THE POTENTIAL SYNERGY BETWEEN IMMUNE CHECKPOINT INHIBITION AND COVID-19

Evaluating the current knowledge and clinical observations of immune checkpoint inhibition in COVID-19 patients

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Foreword

Before introducing you to the topic of this thesis, I would like to provide you with a short context of the time that this report is written. From December, 2019, SARS-CoV-2 emerging from Wuhan, China, has spread globally and the WHO officially called COVID-19 a pandemic by March 12, 2020. In the meanwhile, COVID-19 has had great medical, social, and financial impacts. Due to the measures that were taken to control this virus, most people are working from home, and so did I.

For my thesis I knew that I wanted to focus on immunotherapy. Previously, I had studied how to improve immune checkpoint inhibition by stabilizing the tumor vasculature. Since then, I am really intrigued by immunotherapy and I wanted to approach this therapeutic strategy from a different angle. Therefore, I contacted prof. dr. C.A.H.H. (Toos) Daemen, who has extensive expertise in activation of the immune system for the immunotherapy of cancer. As she was willing to supervise my thesis, I was contented that I had the opportunity to further elaborate my knowledge in immunotherapy. With the proposal to study immune checkpoint inhibition in the context of COVID-19, I found a compelling research question to write about in my thesis. I am glad that I could study this topic, thereby contributing to our understanding of immunotherapy in relationship with COVID-19, currently a major threat to public health.

The topic of this thesis focuses on cancer immunotherapy during this COVID-19 pandemic. We face many medical questions that require extensive research to be answered. Are cancer patients more vulnerable to COVID-19? Does immunotherapy affect COVID-19 vulnerability? And is it safe for cancer patients to continue their immunotherapies while we are facing a pandemic? These questions will be discussed in this report, and I think that this gives a valuable overview of the knowledge we have so far regarding the relationship between immune checkpoint inhibition and COVID-19.

Summary

In the last six months, SARS-CoV-2 has globally spread making COVID-19 a pandemic. COVID-19 can have mild symptoms, but some patients develop severe conditions with fatal outcomes. Cancer patients on immunotherapy are considered to be highly vulnerable to COVID-19, since unrestrained immune and cytokine activation is detrimental to the course of this disease. However, the impact of immune checkpoint inhibition (ICI), including checkpoint inhibitors targeting the CTLA-4 or the PD-1/PD-L1 pathway, on COVID-19 severity has remained elusive, and continuation of ICI in cancer patients during this pandemic is under great debate. This review provides an overview of the current knowledge regarding the relationship between ICI and COVID-19, and clinical observations of COVID-19 patients with cancer as co-morbidity receiving prior checkpoint inhibitors are discussed. Although data is limited, including one single-center study, one multi-center study, and eight single-case reports, first results do not demonstrate negative effects of PD-1/PD-L1 inhibitors on severity of COVID-19. Instead, lung cancer and smoking tend to increase COVID-19 vulnerability. Additional studies are required to generalize recent outcomes and to further evaluate safety of ICI. It is also of great importance to further elaborate on the relationship between COVID-19 severity, lung cancer and smoking. These efforts will help to improve our understanding of COVID-19 in relationship to cancer, and to improve cancer treatment guidelines during this pressing pandemic on healthcare systems worldwide.

Key words; COVID-19, SARS-CoV-2, immune checkpoint inhibition, PD-1, PD-L1.

Introduction

The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing corona virus disease-19 (COVID-19) has resulted in a pandemic with more than 7 million cases and over 400 000 reported deaths worldwide (1,2). While the number of new cases still increases, there is not yet a specific SARS-CoV-2 vaccine available (3). This is a major threat to global health, since various reports have demonstrated severity and fatality of COVID-19, also in cancer patients (4–8). COVID-19 patients with cancer as co-morbidity have a case-mortality rate of 5.6% (7), and a single-center study from New York reported that 55% of COVID-19 patients with lung cancer died due to infection (5). According to the NHS, cancer patients receiving immunotherapy are highly vulnerable to developing severe COVID-19 (9).

