^+$), cytotoxic T cells ($CD4^+$), helper T cells ($CD8^+$), T follicular helper cells, γδ-T cells, and regulatory T cells (T_{regs}). Similarly, changes in B cells have been observed, such as decline of transitional cells, double-negative B cells and antibody-secreting cells (Hasan *et al.* 2021).

Lymphopenia associated with SARS-CoV-2 infection can be considered an important pathological finding and a sign of disease severity (Diao *et al.* 2019). Several authors report that lymphopenia affects $CD4^+$ T cells, $CD8^+$ T cells, B cells and NK cells (Kuri-Cervantes *et al.* 2020, Liao *et al.* 2020, Giamarellos-Bourboulis *et al.* 2020). In a comparison of all the analyses, the highest proportion of patients with lymphopenia was observed in a study by Guan *et al.* (2020). At admission, significant lymphocytopenia was observed in nearly 83.2 % of a total cohort of 1.099 patients. It has been observed that lymphocytopenia occurred in 96.1 % of patients with a severe course of the disease, compared to 80.4 % of patients with less symptomatic or asymptomatic infection (Guan *et al.* 2020).

In a retrospective cohort study, Wu *et al.* (2020) observed lymphocytopenia in 64 % of 201 patients with confirmed COVID-19 pneumonia (Wu *et al.* 2020). Regarding the above-mentioned results of the analyses, the percentage of lymphocytes was proposed as a predictive biomarker of disease severity (Tan *et al*. 2020).

Detailed analysis of two major subtypes of T cells revealed increased levels of $CD4^+$ naïve T cells ($CD3^+$ $CD4^+$ $CD45RA^+$) in severe cases, whereas the percentage of memory helper T cells ($CD3^+$ $CD4^+$ $CD45RO^+$) was reduced in samples of peripheral blood patients with COVID-19. The reduction of T_{regs} ($CD3^+$ $CD4^+$ $CD25^+$ $CD127^{low+}$) in SARS-CoV-2 infection also contributes to an excessive inflammatory response (Guan *et al.* 2020).

In addition, examination and evaluation of immune parameters can be a suitable prognostic marker for the early diagnosis of unexpected complications and different degrees of disease severity. Similarly, a better understanding of changes in the immunoprofile of patients with COVID-19 may lead to new possibilities in therapeutic intervention or immune support (Jesenak *et al.* 2020).

Besides the above-mentioned changes which occur in the immune profile, another possible consequence of insufficient immune control over COVID-19 pneumonia may be T cell exhaustion and increased expression of the inhibitory immune checkpoint molecules on their surfaces (Zheng *et al.* 2020). To design therapeutic strategies for the treatment of COVID-19, it is necessary to investigate the signalling pathways of the inhibitory checkpoint molecules which regulate the immune system (Bersanelli *et al.* 2020).

PD-1

Programmed cell death protein 1 (PD-1; CD279) is a protein which is present on the surface of T cells and pre-B cells and acts as an immune checkpoint molecule. After binding PD-1 of soluble ligands PD-L1 (B7-H1; CD274) and PD-L2 (B7-DC; CD273), they commute to the downregulation of excessive immune response, and thus maintains self-tolerance by suppressing the inflammatory activity of T cells. This mechanism protects an organism against the development of autoimmune diseases. PD-1 also causes the escape of cancer cells from the host immune system control (Syn *et al.* 2017). Furthermore, PD-1 supports apoptosis of specific T cells in lymph nodes. However, it reduces the apoptosis of regulatory T cells (anti-inflammatory, suppressive T cells) and thus indirectly increases the regulation of immune cells (Fife *et al.* 2011). Increased expression of PD-1 is one of the main indicators of T cell exhaustion (e.g. in chronic infection or cancer) (Syn *et al.* 2017, Pauken *et al.* 2015).

Table 1. Mechanism of action of immune checkpoint inhibitors (ICI) in COVID-19.

Molecule		Pathway of ICI in global immune responses during SARS-CoV-2 infection	References
PD-1	↑	Expression on $CD4^+$ T cells in severe cases	Kuri-Cervantes *et al.* 2020
	↑	Expression on $CD4^+$ and $CD8^+$ in severe cases	Diao *et al.* 2019
	↑	Expression on $CD4^+$ and $CD8^+$ in non-survivors	Bobcakova *et al.* 2021
	↑	Concentration sPD-1 in severe cases	Kong *et al.* 2020
	↑	Expression on NK cells in critical cases	Demaria *et al.* 2020
TIM-3	↑	Expression on $CD8^+$ in non-survivors	Shahbazi *et al.* 2021
	↑	Expression on $CD4^+$ and $CD8^+$ in severe cases	Martín-Quirós *et al.* 2021
	↑	Expression on CD4+, CD8+ and NK cells in severe cases	Varchetta *et al.* 2021
	-	No significant difference in expression between survivors and non-survivors	Bobcakova *et al*. 2021
	↑	Expression on $CD4^+$ and $CD8^+$ in severe cases	Herrmann *et al.* 2020
LAG-3	↑	Expression in macrophages and $CD8^+$ from BALF	Saheb Sharif-Askari *et al.* 2021
	↑	Expression of mRNA LAG-3 in nasopharyngeal swabs and lung autopsies	Saheb Sharif-Askari *et al*. 2021
	↑	Concentration sLAG-3 in severe cases	Kong *et al*. 2020
	↑	Expression on $CD4^+$ and $CD8^+$ T cells in different severity groups	Herrmann *et al*. 2020
	↑	Expression on $CD4^+$ in mild and severe cases	Rendeiro *et al*. 2020
CTLA-4	↑	Expression on $CD4^+$ and T_{regs}	Jeannet *et al*. 2020
	-	No significant differences between mild versus severe cases	Zheng *et al.* 2020
	↑	Serum levels of sCTLA-4	Kong *et al*. 2020
TIGIT	-	No difference in expression between healthy controls, mild and severe cases	Herrmann *et al.* 2020
	-	Expression on $CD4^+$ cells and $CD8^+$ cells was similar to that of healthy controls	Schultheiß *et al.* 2020
	-	Expression on $CD4^+$ and $CD8^+$ was lower in non-ICU than in healthy controls and ICU patients	Gutiérezz-Bautista *et al.* 2020
	↑	Expression on $CD4^+$ in mild and severe cases	Zheng *et al.* 2020
	↑	Expression on $CD8^+$ in healthy and mild cases compared to severe patients	
	↑	Expression on $CD4^+$ cells in hospitalized and non-hospitalized COVID-19 patients compared to healthy controls	Files *et al.* 2021
	↑	Expression on $CD8^+$ over time in non-hospitalized patients	
	↓	Expression on $CD8^+$TCM, $Ki67^+CD4^+$ and $Ki67^+CD8^+$ in COVID-19 patients	Breton *et al.* 2021
BTLA	↑	Expression on $CD4^+$ and $CD8^+$ in COVID-19 patients with active disease	Schultheiß *et al.* 2020
	↓	Expression in transient and effector memory $CD8^+$ cells pronounced less significantly in COVID-19 patients than in healthy controls	Herrmann *et al.* 2020

