COMMENTARY

Considerations for cancer immunotherapy biomarker research during COVID-19

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The COVID-19 pandemic continues to profoundly impact the function of medical oncology practices across the globe. With constantly evolving recommendations, clinicians are pushed to extrapolate data and apply foundational principles of oncology to balance risks and benefits for each individual and their treatment options (Lewis 2020). Large registries have been established to collect clinical and outcome data on patients with cancer who become infected with the novel SARS-CoV-2 including the American Society of Clinical Oncology Survey on COVID-19 in Oncology Registry, the American Society of Hematology Research Collaborative COVID-19 Registry, and the COVID-19 and Cancer Consortium (https://www.asco.org/asco-coronavirus-information/coronavirus-registry; https://www.ashresearchcollaborative.org/covid-19-registry; https://ccc19.org). While patient safety and clinical care remain paramount, the immune repercussions of SARS-CoV-2 viral infection on cancer immunology research efforts need to be carefully considered. Herein, we review the T-cell response to acute viral infection, explore the overlap among markers of T-cell activation in response to immunotherapy and in viral infections, highlight similarities in systemic inflammatory profiles in these two settings, and discuss practical considerations for clinical and research programs.

Strategies for and considerations in monitoring the *in vivo* T-cell response during COVID-19

A significant limitation in studying the T-cell response in cancer patients is not knowing the antigen specificity of the cells being monitored. To overcome this challenge, we use markers that have previously been defined to mark T-cell activation in diseases, such as viral infections, where we are much more certain of the timing and kinetics of the response. Details of what these markers mean in viral infections, how they have been used for to monitor the immune response to cancer, and how SARS CoV-2 might disrupt our use of these markers are discussed subsequently.

During viral infections, naive CD8 T cells that are specific to the invading pathogen are activated and undergo 15–20 rounds of cell division, resulting in a rapid, large expansion in the number of virus-specific CD8 T cells (Doherty 1998, Murali-Krishna *et al.* 1998, Butz & Bevan 1998, Kalia *et al.* 2006). These newly expanded virus-specific CD8 T cells express effector molecules such as granzymes and perforin, which contribute to the targeted killing of infected cells. This period of proliferation and infected cell killing is often referred to as the expansion phase of the T-cell response (Fig. 1). When the pathogen has been cleared, the majority of the virus-specific CD8 T cells die during the contraction phase of the T-cell response. A small subset of these cells is able to persist and become memory CD8 T cells, which are critical for providing a rapid, antigen-specific T-cell response in the event of reinfection with the same virus (Kaech *et al.* 2002). The maintenance of these cells is known as the memory phase of the T-cell response.

A strong body of work has confirmed and expanded upon these findings in human studies. One tool for describing the human T-cell response to acute viral

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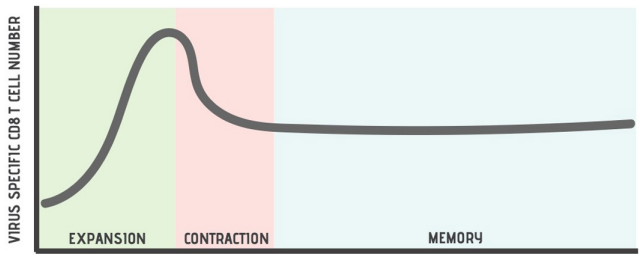

Figure 1
An illustration of the three phases of the CD8 T-cell response to acute viral infection. During the expansion phase, the number of virus-specific CD8 T cells increases. This is followed by the contraction phase, when the number of virus-specific CD8 T cells decreases. In the memory phase, this number of virus-specific CD8 T cells plateaus and remains above the baseline level.

infection is measuring T-cell responses after immunization with a live attenuated vaccine, such as the yellow fever vaccine or the Dry-Vax smallpox vaccine. In patients receiving either of these vaccines, expression of activation markers such as PD-1, HLA-DR, CD38, or Ki67 (Fig. 2) on peripheral blood mononuclear cells (PBMCs) is extremely low at baseline. Following immunization, the proportion of CD8 T cells expressing these markers greatly expands and their expression is largely limited to virus-specific CD8 T cells, indicating a rapid, large increase in newly generated, activated, virus-specific effector T cells. While virus-specific cells can be detected >30 days after immunization, they no longer express these activation markers (Miller *et al.* 2008, Akondy *et al.* 2009). Consequently, measuring the surge in T cells expressing these activation markers allows for identification of only newly generated effector CD8 T cells (Callan *et al.* 1998, Lechner *et al.* 2000, Appay *et al.* 2002).

Antigen-specific studies of viral infection have provided a strong foundation for establishing these measures of T-cell activation. In turn, applying this understanding more broadly allows for study of T-cell activation in settings where antigens may be unknown or too numerous, such as in cancer. Given the lack of known cancer antigens and the heterogeneous nature of human cancer, this activation marker-based approach is also commonly employed to observe the expansion phase of the tumor-related T-cell response, thereby functioning as a biomarker of response to immunotherapy. Examples of successful use of this tactic and the potential impact of COVID-19 on this approach is reviewed subsequently.