Immunotherapy has become the fourth pillar in cancer treatment and immune checkpoint inhibition (ICI) demonstrates promising therapeutic potentials in several malignancies. Antibodies targeting immune checkpoints, such as the PD-1/PD-L1 or the CTLA-4 pathway, are able to activate cellular immunity and increase cancer cell elimination (10). The first immune checkpoint inhibitor approved by the FDA was ipilimumab in 2011, a CTLA-4 inhibitor as treatment option for metastatic melanoma. Ipilimumab demonstrated significant improvements in outcome of disease in melanoma patients, and later more checkpoint inhibitors were developed (11,12). Nowadays, many CTLA-4 and PD-1/PD-L1 checkpoint inhibitors are available as first- and second-line cancer treatment, also in combination with chemotherapy.

Immune checkpoint inhibitors have great efficacy in cancer treatment, but besides their anti-tumor effects, they can also lead the onset of highly toxic immune related adverse events (irAEs) (13). It is now under great debate whether ICI treatment should be continued with this ongoing pandemic considering possible diverse effects on COVID-19. On the one hand, ICI enhances anti-viral immunity by reinvigorating the cellular immune response required for viral clearance. On the other hand, unrestrained immune activation and ICI-related irAEs can synergize with the course of COVID-19, thereby increasing severity of disease (14–16). This review focusses on the question to what extent immune checkpoint inhibition impacts severity of COVID-19 illness in cancer patients. Firstly, the course of COVID-19, the cellular immune response against SARS-CoV-2 infection, and the similarities of COVID-19 and ICI will be discussed. Subsequently, clinical observations of COVID-19 patients on ICI will be reviewed. I hypothesize that evaluation of ICI in the context of COVID-19 helps to direct and improve cancer treatment guidelines during this pandemic.

Clinical course of COVID-19

SARS-CoV-2 primarily infects the lungs inducing an inflammatory response. This virus enters pneumocytes via the ACE2 cell entry receptor (17). Here, tissue-resident macrophages become activated by recognition of either damage-associated molecular patterns or pathogen-associated molecular patters (16). Activated macrophages release various cytokines to stimulate the innate and adaptive immune response. For instance, interferons type I and III are required for induction of an anti-viral response in neighbor cells to protect themselves from viral infection. Moreover, interleukin (IL) 6 and IL-1β are necessary for recruitment of natural killer cells and T cells, and subsequent activation of adaptive anti-viral immunity. The incubation time of COVID-19 is five to seven days (18,19) and first symptoms are cough, fever, diarrhea, and headache (16). Most people infected with SARS-CoV-2 develop mild symptoms and recover without medical intervention (7,20,21), but some patients suffer from pneumonia and require hospitalization (22).

Severe COVID-19 is characterized by acute respiratory distress syndrome (ARDS) and multiple organ failure following cytokine release syndrome (CRS), and these patients are often admitted to ICU (23,24). CRS in late-phase COVID-19 is related to the cytokines IL-6, IL-10, and tumor necrosis factor (TNF) α, which are significantly upregulated in ICU-patients compared to non-ICU patients (25). Excessive IL-6 production can be due to increased macrophage activation after SARS-COV-2 infection

(26). Research to improve COVID-19 treatment has focused on IL-6 inhibition and some positive outcomes of treatment with tocilizumab, a monoclonal antibody against the IL-6 receptor, for CRS in COVID-19 have been reported (27). CRS can lead to the development of ARDS and multiple organ failure approximately eight days after onset of first clinical symptoms (28,29). These patients are often in urgent need of mechanical ventilation. 81% of succumbed COVID-19 patients suffered from ARDS and only 9% of patients with ARDS during their course of COVID-19 were discharged (24).