CD224	↑	Expression on $CD4^+$ and $CD8^+$ in severe and mild cases	Li *et al.* 2020
VISTA	↑	Expression on $CD4^+$ and $CD8^+$ in mild and severe COVID-19 patients	Rendeiro *et al.* 2020
IDO	↑ ↓	Percentage in severe versus both mild COVID-19 patients and healthy donors Expression on monocytes and polymorphonuclear myeloid-derived suppressor cells in severe patients	Tomić *et al.* 2021

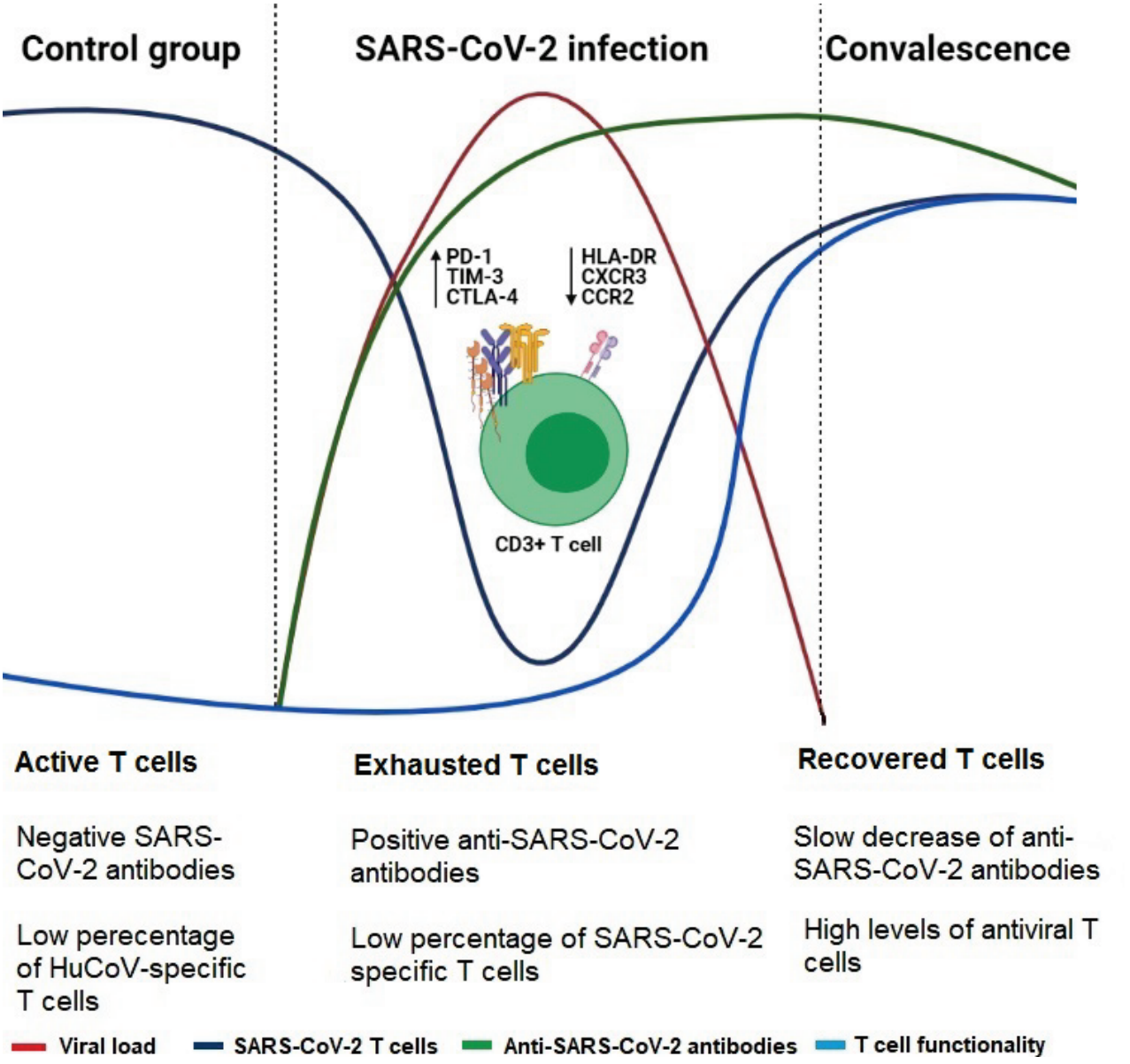

Fig. 1. Graphical representation of T cell functionality and immune response during SARS-CoV-2 infection. The immune response is dysregulated and involves innate and adaptive immunity. During the severe-to-critical form of SARS-CoV-2 infection, an impaired cellular immune response is observed: decreased T cells including $CD4^+$ and $CD8^+$; dysfunctional T cells express marker of exhaustion PD-1, TIM-3, LAG-3; anti-SARS-CoV-2 antibodies and SARS-CoV-2 specific memory T cells (huCoV-human coronavirus) are progressively produced (adapted from Bonifacius *et al.* 2021). (Created in BioRender.com).

Considering physiological conditions, PD-1 negatively regulates the immune response. Signal transmission occurs only if it is associated with a T cell or a B cell receptor. PD-1 inhibits the PI3K/AKT/mTOR intracellular signalling pathway and MAP kinase pathway (Ras/MEK/Erk; inhibition of Ras protein). Inhibition of these signalling pathways leads to a blockade of the cell cycle. It is known that inhibition of the PI3K pathway stimulates apoptosis (Parry *et al.* 2005). This leads to a reduction in the expression of the apoptosis inhibitor B-cell lymphoma-extra large (BcL-xl), which is otherwise expressed in costimulatory signaling *via* CD28 (Chemnitz *et al.* 2004). Blockade of the PI3K/AKT/mTOR pathway also results in activation (absence of inhibitory phosphorylation) of the transcription factor forkhead box protein O1 (FoxO1), leading to an increased expression of PD-1 by positive feedback. This contributes to the exhausted phenotype of T cells (Staron *et al.* 2014).

The role of PD-1/PD-L1 axis have been studied especially in cancer immunotherapy. PD-L1, the ligand for PD-1, is highly expressed in several types of cancer such as lung cancer, kidney cancer or melanoma. Therefore, the role of PD-1 in cancer immune evasion is well established. Inhibition of the interaction between PD-1 and PD-L1 may increase T cell-mediated immune response and preclinical antitumor activity. Pembrolizumab and nivolumab, monoclonal antibodies targeting PD-1 receptor, are used in clinical practice to treat metastatic melanoma, non-small cell lung cancer, head and neck squamous cell carcinoma, urothelial carcinoma and Hodgkin lymphoma (Alsaab *et al.* 2013).