Expression of programmed cell death protein 1 (PD-1) on T cells during chronic antigen exposure and subsequent 'exhaustion' is widely appreciated (Hashimoto *et al.* 2018), but it should be emphasized that PD-1 is expressed by recently activated cells upon exposure to their cognate antigen (Ahn *et al.* 2018). Accordingly, PD-1+ T cells are often evaluated in assessing the anti-tumor immune response (Jansen *et al.* 2019), as a readout of cells that have recently encountered their cognate antigen, in which tumor tissue is likely to enrich for tumor-specific T cells. Importantly, PD-1+ T cells in the blood are measured in response to immunotherapy. These PD-1+ cells represent a readout of T cells that are recently activated, and an expansion of these cells has been associated with a clinical response to immunotherapy (Kamphorst *et al.* 2017, Huang *et al.* 2017).

Much like monitoring expression of PD-1 on CD8 T cells, Ki67 expression is also used as a biomarker for assessing response to immunotherapy. Ki67 is a nuclear protein that is active in all phases of the cell cycle, but is absent in quiescent (G0) cells (Bruno & Darzynkiewicz 1992), and thus, it is commonly used as a readout of cell proliferation. Newly activated T cells are known to undergo several rapid, obligatory rounds of proliferation following antigenic stimulus (Kaech & Ahmed 2001). Accordingly, measurement of Ki67+ proliferating cells can be used as an indicator of recent CD8 T-cell activation and thus can approximate the anti-cancer immune response.

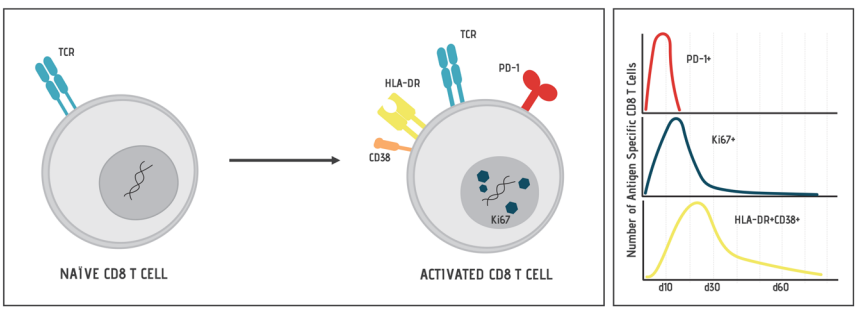

Figure 2
An illustration of the dynamic expression of proteins that indicate CD8 T-cell activation. Activation markers PD-1, Ki67, HLA-DR, and CD38 are not present on naive cells, but are upregulated upon CD8 T-cell activation. Expression of PD-1 is most acute, rising and falling rapidly. The number of Ki67 expressing cells rises and falls as the antigen-specific CD8 T-cell population grows and then shrinks during the expansion and contraction phases of the acute immune response. The number of HLA-DR+ CD38+ cells also increases following antigen exposure, gradually decreasing as the immune response resolves.

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For example, an increase in Ki67+ CD8 T cells in the blood following PD-1 blockade is associated with clinical response (Kamphorst *et al.* 2017, Huang *et al.* 2017). In addition to use as a readout for tumor-specific T cells in the blood following immunotherapy (Huang *et al.* 2017), measurement of Ki67+ T cells in tissue has been used to predict patient outcomes in renal cell carcinoma (Nakano *et al.* 2001).

Similarly, the co-expression of HLA-DR and CD38 on CD8 T cells can be used as a marker of recent activation (Appay *et al.* 2002, Miller *et al.* 2008, Akondy *et al.* 2009). The co-expression of these molecules closely mirrors expression of Ki67(Wieland *et al.* 2018), indicating that these recently activated cells are proliferating and suggesting they are likely responding to their cognate antigen. Indeed, expression of HLA-DR and CD38 has been reported on Ki67+ CD8 T cells in the blood of immunotherapy-responsive patients (Kamphorst *et al.* 2017, Huang *et al.* 2017), as well as on tumor-infiltrating lymphocytes in cancers such as glioma, ovarian cancer, melanoma, and colorectal cancer (Kovacsovics-Bankowski *et al.* 2014, Chen *et al.* 2019). Similarly, an increase in HLA-DR expression on tumor-infiltrating CD8 T cells has been observed following immunotherapy (Daud *et al.* 2016).

Understanding the role of each of these markers in the biology of the T-cell response has enabled powerful analysis of the anti-tumor immune response and the response to immunotherapy in the absence of robust and accessible techniques for assessing cancer antigen specificity. However, it is important to note that expression of these markers is not specific to activation of the cancer-related immune response. For example, the surge in activation marker expression 14 days following yellow fever vaccination can be attributed to the generation of many new effector CD8 T cells specific for the virus (Miller *et al.* 2008, Akondy *et al.* 2009). Similarly, assessment of activated CD8 T cells (HLA-DR+, CD38+) was used to describe the immune response of patients acutely infected with Ebola virus, prior to specific major histocompatibility complex tetramer development (McElroy *et al.* 2015). Thus, assessment of the response to viruses as they emerge, such as Ebola in 2014 and SARS-CoV-2 presently, often relies on these more general readouts of immune activation. This lack of inherent specificity is an increasingly important consideration amidst the SARS-CoV-2 pandemic, particularly in the context of currently utilized biomarkers of the response to immunotherapy.