T cell immunity during SARS-CoV-2 infection

Analyses of COVID-19 patients' blood samples reveals reduced lymphocyte counts, referred to as lymphopenia (22,24,25,28,30,31). *Diao et al.* report significantly decreased numbers of $CD4^+$ and $CD8^+$ T cells in ICU-patients compared to non-ICU patients, and the lowest lymphocyte counts were found in patients ≥ 60 years old (25). It is suggested that this could explain the observation that elderly are more susceptible to COVID-19. Additionally, one report demonstrates that COVID-19 patients that succumbed to the disease have a lymphocyte count of $0.60 \cdot 10^9$/L compared to $1.42 \cdot 10^9$/L for discharged patients (24). Interestingly, a negative correlation in non-ICU patients exists between total T cells counts, $CD4^+$ counts, and $CD8^+$ counts and the cytokines IL-6, IL-10, and TNF-α (25). Therefore, it is unlikely that lymphocytes are responsible for increased cytokine production, and these upregulated cytokines might even be involved in T cell depletion. It is also noteworthy that T cells do not express ACE2, and can therefore not be infected by SARS-CoV-2 resulting in depressed T cell counts (25). Although the exact cause of lymphopenia remains elusive, it is proposed that TNF-α can play a role in T cell depletion (25). High concentrations of TNF-α are present in severely ill COVID-19 patients, consisting mainly of elderly. It is known that aging T cells have increased TNFR1 expression (32,33), which makes them more sensitive to TNF-α induced apoptosis. Consequently, it could be suggested that high concentrations TNF-α induce T cell apoptosis in elderly suffering from COVID-19. Because adaptive cellular immunity and especially $CD8^+$ T cells play a pivotal role in the anti-viral immune response, T cell depletion could be accounted for prolonged duration of disease in comparison with other viral infections (16).

Besides reduced lymphocyte counts, T cells in COVID-19 patients express exhaustion markers, such as PD-1 and Tim-3 (25,31). In a variety of chronic viral infections T cells reach an 'exhausted' state (34). These T cells lose their effector functions, have limited proliferative capacity, and express inhibitory receptors. Expression of these markers is regulated by IL-10, which is highly present in COVID-19 ICU-patients (25,31). COVID-19 patients admitted to ICU have increased expression of PD-1 on $CD4^+$ and $CD8^+$ T cells, and in some patients increasing expression of PD-1 and Tim-3 on $CD8^+$ T cells is associated with progression to overtly symptomatic COVID-19 (25). $CD8^+$ T cells with low or intermediate expression of PD-1 can be reinvigorated by PD-1 inhibition. On the contrary, terminally exhausted $CD8^+$ T cells highly expressing PD-1 are not responsive to PD-1 blockade and their cytotoxic potential cannot be restored (31). $CD8^+$ lung-infiltrates of COVID-19 patients express transcriptional hallmarks, such as *CCL4* and *GZMB*, pointing to terminal exhaustion. Moreover, as found in extracted terminally exhausted $CD8^+$ T cells from melanoma, these cell infiltrates express terminal exhaustion markers *MKI67* and *TYMS* (16). Expression of a wide range in exhaustion markers is suggestive for limited cellular capacity to reduce viral load in COVID-19.

Although the cellular immune response is not in every patient adequate enough to disseminate SARS-CoV-2 infection, T cells show hallmarks of hyperactivation during COVID-19 (35,36). The $CD4^+$ T cell population consists of an increased percentage of pro-inflammatory $CCR6^+$ Th17 cells. Furthermore, $CD8^+$ T cells contain high concentrations of cytotoxic granules, suggesting increased cytotoxicity (37). So, despite reduced lymphocyte counts in COVID-19 patients, part of the lymphocytes that are present in the peripheral blood can be characterized as hyperactive. It is hypothesized that initial SARS-CoV-2 infection is associated with rapid $CD8^+$ T cell expansion resulting in immunopathology of the lung. Subsequently, T cells become exhausted, they cannot clear

the viral infection, and a persistent infection develops with CRS as main contributor to severity of disease. The virus-induced hyperinflammatory syndrome, instead of direct effects of viral infection, can eventually be considered as cause of death (16,38).

Overlap between symptomatic COVID-19 and immune checkpoint inhibition

By reinvigorating T cell function, ICI aims to increase cancer cell elimination in patients. Equally, this adaptive cellular immune response is also required for an adequate and effective SARS-CoV-2 clearance in COVID-19. Unfortunately, some toxic and fatal irAEs are associated with ICI, for instance pneumonitis, a severe and life-threatening condition (13). Moreover, even if COVID-19 coincides with lymphopenia, lymphocytes demonstrate to be highly active possibly causing immunopathology. Hence, it can be questioned whether continuation of ICI during this pandemic is safe for cancer patients.