In chronic viral infections, the principal role of PD-1 in the regulation of T cell exhaustion was well-defined in profiling of the gene expression of virus-specific $CD8^+$ T cells in chronic lymphocytic choriomeningitis (LCMV) (Barber *et al.* 2006).

The effect of dysfunction or physical destruction of antigen-specific T cells has been also found in HIV infection. PD-1 expression on $CD8^+$ T cells has been positively correlated with high viral load, disease progression, decreased $CD4^+$ counts, and impaired of $CD8^+$ T cell function. *In vitro* studies have reported that blocking of the PD-1 signaling pathway has led to the recovery of T cell functions (Day *et al.* 2006). Control of the immune system also improved by increasing T cell proliferation and cytokine production (Palmer *et al.* 2013). In addition, after *in vivo* administration of anti-PD-L1 antibodies, the percentage of $CD4^+$ and $CD8^+$ T cells increased. It led to a blockade of viral replication in HIV-infected mice (Di Cosimo *et al.* 2020).

Importantly, it has been demonstrated that in SARS-CoV-2 alveolar infection, early anti-PD-1 therapeutic intervention is required and may decrease the number of exhausted T cells and block the development of ARDS (Fig. 2) (Vivarelli *et al.* 2021).

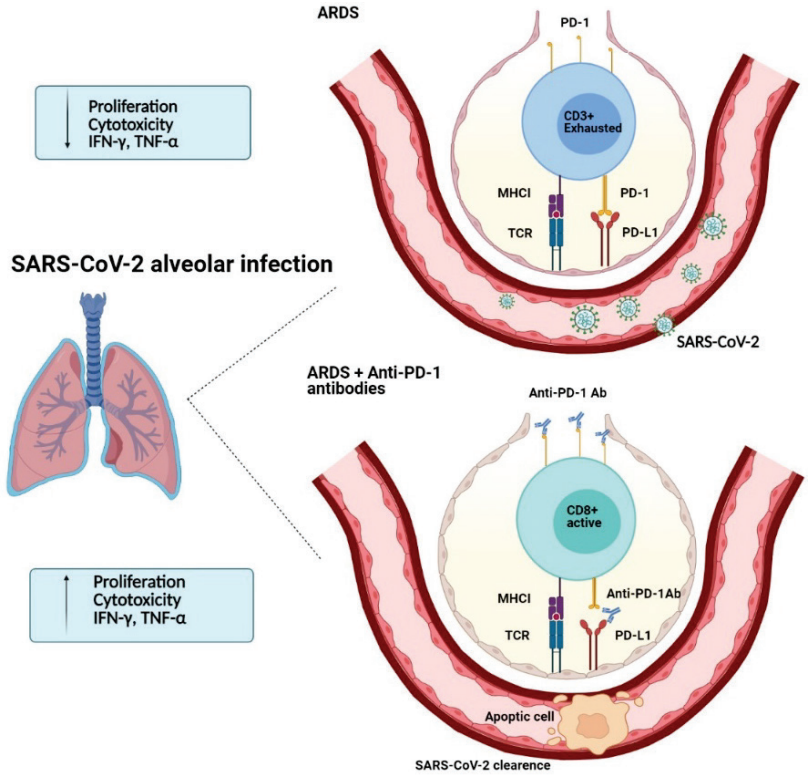

Fig. 2. SARS-CoV-2 alveolar infection in severe-to-critical degree of the disease could promote ARDS. Immunotherapy with anti-PD-1 antibodies can recover the function of cytotoxic T cells and maintain optimal viral clearance (adapted from Vivarelli *et al.* 2021). (Created in BioRender.com).

Kuri-Cervantes *et al.* (2020) observed increased activation of $CD4^+$ and $CD8^+$ T cells in patients with severe course of COVID-19 compared to the control groups. In summary, the frequency of PD-1 on $CD4^+$ T cells, but not $CD8^+$ T cells, were higher in the severe group of COVID-19 than in the healthy donors (HD). Activation of $CD4^+$ and $CD8^+$ T cells in recovered patients was equivalent to the HD group. In mild or severe COVID-19, PD-1^+ $CD4^+$ T cells, but not PD-1^+ $CD8^+$ cells, correlated with donor age. The percentage of PD1^+ $CD4^+$ T cells also correlated with acute physiology and chronic health evaluation (APACHE) III score (Kuri-Cervantes *et al.* 2020). Another retrospective study analysed 522 patients with COVID-19. The percentage of total $CD8^+$ $CD4^+$ T cells was significantly lower, especially in patients who required intensive care unit (ICU) care. The study also examined whether $CD4^+$ and $CD8^+$ had exhaustion phenotypes. Flow cytometry analysis showed that compared to healthy individuals, patients with severe COVID-19 syndrome had considerably high percentages of PD-1^+ $CD8^+$ and PD-1^+ $CD4^+$. PD-1 expression on the surface of T cells was also monitored in three patients during hospitalization. These patients had a very low percentage of PD-1 on $CD4^+$ and $CD8^+$ in the initial stage of the disease. However, the expression of PD-1 on $CD8^+$ increased gradually, depending on the disease progression and ICU period stages. Otherwise, the percentage of PD-1 on $CD4^+$ T cells was not obviously affected during disease progression. These results clearly indicated that T cells are exhausted in patients with COVID-19 during SARS-CoV-2 infection (Diao *et al.* 2019).

A Slovak study demonstrated the expression of immune cell exhaustion markers in 21 patients sorted into four groups according to disease severity. Overall, the results showed notably lymphopenia and the depletion of $CD3^+$, $CD4^+$, $CD8^+$ and $CD19^+$ T cell subpopulations in correlation with the progress of the disease. Patient recovery was correlated with a significant increase in $CD3^+$ and $CD3^+$ $CD4^+$ T cells. Significantly higher expression levels of PD-1 on $CD4^+$ and $CD8^+$ were found in non-survivors than in survivors. Patients with a severe degree of infection which led to death also had a significantly higher percentage of activated $CD38^+CD8^+$ cells and a lower proportion of $CD38^+$HLA-DR^+CD8^+. Clinical improvement was associated with a significantly decreased expression of $CD38^+CD8^+$ T cells. Furthermore, AUC values derived from the ROC curve were also analysed. The highest AUC values within univariate and multivariate logistic regression were recorded for $CD38^+$ on $CD8^+$ and PD1 on $CD4^+$ T cells. This suggests that co-expression of $CD38^+$ on $CD8^+$ and PD-1 on $CD4^+$ T cells or their independent expression may represent a promising predictive biomarker of unfavorable prognosis of COVID-19 (Bobcakova *et al.* 2021).