The risk to develop pneumonitis in PD-1/PD-L1 monotherapy is 2.5-5.0%, and 7-10% in CTLA-4/PD-1 combination therapy (39). In most cases, pneumonitis improves when holding treatment and resolves with immunosuppression. In rare cases, pneumonitis worsens or progresses in spite of immunosuppression and can be fatal. Pneumonitis is accounted for 35% of all irAE-related deaths, thereby the most fatal irAE in comparison with others (40,41). irAEs often occur within the first three to six months after initiation of treatment. If treated for a longer period, it is unlikely that ICI-related pneumonitis will develop. Pneumonitis mimics COVID-19 symptoms with great overlap in clinical and radiological characteristics. This makes differentiation in diagnosis between COVID-19 and ICI-related pneumonitis very difficult (42). A case-report discusses late diagnosis of COVID-19 due to symptomatic interference with prior chemotherapy and atezolizumab, a PD-L1 monoclonal antibody (43). At first examination after entering the clinic with symptoms, such as cough, dyspnea, and hypoxia, the patient was diagnosed with small cell lung carcinoma. Combined treatment of chemotherapy plus atezolizumab was initiated, and two days later, the patient returned to the clinic with progression of dyspnea. CT imaging resulted in diagnosis of pneumonitis and clinicians related this to atezolizumab treatment. ICI-related pneumonitis is treated with steroids (44), while COVID-19 treatment includes chloroquine to control pneumonia (45). So, with his diagnosis this patient was given steroids, but symptoms did not improve. Five days later, IgG to SARS-CoV-2 was detected in blood samples, which was 22 days after onset of first symptoms. This example demonstrates the difficulty of differentially diagnosing between COVID-19 and ICI-related pneumonitis, resulting in inadequate treatment.

Next to similar primary clinical presentation, ICI and SARS-CoV-2 infection can both possibly lead to the onset of CRS (43). Severe inflammatory response syndrome, corresponding to CRS, is one of the immune-mediated toxicities of ICI. This originates as a consequence of excessive T cell proliferation and production of pro-inflammatory cytokines, such as IL-6. IL-6 is indicative for this syndrome with C-reactive protein as reliable surrogate for IL-6 expression (46). Cancer patients having immunotherapy and developing severe inflammatory response syndrome show increased C-reactive protein levels. Tocilizumab has demonstrated to improve of a diverse set of irAEs, and is a therapeutic option for management of ICI-associated CRS (47). IL-6 modulating therapy and use of steroids are able to restore C-reactive protein levels back to baseline values (46). CRS in COVID-19 can induce ARDS and multiple organ failure with fatal outcomes, so management of CRS is crucial to prevent severity of COVID-19. CRS is mainly present in late-phase COVID-19 and cancer patients do not receive checkpoint inhibitors during SARS-CoV-2 infection (40). Thus, there is very little chance that ICI-related CRS synergizes with COVID-19 related CRS.

ICI can also positively affect the course of COVID-19. Checkpoint inhibitors, for instance inhibitors targeting the PD-1/PD-L1 pathway, prevent suppression of the adaptive immune system. There is strong evidence that T cell-mediated adaptive immunity is essential for viral dissemination (16). The early phase of the anti-viral cellular immune response is associated with low activity of the PD-1/PD-L1 checkpoint pathway. In the late phase, plasmacytoid dendritic cells secrete high amounts of IFN type I, and activated $CD8^+$ T cells secrete IFN type II and TNF-α. These induce PD-L1 expression and activate

the PD-1/PD-L1 pathway to control the immune response and prevent excessive inflammation and immunopathology (15). Blockade of the PD-1/PD-L1 pathway can stimulate activity of exhausted T cells in chronic HIV, HBV, and HCV infections to control viral replication and reduce viral load (48). Notably, PD-1 blockade is only effective during T cell effector phase and not during activation of naive $CD8^+$ T cells (49). It is unknown whether inhibition of PD-1/PD-L1 affects exhausted $CD8^+$ T cell populations observed in COVID-19 patients, thereby possibly favoring viral dissemination.

Clinical observations of COVID-19 patients with cancer receiving prior PD-1/PD-L1 inhibitors