Although above mentioned membrane-bound checkpoint inhibitors are of major importance and have become the scientific topic worldwide, concentration of their soluble isoforms should also be of interest, as they are either the product of membrane cleavage or alternative mRNA splicing and participate in competitive regulation of their membrane-bound counterparts (Gu *et al.* 2018).

A Chinese study investigated the relationship between soluble checkpoint molecules and the progression of COVID-19. The immune profile was evaluated in 109 patients with a confirmed diagnosis of COVID-19. The patients were divided into groups according to clinical symptoms and disease severity. Serum concentrations of 14 soluble checkpoint inhibitors (sBTLA, sGITR, sHVEM, sIDO, sLAG-3, sPD-1, sPD-L1, sPD-L2, sTIM-3, sCD28, sCD80, s4-1BB, sCD27 and sCTLA-4) were evaluated within three days of admission to the hospital. It was found, that serum levels of all tested molecules, except PD-L2, were significantly higher in critical cases than in mild-to-moderate cases and asymptomatic groups. Additionally, dynamic analysis showed that 11 molecules (sGITR, s4-1BB, sTIM-3, sCD27, sLAG-3, sPD-1, sCD28, sCTLA-4, sBTLA, sHVEM and sCD80) had higher concentrations in patients with a severe course of the disease compared to moderate cases during hospitalization. The result of flow cytometric analysis was higher levels of the glucocorticoid-induced tumor necrosis factor receptor (GITR), CD137, TIM-3, CD27, PD-1, and LAG-3 on $CD4^+$ and $CD8^+$ T cells in critical patients than in milder patients (Kong *et al.* 2020).

TIM-3

TIM-3 is a negative regulator of immune response, and together with PD-1 and lymphocyte activation gene 3 protein (LAG-3), is highly expressed on dysfunctional or exhausted $CD8^+$ T cells (Blackburn *et al.* 2009). The key tasks of TIM-3 are the inhibition of Th1 responses and the expression of cytokines such as INF-γ and TNF. The role of TIM-3 in of T cell exhaustion was

first identified in patients with HIV infection (Jones *et al.* 2008). As a checkpoint receptor, TIM-3 controls T cell response against different chronic viral infections such as HBV, HCV and Friend virus (Takamura *et al.* 2010).

The study of co-expression of exhaustion markers PD-1, TIM-3 and CD39 on $CD8^+$ involved 44 patients, specifically 17 subjects in a critical group, 27 patients in a non-critical group, and 14 healthy controls. Their findings showed that the percentage of $CD8^+$ T cells was significantly lower in patients with mild and severe COVID-19 pneumonia than in a healthy group (Shahbazi *et al.* 2021). This finding is consistent with a previous report and confirms that the percentage of T cells could be used as a diagnostic marker of COVID-19 (Chen *et al.* 2020). In this univariate analysis, critical patients had a higher number of $CD8^+$ $TIM\text{-}3^+$ than patients with a mild course of the disease or the healthy controls. Similarly, the percentage of $CD8^+$ $TIM\text{-}3^+$ cells was significantly higher in non-critical patients than in a healthy subjects. This analysis showed that SARS-CoV-2 activates high cytotoxic activity of T lymphocytes in the initial stage of the infection, which continues in their exhaustion. Presumably, the excessive depletion of $CD8^+$ T cells in critical patients attenuates the cellular response to SARS-CoV-2 (Shahbazi *et al.* 2021).

An interesting case report was presented by Martín-Quirós *et al.* (2021), who analysed the Galactin-9/TIM-3 axis in different clinical courses of SARS-CoV-2. The cohort involved 57 patients, including one married couple. The married couple was exposed to the same dose of SARS-CoV-2 before the onset of the infection and had the same clinical characteristics with bilateral interstitial pneumonia, respiratory failure and almost similar laboratory findings. Patient 1 was a 60-year-old overweight male and his anamnesis showed hypertension, type 2 diabetes and hemochromatosis type 1 with no hepatic or systemic involvement. The course of the infection was moderate, but the patient was hospitalized after 10 days due to worsening of dyspnea. The physical examination showed tachycardia, moderate tachypnea and disseminated crackling rales on pulmonary auscultation. An RT-PCR for SARS-CoV-2 confirmed the COVID-19 infection. Patient 2 was a non-obese 60-year-old woman without previous comorbidities. The course of infection progressed with moderate symptoms, such as asthenia myalgia, low-grade fever, and nausea with episodes of vomiting. Similarly, COVID-19 was confirmed by SARS-CoV-2 RT-PCR. The immune profile of patients was repeatedly monitored by flow cytometry until the exitus of patient 1 and the discharge of patient 2. The expression of TIM-3 on $CD3^+$ cells was significantly increased in patient 1, while in patient 2, the percentage of TIM-3 was the same as in the control group. Low levels of $CD4^+$ and $CD8^+$ subpopulations were confirmed in the critical patients (patient 1) and remained unchanged throughout the infection. In addition, TIM-3 levels were remarkably higher on the $CD4^+$ and $CD8^+$ T cells of patient 1 than patient 2. Analysis of soluble Gal-9 in plasma at the onset of infection were increased in both patients. However, eight days after the initial stage of the infection, a significantly different level of sGal-9 was observed between patients 1 and 2. This difference was correlated with different clinical disease progression. Levels of sGal-9 were significantly higher in non-surviving patients than survivors. The data obtained suggest that the concentration of sGal-9 and the expression of TIM-3 on T cells could represent a prognostic biomarker for patients at high risk of developing secondary bacterial infections, even sepsis (Martín-Quirós *et al.* 2021).

An extensive analysis of the immune response was performed in 32 patients with radiologically confirmed mild-to-severe pneumonia and a SARS-CoV-2 RNA positive nasopharyngeal swab. It has been found that in COVID-19 infection, not only T cells but also NK cells showed an exhausted phenotype. The absolute count of NK cells was significantly lower in patients with COVID-19 while the control group had the reference number. In brief, increased expressions of CD69 and TIM-3 on NK cells, $CD4^+$ and $CD8^+$ T cells were observed in patients with poor outcome compared to healthy subjects. NK cell exhaustion was confirmed by increased frequencies of $PD\text{-}1^+$ and reduced frequencies of natural killer group 2 member D (NKG2D), DNAX accessory molecule-1 (DNAM-1) and sialic acid-binding Ig-like lectin 7 (Siglec-7) expressing NK cells, associated with a reduced ability to secrete IFN-γ (Varchetta *et al.* 2021). In addition, Demaria et al. (2020) identified a depleted NK cells phenotype by increased PD-1 expression on NK cells in critical patients (Demaria *et al.* 2020). Increased $CD8^+$ T cell frequencies were also observed in 7 patients who underwent regular testing after recovery. Simultaneously, there was a significant reduction in expression of TIM-3 on $CD8^+$ and NK cells. By contrast, no functional renewal of the NK cells was observed, suggesting that functional changes in COVID-19 were more pervasive than expected. In

general, this study confirmed that patients with unfavorable prognosis have functionally exhausted NK cells and hyperactivated/exhausted T cells (Varchetta *et al.* 2021).