Literature discussing COVID-19 patients with cancer as co-morbidity and receiving ICI is scarce, and mainly consists of single-case reports. All available literature relating to the MeSH terms 'COVID-19', 'checkpoint inhibitor', 'PD-1', and 'CTLA-4' in the PubMed Database is collected between June 3, 2020, and June 9, 2020 (50). An overview of these reports is provided in Table 1. All patients described in Table 1 received a prior PD-1 inhibitor (n=8), for instance nivolumab or pembrolizumab, or a PD-L1 inhibitor (n=1), atezolizumab, as treatment of lung cancer (n=4), melanoma (n=4), or Hodgkin lymphoma (n=1). Six patients were alive after recovering from COVID-19 (14,51–54), and three died with COVID-19 defined as cause of death (43,55,56). Notably, one patient (75 yr.) survived SARS-CoV-2 infection, while also having multiple co-morbidities, such as hypertension, diabetes mellitus type 2, atrial fibrillation, coronary artery disease, and chronic obstructive pulmonary disease (53). Ten days after being discharged from hospital, she died due to her chronic cardiac problems. Remarkably, all three succumbed COVID-19 patients had lung cancer as co-morbidity, while all non-lung cancer patients survived this disease. Moreover, only one lung cancer patient survived COVID-19. Taken together, most single-case reports demonstrate positive disease outcomes in COVID-19 patients on ICI.

The first large cohort study of cancer patients confirmed with COVID-19 is a systemic multi-centered analysis by *Dai et al.* published on April 28, 2020 (57). They included 105 COVID-19 patients with cancer as co-morbidity and 536 age-matched noncancer COVID-19 patients from 14 hospitals in Wuhan for the time period January 1, 2020, to February 24, 2020. Six patients (5.71%) in their cohort were on immunotherapy for lung cancer and received last treatment within 40 days of COVID-19 development. All patients were male, (former) smokers, and received PD-1 monotherapy (n=2), chemotherapy plus PD-1 immunotherapy (n=3), or targeted therapy (EGFR-TKI) plus PD-1 monotherapy (n=1). Four (66.67%) patients suffered from severe events and two (33.33%) patients died due to COVID-19; one at age 61 without co-morbidities, and one at age 81 with hypertension. Both succumbed patients were current smokers. Compared to other cancer therapies, immunotherapy tended to have higher chances to develop critical symptoms and to cause death.

In addition, *Luo et al.* performed a single-center study in New York including 69 lung cancer patients diagnosed with COVID-19 between March 12, 2020, and April 13, 2020 (58). Of these patients, 41 (59%) were on prior PD-1 inhibition. All patients on ICI have a history of smoking; 8 (20%) patients as < 5 pack-year smokers and 33 (80%) as ≥ 5 pack-year smokers. After adjusting for the imbalance in smoking history between patients with and without prior PD-1 blockade, no significant effect of prior PD-1 inhibition on COVID-19 severity is found. There is also no effect of the period between last time of treatment and COVID-19 diagnosis on severity of disease. Furthermore, the peaks in IL-6 levels during the course of COVID-19 were similar in both groups. The risk to develop severe COVID-19 does not seem to be related to prior PD-1 treatment, however, smoking pretends to be associated to severity of disease.

Taken together, one multi-center study (57) demonstrates high vulnerability to COVID-19 in patients on receipt of prior PD-1/PD-L1 blockade and some single-case reports present fatal COVID-19 outcomes (43,55,56). On the contrary, single-case reports about cancer patients on checkpoint inhibition also describe positive outcomes of COVID-19 (14,51–54), supported by the conclusion of one single-center study (58) that prior PD-1 inhibition does not impact COVID-19 severity. Remarkably, all cancer

patients in these studies receiving ICI were on one of the PD-1/PD-L1 inhibitors atezolizumab, nivolumab, or pembrolizumab, and none were on CTLA-4 inhibition. Moreover, two patients showed rapid development of severe and fatal COVID-19 symptoms and died within one or five days (55,56). Normally, severe conditions develop over roughly eight days after the onset of first symptoms, characterizing COVID-19 as a subacute infectious disease. However, SARS-CoV-2 infection exacerbated very rapidly in these two cases, thereby indicating to a hyperactive form of COVID-19.

Discussion

PD-1/PD-L1 inhibition does not seem to have great impact on the outcome of COVID-19, although available data on ICI in combination with COVID-19 is scarce and no general conclusions can be made. Therefore, large cohort studies are required to assess the risk *versus* benefit of ICI continuation during this COVID-19 pandemic. Some studies report severe and fatal COVID-19 outcomes in cancer patients with prior PD-1/PD-L1 inhibition. It must be noted that increased severity of COVID-19 and being on ICI share similar risk factors, for instance lung cancer and prior smoking (58).