In another retrospective observational study that contributed to characterization of the immunological profile, a significantly increased PD-1 expression was observed on $CD4^+$ and $CD8^+$ T cells in non-survivors compared to survivors. However, no significant difference in TIM-3 expression was observed, which showed possible reversibility of immune paralysis in the most severe group of patients with COVID-19. Expression of TIM-3 on both $CD4^+$ and $CD8^+$ was significantly reduced during clinical recovery (Bobcakova *et al.* 2021).

LAG-3

Lymphocyte-activation gene 3 (LAG-3; CD223) is a CD4-related, activation-induced cell surface molecule which is expressed upon antigen stimulation on activated $CD4^+$ and $CD8^+$ T cells (Huard *et al.* 1995), NK cells (Triebel *et al.* 1990), B cells (Kisielow *et al.* 2005) and plasmacytoid dendritic cells (Workman *et al.* 2009). The expression of LAG-3 on DCs may result in signalling pathways which upregulate TNF-α and IL-12. Sharing several structural similarities with CD4, it also binds to MHC class II molecules, but with significantly higher affinity, possibly acting as a natural competitor of CD4 (Huard *et al.* 1995), and was shown to downregulate the expansion of activated T cells (Workman *et al.* 2002). LAG-3 negatively regulates cellular proliferation, activation, and homeostasis of T cells (Workman *et al.* 2003, Huang *et al.* 2004).

In 2010, LAG-3 was defined as a marker of $CD8^+$ T cell depletion in lymphocytic choriomeningitis (LCM). However, in this case, LAG-3 did not significantly contribute to T cell exhaustion on it is own (Lucas *et al.* 2011). Multiple transcriptional regulators such as thymocyte selection-associated high mobility group box protein (TOX), interferon regulatory factor 4, nuclear receptor subfamily 4 group A (NR4A), B lymphocyte-induced maturation protein-1, and nuclear factor of activated T cells (NFAT) are also known to be involved in T cell depletion. Based on preclinical data, the therapeutic benefit of agents which block or stimulate LAG-3 functions is expected, especially in the treatment of cancer and autoimmune diseases in combination with substances aimed at inhibiting PD-1 (Maruhashi *et al.* 2020).

Currently, only a few studies have examined the role of LAG-3 in SARS-CoV-2 infection. A transcriptomics study and subsequent *in silico* analysis evaluated the expression profiles of 38 selected immune inhibitor receptors (IRs) during SARS-CoV-2 infection. The expression levels of IRs were analyzed in nasopharyngeal swabs (NPSs) in 430 RT-PCR-confirmed COVID-19 patients and 54 negative controls by RNA sequencing. RNA-seq analyses revealed an overall upregulation of IRs mRNA during the acute phase of SARS-CoV-2 infection. Accordingly, eight genes (*BTLA, LAG3, FCGR2B, PDCD1, CEACAM1, CTLA4, CD72*, and *SIGLEC7*) shared by NPSs and autopsies were more expressed in autopsies and directly correlated with viral levels. The upregulation of PD-1, CTLA4, BTLA and LAG-3 was also observed on macrophages and $CD8^+$ T cells isolated from bronchoalveolar lavage fluid in patients with severe COVID-19. The observed upregulation of LAG-3 mRNA in NPS may cause a negative feedback mechanism in response to immune activation, while increased expression of LAG-3 in lung autopsies and macrophages could most likely cause terminal differentiation of these cells (Saheb Sharif-Askari *et al.* 2021). Thus, epigenetic modulation of immune regulatory genes has also been reported in other viral infections and may represent a mechanism of SARS-CoV-2 immune escape (Menachery *et al.* 2018). Activation of these IRs could suppress innate and adaptive immune responses, resulting in a defect in viral clearance (Saheb Sharif-Askari *et al.* 2021). A study of soluble checkpoint molecules in 109 patients with confirmed COVID-19 infection showed that sLAG-3 was persistently higher in severe cases than in moderate cases during hospitalization. Simultaneously, flow cytometric analysis revealed greater levels of LAG-3 on $CD4^+$ and $CD8^+$ T cells in severe case patients than in those from mild-to-moderate patients (Kong *et al.* 2020).

An increased expression of LAG-3 and TIM-3 on both $CD4^+$ and $CD8^+$ T cells was observed across all severity groups of COVID-19 patients, with the level of their expression depending on disease severity; higher expression was described in severe cases than in cases with milder forms. Recovery was associated with a rapid decline in PD-1, Tim-3 and LAG-3 expression. Compared to healthy controls, significantly higher frequencies of single positive $PD1^-LAG3^+$ and $PD1^-Tim3^+$ $CD4^+$ and $CD8^+$ cells, and double positive $PD1^+LAG3^+$ and $PD1^+Tim3^+$ $CD4^+$ and $CD8^+$ cells were present. The level of activation markers expression

(CD69, CD38 and HLA-DR) was related to the co-expression of inhibitory receptors (PD1, LAG-3, TIM-3) (Herrmann *et al.* 2020).

A study by Rendeiro *et al.* (2020) revealed that both mild and severe COVID-19 patients had increased frequencies of LAG-3 positive $CD4^+$ cells (Rendeiro *et al*. 2020). It therefore remains necessary to investigate whether upregulation of inhibitory receptors on T cells may have a beneficial or adverse effect on the course of infection (Dookie *et al.* 2020). There are also discussions whether T cell exhaustion can occur during acute viral infections, as expression of inhibitory receptors is also induced by T cell activation and differentiation. However, LAG-3, either alone or in combination with other immune checkpoints, is an ideal target for immunotherapy (Legat *et al.* 2013).

CTLA-4

Cytotoxic T Lymphocyte Antigen 4 (CTLA-4; CD152) is expressed on both $CD4^+$ and $CD8^+$ cells, and its interaction with CD80 and CD86 on antigen presenting cells (APCs) is responsible for the inhibition of T cell responses, in contrast to CD28, which, sharing the same ligands CD80 and CD86, in turn leads to stimulation (Rowshanravan *et al.* 2018).

Only a small number of studies have analyzed this inhibitory receptor in COVID-19 patients. Jeannet *et al.* (2020) noticed a transient increase in CTLA-4 expression on $CD4^+$ during the first three days of ICU stay and its over-expression on regulatory T cells (Jeannet *et al.* 2020). However, CTLA-4 expression on $CD8^+$ cells did not differ from the healthy controls. By contrast, in a study by Zheng HY *et al.* (2020), no significant differences between CTLA-4 expression in healthy versus mild, healthy versus severe, nor mild versus severe COVID-19 patients were observed (Zheng *et al.* 2020).