Some shortcomings of this review need to be mentioned for proper interpretation of the results and conclusions. For evaluation of the available literature in the PubMed Database on clinical observations of COVID-19 patients with cancer receiving prior ICI, a systemic search is performed demonstrating limited available data on this topic. Studies contributing to this review describe varying patient characteristics leading to incompleteness of the data discussed. Whereas one study only includes lung cancer patients, others include patients with differing forms of cancer. Single-case reports do also not provide all information about important parameters for the course of COVID-19. Likewise, to provide a good evaluation of the effect of ICI on severity of COVID-19, the definition of 'severe' needs to be well-defined. Unfortunately, differences exist between reports making comparisons between studies less accurate. These remarks can be considered as most notable flaws of this report.

An interesting finding is the observed entanglement of lung cancer and smoking in relationship with severity of COVID-19. All succumbed cancer patients on ICI described in single-case reports had lung cancer (43,55,56). Additionally, it is observed that the COVID-19 mortality-rate for lung cancer patients is 55% (5). *Luo et al.* (58) report no different COVID-19 outcomes between prior and no prior PD-1 blockade in lung cancer patients. Instead, they describe a negative effect of smoking on COVID-19 severity. *Dai et al.* (57) report two deaths in COVID-19 patients on ICI, both being characterized as current smokers. Regarding these findings, it can be hypothesized that lung cancer and smoking seem to be important risk factors for COVID-19 severity, and their specific role in COVID-19 severity should be further investigated. Furthermore, studies investigating the effect of ICI on COVID-19 severity should adjust for patient characteristics, such as lung cancer and smoking, that could possibly confound the relation with severity of COVID-19.

Although the PubMed Database is searched for both CTLA-4 and PD-1/PD-L1 checkpoint inhibitors, no cancer patients suffering from COVID-19 and treated with a CTLA-4 inhibitor were found. Most patients described in the studies mentioned above suffer from lung cancer or melanoma. Non-small cell lung cancer accounts for 80-85% of all lung cancers (59). Many of these patients respond to first-line chemotherapy, but almost half of them also receive second-line treatment for progression of disease. Second-line treatment for this form of cancer mainly consists of checkpoint inhibitors targeting the PD-1/PD-L1 pathway (60). Additionally, most checkpoint inhibitors in clinical trials for treatment of small cell lung cancer are PD-1/PD-L1 inhibitors as well, such as nivolumab, pembrolizumab, and atezolizumab (61). Melanoma is another cancer occurring in almost half of all patients described in the single-case reports. First-line treatment of melanoma is often nivolumab, since this therapeutic has demonstrated significantly longer progression-free survival in metastatic melanoma compared to either ipilimumab monotherapy or nivolumab plus ipilimumab combination therapy (62). So, almost all patients discussed in the studies to the relationship between ICI and COVID-19 suffer from lung cancer or melanoma. These cancers are mainly treated with PD-1/PD-L1 checkpoint inhibitors. Therefore, I

hypothesize that this is the reason that only PD-1/PD-L1 inhibitors, and no CTLA-4 inhibitors, are discussed in the literature on this topic.

Most cancer patients discussed in this review received ICI in combination with chemotherapy (14,51,52,54–57). It is known that chemotherapy has strong effects on immunity. It impacts rapidly dividing cells, such as myeloproliferative cells, in this way compromising the immune system (63). Consequently, it is plausible that cancer patients on chemotherapy have increased vulnerability to COVID-19. If the effect of ICI on severity of COVID-19 is assessed in cancer patients on combined chemotherapy plus ICI, it must be taken into consideration that prior chemotherapy can interfere with the impact of ICI on COVID-19 severity. Therefore, the attribution of ICI to severity of disease in those cases cannot be determined with great accuracy. Another important note made by England foresee is that they expect that cancer mortality will increase with 20% during this COVID-19 pandemic (64). This expectation is based on delayed cancer diagnosis and treatment at the time of this pressing situation on healthcare systems.

In conclusion, ICI does not seem to influence severity of COVID-19, while first, although limited, results point to a negative impact of lung cancer and smoking on outcome of COVID-19. Since data on the relationship between ICI and COVID-19 is scarce, follow up studies directed at the impact of ICI on susceptibility to COVID-19 in cancer patients are required. Those could focus on generalization of recent findings, further safety evaluation of ICI, and improvement of cancer treatment guidelines during this COVID-19 pandemic. Additionally, the independent effects of lung cancer and smoking on COVID-19 vulnerability need further investigations. Lastly, additional studies are required to provide greater insights to pathology of SARS-CoV-2 infection and reciprocal interplay with cancer immunotherapy. In the meanwhile, I ask clinicians to share experiences, so that we can handle this global and threatening disease with globally available knowledge.