A Chinese study compared serum concentrations of soluble forms of 14 checkpoint inhibitors (sBTLA, sGITR, sHVEM, sIDO, sLAG-3, sPD-1, sPD-L1, sPD-L2, sTIM-3, sCD28, sCD80, s4-1BB, sCD27, and sCTLA-4) in patients with asymptomatic versus mild-to-moderate and severe-to-critical COVID-19 patients. Severe-to-critical cases had significantly higher serum levels of all tested molecules, with the exception of PD-L2; this pattern could be observed during the entire hospitalization period for most of the examined molecules, including sCTLA-4 (Kong *et al.* 2020).

TIGIT

T cell immunoglobulin and ITIM domain (TIGIT) is an inhibitory molecule expressed on NK cells and T cells ($CD4^+$, $CD8^+$ and T_{regs}); its expression increases upon cell activation. TIGIT binds to CD155, CD112 and CD113 (in order of decreasing affinity). CD155 can be found on various hematopoietic (DCs, T cells, B cells, macrophages) and non-hematopoietic cells. Similarly to the CTLA-4/CD28 pathway, TIGIT competes for its main ligand (CD155) with other receptors (DNAM-1 and CD-96), which upon binding to CD155, in contrast, induce activation responses (Harjunpää *et al.* 2020).

Studies which examined TIGIT expression in COVID-19 patients offer conflicting results. No difference in TIGIT expression between healthy controls, mild and severe COVID-19 patients was described by Herrmann *et al.* (2020), instead even a decreased trend of its expression (significantly in subset of effector memory $CD4^+$ cells) in COVID-19 patients compared to healthy controls was observed (Herrmann *et al.* 2020). Similar results were reported by Schultheiß *et al.* (2020): the level of expression of TIGIT on $CD4^+$ cells and $CD8^+$ cells was similar to that of healthy controls, however, it was significantly decreased on NK cells in COVID-19 patients with active disease (Schultheiß *et al.* 2020).

Conflicting results were published by Spanish authors. In a study which followed COVID-19 patients with different disease severity (asymptomatic, non-ICU and ICU) and compared the results to healthy controls, TIGIT expression on $CD4^+$ cells was significantly lower in non-ICU patients than in both healthy controls and ICU patients, and on $CD8^+$ cells in non-ICU and asymptomatic patients than in the control group. Control tests were performed on a subgroup of hospitalized patients and confirmed lower levels of $TIGIT^+CD4^+$, $PD1^+CD4^+$ and $PD1^+CD8^+$ cells in recovered patients than in healthy donors. The expression of activation markers HLA-DR and CD38 correlated positively with $CD4^+TIGIT^+$ and $CD4^+PD\text{-}1^+$ cells, while $CD45RA^+$ and $TIGIT^+CD4^+$ and $TIGIT^+CD8^+$ cells correlated negatively. No significant difference was found in TIGIT expression between survivors and non-survivors in non-ICU groups of patients (Gutiérrez-Bautista *et al*. 2020).

In contrast to these findings, Zheng *et al.* (2020) detected increased expression of TIGIT on $CD4^+$ cells in both mild and severe COVID-19 patients compared to

healthy controls and on $CD8^+$ cells in healthy and mild cases compared to severe COVID-19 patients. In a correlation network analysis, increased expression of TIGIT on $CD8^+$ cells was significantly related to disease progression (Zheng *et al.* 2020). TIGIT was also over-expressed on $CD4^+$ cells in both hospitalized and non-hospitalized COVID-19 patients compared to healthy controls, and its expression remained increased on $CD4^+$ cells during the convalescent phase in a non-hospitalized subpopulation. Simultaneously, the proportion of $CD8^+$ cells expressing TIGIT increased over time in non-hospitalized patients. TIGIT frequencies on $CD4^+$ and $CD8^+$ cells in a non-hospitalized group, correlated with age (Files *et al.* 2021).

In a longitudinal study which investigated the COVID-19 recovery period, numerous parameters were examined in patients with a history of COVID-19 infection at two time points: 1.3 and then 6.1 months after infection. Even if the disease course in the studied population was skewed towards milder forms, various perturbations in the immune profile persisted. Among other changes, the MFI of $TIGIT^+CD4^+$ and $TIGIT^+CD8^+$ cells was lower at both time points in COVID-19 patients than in healthy controls (hence significant differences obtained only for time point 6.1 months after the infection). Regarding longitudinal variations, the MFI of $TIGIT^+CD4^+$ and $TIGIT^+CD8$ was significantly lower 6.1 months after infection than 1.3 months after infection. Significantly lower MFI of TIGIT expression on central memory $CD8^+$ cells ($CD8^+T_{CM}$), cycling $CD4^+$ ($Ki67^+CD4^+$) and $CD8^+$ ($Ki67^+CD8^+$) cells was seen at both time points in patients with history of COVID-19 than in healthy controls. However, the described abnormalities did not correlate with persistence of symptoms nor overall health status (Breton *et al.* 2021).

BTLA

B and T lymphocyte attenuator (BTLA; CD 272) is a member of co-inhibitory receptors. Its expression on $CD4^+$ and $CD8^+$ cells is constitutive and down-regulated during T cell activation and differentiation, and hence, typically, naive cells express it more than memory cells (Otsuki *et al.* 2006).

The expression of BTLA was significantly higher on both $CD4^+$ and $CD8^+$ cells in COVID-19 patients with active disease than in healthy controls, but it remained unchanged on NK cells (Schultheiß *et al.* 2020).

Focusing on the subsets of $CD8^+$ cells, Herrmann *et al.* (2020) reported that the decrease in BTLA expression in transient and effector memory $CD8^+$ cells was significantly less pronounced in COVID-19 patients than in healthy controls.

CD224

Cluster of differentiation 244 (CD244) is an immunoregulatory receptor expressed on selected cells from both lymphoid and myeloid lineages (T cells, NK cells, basophils, monocytes, DCs, MDSC) and binds CD48. Based on the interactions of its intracellular domain with different cytoplasmic adaptor molecules, depending on their concentration, availability and the level of expression of the receptor itself, it may transfer either inhibitory or activating signals (inhibitory signals in the case of high CD244 expression, activating signals in the case of low CD244 expression). Similarly to other immune checkpoint inhibitors, variances in CD244 expression were associated with pathologic conditions, i.e. chronic infections and cancer (Agresta *et al.* 2018).

The level of expression of CD244 on $CD4^+$ and $CD8^+$ cells was significantly higher in both severe and mild COVID-19 patients than in healthy donors; however, no significant difference between the two severity groups of COVID-19 patients was observed (Li *et al.* 2020).

VISTA

V-domain Ig suppressor of T cell activation (*V-set immunoregulatory receptor*, VSIR, VISTA) is a transmembrane protein acting as an immune checkpoint with suppressive effects on $CD4^+$ and $CD8^+$ cells (Lines *et al.* 2014). VISTA was up-regulated on $CD4^+$ and $CD8^+$ in mild and severe COVID-19 patients compared to healthy patients (Rendeiro *et al.* 2020).

IDO-1

Indoleamine 2,3 dioxygenase (IDO) is an intracellular enzyme recognized for its participation in metabolic immune regulation by catabolising tryptophan. Expressed in various cells, including APC, its effects are not limited to cells producing IDO, but through secreted metabolites and restricted access to tryptophan, it spreads to neighboring cells, leading to suppression of immune responses with important consequences under physiological and pathological circumstances (Munn *et al.* 2013).

Tomić *et al.* (2021) detected higher percentages of ILT3$^+$, PD-L1$^+$ and IDO-1$^+$ Mo-MDSC (Monocytic Myeloid-Derived Suppressor Cells) in severe COVID-19 patients than in mild cases and healthy donors. However, in severe patients, the expression of PD-L1, ILT-3 and IDO-1 was decreased in monocytes and low in neutrophils and polymorphonuclear myeloid-derived suppressor cells (PMN-MDSC) and eMDSC (Tomić *et al.* 2021).

Therapeutic perspectives

Regarding the increasing number of reports on the role of immune checkpoints in the pathophysiology of cancer, they have become an important therapeutic target in the management of several malignant diseases since 2011 (Gambichler *et al.* 2020). The COVID-19 pandemic has raised several concerns regarding the safety of their use and their possible usefulness in the treatment of COVID-19 patients (Fig. 3) (Bersanelli *et al.* 2020, Vivarelli *et al.* 2021, Gambichler *et al.* 2020, Chiappelli *et al.* 2020).

The risk of ICI pneumotoxicity and viral pneumonia overlap, possible difficulties of their differential diagnosis and eventual triggering or enhancement of cytokine release syndrome due to inhibition of immune checkpoint molecules should be taken into account on one hand. On the other hand, the possibility of reversing exhausted phenotype of lymphocytes supports the idea of ICI use in these patients (Pezeshki *et al.* 2021, Shen *et al.* 2020). Clinical studies are needed to resolve these questions. In the following text, we summarize available evidence.

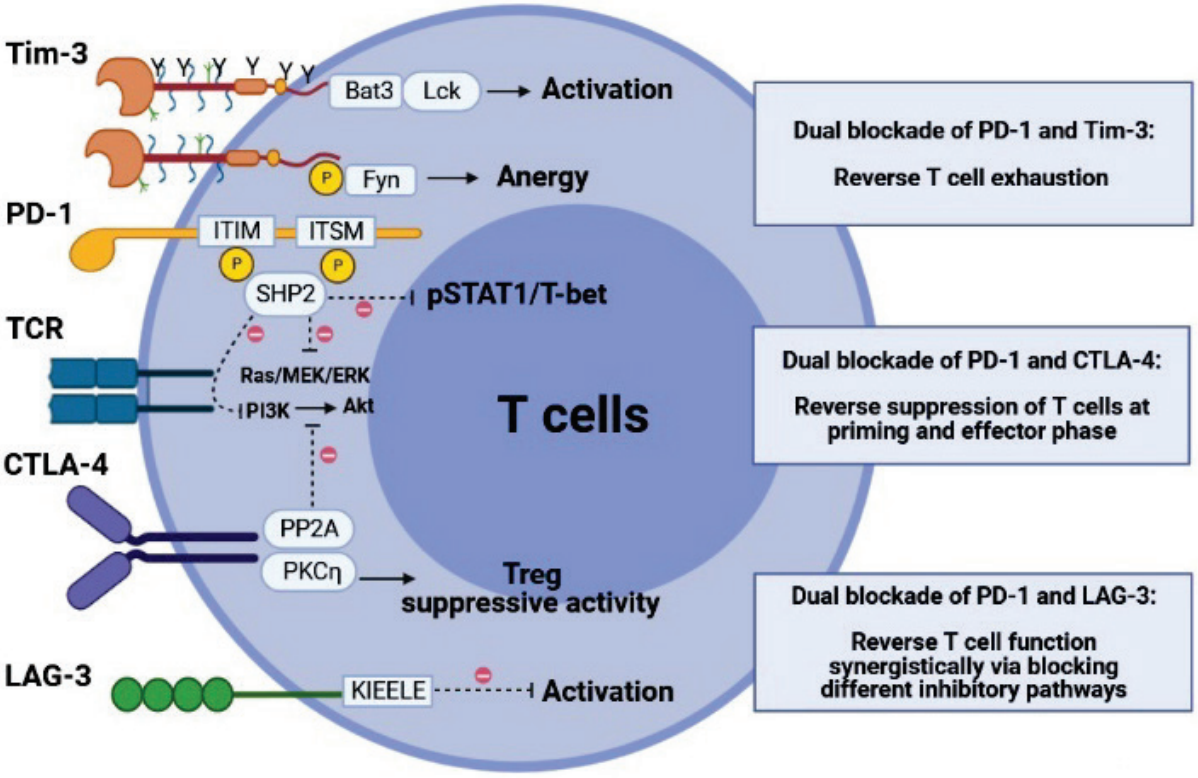

Fig. 3. Potential therapeutic intervention of immune inhibitory receptors in association with cancer and chronic infection. Blockade of the signaling pathway of representative IRs can improve the function and activation of T cells. Bat3, which promotes activation of TCR and acceptance of Fyn, which induces T cell energy. The interaction between phosphatases induced by PD-1 and kinases recruited by Tim-3 might induce T cell exhaustion. PD-1 inhibits PI3K/Akt, Ras/MEK/ERK and p-STAT1/T-bet pathways *via* SHP-2 to its phosphorylated ITSM motif. CTLA-4 inhibits PI3K/Akt downstream of TCR activation *via* PP2A in conventional T cells and suppresses activity to T_{reg} *via* PKC-h. Inhibition of T cell activity by LAG-3, which contains the KIEELE motif, occurs in an unknown signalling path (adapted from Li *et al.* 2017). (Created in BioRender.com).

Up to now, monoclonal antibodies against CTLA-4 (ipilimumab), PD-1 (pembrolizumab, nivolumab, cemiplimab) and its ligand PD-L1 (durvalumab, atezolizumab, avelumab) have already been in use with cancer patients (Gambichler *et al.* 2020). Since immunotherapy with immune checkpoint inhibitors

(ICI) is associated with restoration of cellular immunocompetence, these patients are likely to be more immunocompetent than those treated with chemotherapy (Bersanelli *et al.* 2018, Bersanelli *et al.* 2020). In general, the therapy with ICI was not associated with an increased risk of acquired infections (Gambichler *et al.* 2020).