Table 1. Overview with single-case reports of cancer patients treated with an immune checkpoint inhibitor and suffering from COVID-19. This data is collected between June 3, 2020, and June 9, 2020, from the PubMed Database (50)*. §: With patient's symptoms, he was first diagnosed with small cell lung carcinoma and later developed pneumonitis. Five days after development of pneumonitis, he was diagnosed with COVID-19, and this was 22 days after onset of first symptoms before diagnosed with small cell lung carcinoma. ‖: Died due to chronic cardiac problems ten days after discharge.*

Author	**Gender**	**Age**	**Cancer type**	**Drug**	**Fever**	**Dyspnoea**	**Cough**	**CT-scan**	**PCR**	**Severe**	**Time between onset of symptoms and diagnosis in days**	**Duration of hospitali -zation in days**	**Survival status**
Bonomi et al.	Male	65	Advanced lung cancer adenocarcinoma	Nivolumab	Yes	Yes	No	Positive	Positive	Yes	0	5	Dead
Di Giacomo et al.	Male	74	Metastatic cutaneous melanoma	Anti-PD-1	Yes	Yes	Yes	Positive	Positive	Yes	4	17	Alive
Di Giacomo et al.	Female	51	Cutaneous melanoma	Anti-PD-1	Yes	No	No	N/A	Positive	No	6	N/A	Alive
Di Noia et al.	Male	53	Metastatic non-small cell lung cancer	Nivolumab	Yes	Yes	No	Positive	Positive	Yes	0	<1	Dead
Lovly et al.	Male	56	Extensive-stage small cell lung cancer	Atezolizumab	No	Yes	Yes	Positive	Positive	Yes	5-22[§]	13	Dead
O'Kelly et al.	Female	22	Hodgkin lymphoma	Pembrolizumab	Yes	Yes	Yes	Positive	Positive	Yes	5	13	Alive
Schmidle et al.	Female	47	Fully resected stage IV melanoma	Nivolumab	Yes	No	Yes	Negative	Positive	No	4	N/A	Alive
Yekedüz et al.	Female	75	Metastatic malignant melanoma	Nivolumab	Yes	Yes	No	Negative	Positive	No	2	16	Dead[‖]
Yu et al.	Male	unknown	Non-small cell lung carcinoma	Pembrolizumab	Yes	No	No	Positive	Negative	No	0	32	Alive

Afterword

Although I wrote this thesis in extraordinary times, I did it with great pleasure and I think that it could be meaningful to others. Although I had imagined that these last three weeks of my Bachelor study would be different, so without quarantine at home being bound to my desk in my student room, I enjoyed working on this report. Since I could work on such a relevant topic, I was really motivated to write a nicely comprehensive review of currently available literature. In previous studies I did during my Bachelor's, I mainly focused on fundamental research investigating cellular pathways to discover the underlying mechanism of pathologies. In contrast, for this report I had to study and describe mostly clinical manifestations of disease, instead of cellular pathways. This offered me a new and valuable perspective to diseases, which I will use in the future to understand processes on smaller scales.

I would like to express my thankfulness to my supervisor prof. dr. C.A.H.H. (Toos) Daemen for introducing me to the topic I discussed in this paper and for her adequate feedback I received. Although I already knew from the start that I wanted to study and write about immunotherapy in my thesis, she proposed to discuss immune checkpoint inhibition in the context of COVID-19. Both a hot and ongoing topic, with many interesting points to be discussed. Moreover, her feedback made me think more critically about this subject, and I am very grateful that I had the opportunity to work under her supervision.

I also thank dr. C.C.M. Schuiling-Veninga for the fruitful discussions we had regarding epidemiological methodology. These provided me with new insights in the interpretation of clinical data and it allowed me to describe and discuss the clinical findings more extensively, thereby contributing to the quality of this report.

All in all, I really enjoyed working on this thesis and I am proud of the result. I have improved my academic skills, such as critical thinking and academic writing, and I have learned much about both the current pandemic as well as immunotherapy in our fight against cancer. With these acquired skills and knowledge, I am looking forward to starting with my Master's degree and continue my path in academia.

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