Pneumotoxicity is a well-known immune-related adverse event of ICI to which patients with underlying lung diseases were shown to be prone. One of suggested risks concerning treatment with ICI in the era of COVID-19 is possible overlap between coronavirus interstitial pneumonia and ICI-related pneumonitis (Bersanelli *et al.* 2020). There is also a need to carefully differentiate between these two entities, as they might have similar clinical and radiographic presentation (Chang *et al*. 2020). However, no link between seasonality (winter months with higher frequencies of respiratory infections) and development of irAE was observed (Shah *et al.* 2020). Another aspect which should not be overseen is the possible contribution of ICI to the development of cytokine release syndrome (CRS) and deterioration of the course of COVID-19 (Rotz *et al.* 2017, Bersanelli *et al.* 2020).

Viral clearance might be facilitated, however, by a blockade of checkpoint inhibitors. This could be beneficial (Sullivan *et al.* 2020) as several studies have reported that increased expression of inhibitory molecules is related to unfavorable disease outcome in COVID-19 patients (Diao *et al.* 2019, Zheng *et al.* 2020, Bobcakova *et al.* 2021, Zheng *et al.* 2020). Restoration of normal T cell function might prevent the disease progression (Vivarelli *et al.* 2021).

Recently, treatment with pembrolizumab lead to decreased viral loads together with increased $CD4^+$ and $CD8^+$ activity in five out of eight patients with progressive multifocal leukoencephalopathy (Cortese *et al.* 2019). In sepsis, administration of immune checkpoint inhibitors did not provoke hypercytokinemia or cytokine storm and suggested restoration of immune status in a Phase Ib study (Hotchkiss *et al.* 2019, Hotchkiss *et al.* 2019). PD-1/PD-L1 pathway was shown to be relevant during persistent viral infection. In HIV patients, its blockade lead to an increase of HIV-specific $CD4^+$ cells and restored $CD8^+$ functions (Day *et al.* 2006).

Up to now, increasing evidence about the impact of ICI on disease course in cancer patients is becoming available. Although a case of rapid progression with fatal outcome in a patient with a previously long-term stable metastatic lung cancer treated by nivolumab was described (Bonomi *et al.* 2020), an uncomplicated COVID-19 course was reported in amelanoma patient with lung metastases undergoing treatment with pembrolizumab (Pala *et al.* 2021). In a study by Luo *et al.* (2020), anti-PD1 treatment did not affect the severity of COVID-19 in patients with lung cancer (Luo *et al.* 2020). Similarly, the first results of the TERAVOLT study do not suggest a difference in survival rate based on the type of therapy (including immunotherapy) in patients with lung cancer (Garassino *et al.* 2020). Likewise, a study based on the Spanish registry of melanoma patients treated with anti-PD1 did not show increased risk of death when infected with SARS-CoV-2 (Gonzalez-Cao *et al.* 2020). In another retrospective study in cancer patients, it was concluded that ICI did not increase the risk of becoming infected with or death from COVID-19 (Klebanov *et al.* 2021). In the German melanoma registry, 13 patients on ICI with COVID-19 were identified; however, most patients had an asymptomatic or a mild-to-moderate disease course, two patients required hospitalization (one in ICU), but none of them died (Moritz *et al.* 2021). A multicenter study, including centers from North America, Europe and Australia, has shown that overall, mortality due to COVID-19 in ICI-treated cancer patients was comparable to mortality due to COVID-19 previously reported in cancer patients. In-hospital mortality was higher than previously reported for cancer patients and related to COVID-19 in 50 % of cases. A combination of ICI was evaluated as an independent risk factor for hospital admission (Rogiers *et al.* 2021). Surprisingly, preliminary data from Italy based on a comparison of seroconversion rates against SARS-CoV-2 in patients with malignant diseases treated with chemotherapy and ICI suggested that ICI might be protective factor against SARS-CoV-2 infection (Isgrò *et al*. 2021).

Unlike major surgery or chemotherapy, age and treatment with ICI were identified as risk factors for hospitalization and severe course of COVID-19 in a population of 432 cancer patients with COVID-19, (Robilotti *et al.* 2020). Dipasquale *et al.* (2021) hypothesized that anti PD-L1 agent acts as a primer for the development of ICI-related pneumonia in patients with squamous head and neck cancer with recent asymptomatic COVID-19 infection. However, lung toxicity was graded as grade 1, and regarding positive impact on malignant disease, immunotherapy was restarted after 6 weeks with no progression of

pneumopathy (Dipasquale *et al.* 2021).

It is not surprising that several studies intend to analyze the effect of ICI in COVID-19 patients with (NCT04333914) or without (NCT04413838; NCT04268537; NCT04343144; NCT04356508) underlying malignant disease (Vivarelli *et al*. 2013). To avoid the risk of cytokine release syndrome, a combination of ICI with anti-IL6R or anti-IL1R was suggested (Bonam *et al.* 2020, Toor *et al.* 2021, Vivarelli *et al.* 2021). Other possible experimental approaches were suggested for inclusion in the management of COVID-19. VISTA is an immunoregulatory receptor constitutively expressed on myeloid and T cells and is downregulated upon activation. VISTA agonists could suppress the cytokine storm mediated mostly by cytokines released from monocytes and macrophages (Eltanbouly *et al.* 2021). Timing of these therapeutic interventions is presumed a key factor in treatment outcome and their effectiveness (Toor *et al.* 2021).

Conclusions

Currently, the terms "immunity" in association with "COVID-19" are one of the most searched terms in bibliographic databases. This is understandable, because immune system is one of the main regulatory systems of the human body and plays a key role in ongoing SARS-CoV-2 infection. Interaction between the virus and an individual's immune system leads to specific clinical manifestation (Paces *et al.* 2020).

A number of clinical studies are trying to elucidate the involvement of the immune system in progression of COVID-19 and reveal early prognostic biomarkers. It was confirmed by several authors, that selected inhibitory immune checkpoint molecules are significantly up-regulated in patients with severe course of the disease. Down-regulation of these markers could hence serve as a positive prognostic marker and possible therapeutic target. However, results vary between different authors and probably depend on exact timing of examination (Bobcakova *et al.* 2021).

An interesting therapeutic approach for patients with COVID-19 would be to target pathways of immunosuppressive modifying molecules to re-activate the exhausted phenotype of T cells, although clinical trials, which investigate the use of ICI in COVID-19 patients, are still ongoing (Vivarelli *et al*. 2021).

To conclude, further studies are needed to exactly specify the function of inhibitory immune checkpoint molecules in the immune response against SARS-CoV-2 and possible use of ICI in COVID-19 patients.

Conflict of Interest

There is no conflict of interest.

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