While much remains unknown about the specific kinetics of the T-cell response to SARS-CoV-2, we can reasonably infer from previous studies of acute viral infections that the immune response to this virus will be associated with an acute T-cell response (Fig. 1). As has been reviewed previously, this acute response will include an expansion in activated T cells, which, without tools for evaluating the antigen-specific immune response, would be indistinguishable from activated T cells responding to a patient's tumor. For example, if a patient receiving immunotherapy became infected with SARS-CoV-2, the viral immune response would obscure accurate measurement of the response to immunotherapy, as an increase in circulating PD-1+, or Ki67+, or HLA-DR+CD38+ T cells could be attributable to either the received immunotherapy, the ongoing viral infection, or both. Accordingly, it will be imperative to know patients' SARS-CoV-2 infection status in order to accurately interpret results from studies that monitor responses to immunotherapy or that include measures of the immune system as biological correlates.

Concurrent use of inflammatory markers in oncology and COVID-19

Long-standing chronic inflammation is known to be a risk factor for the development of cancer, and measurement of inflammatory markers – such as IL-6, neutrophil to lymphocyte ratio (NLR), and C-reactive protein (CRP) – is increasingly appreciated as predictors of prognosis and/or the response to therapy in a variety of tumor types (Table 1). As sustained inflammation is suggested to mediate some severe complications of COVID-19, many of these markers are also being investigated in the setting of SARS-CoV-2 infection. The overlap between these two applications of blood inflammatory markers offers both reason for special consideration of cancer patients as 'high risk' amidst the COVID-19 pandemic and potential insight into understanding the pathogenesis of these inflammatory states.

Neutrophil to lymphocyte ratio (NLR) has been used as an inflammatory marker for a variety of conditions, including cardiovascular disease, stroke, metabolic syndrome, rheumatoid arthritis, and chronic obstructive pulmonary disease (Gibson *et al.* 2007, Chandrashekara *et al.*, 2017, Goyal *et al.* 2018, Ye *et al.* 2019). NLR has also been investigated as a biomarker for cancer-related prognosis, disease recurrence, and therapeutic response.

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Table 1 Blood inflammatory markers.

		Use in oncology	Use in COVID-19
Neutrophil to lymphocyte ratio (NLR)	Ratio of absolute neutrophil count (ANC) to absolute lymphocyte count (ALC)	Associated with poor prognosis, disease recurrence in multiple tumor types	Reported to identify severe COVID-19 illness at an early stage
C reactive protein (CRP)	Acute phase protein secreted in the liver in response to signals such as IL6. Activates complement system	Associated with increased risk of cancer, disease progression, and reduced survival in cancer patients	Elevated CRP associated with increased need for mechanical ventilation in COVID-19 patients
Interleukin 6 (IL-6)	Pro-inflammatory cytokine. Mediator of acute phase response and of fever	Elevated baseline levels present in cancer patients, associated with poor response to immunotherapy	Elevated serum levels associated with severe outcomes in COVID-19 patients

For example, elevated NLR has been associated with a poor prognosis in a number of tumor types (Templeton *et al.* 2014, Baum *et al.* 2016, Diem *et al.* 2017, Bilen *et al.* 2018, Duan *et al.* 2018, Yin *et al.* 2019), as well as with inferior responses to checkpoint blockade or CAR-T therapy (Saied *et al.* 2014, Diem *et al.* 2017, Bilen *et al.* 2019).

C-reactive protein (CRP) is another serum inflammatory marker studied in numerous infections (e.g. malaria, influenza) (Vasileva & Badawi 2019, Addai-Mensah *et al.* 2019), and CRP has been reported to correlate with risk for and outcomes in chronic conditions such as diabetes, hypertension, and cardiovascular disease (King *et al.* 2003, Ridker *et al.* 2005, Jain *et al.* 2011, Hage 2014). Indeed, CRP has also been employed as an inflammatory biomarker in patients with cancer. Elevated CRP has been associated with increased risk of cancer (Siemes *et al.* 2006), with cancer progression (Hall *et al.* 2013, Weber *et al.* 2019), and with reduced survival in cancer patients (Allin *et al.* 2011, Weber *et al.* 2019, Iivanainen *et al.* 2019).

While there are numerous mechanisms that may cause elevation of CRP, increased IL-6 related signaling is well established as a driver of elevated CRP (Moore & June 2020). As such, IL-6 levels are followed as an inflammatory biomarker. Elevated serum IL-6 levels have been reported in patients with breast, cervical, esophageal, head and neck, ovarian, pancreatic, prostate, and renal cancers (Johnson *et al.* 2018). Moreover, elevated serum IL-6 predicted poor prognosis in a number of cancer types (Johnson *et al.* 2018, Weber *et al.* 2019) and has been associated with poor response to immune checkpoint blockade (Weber *et al.* 2019). Conversely, low levels of IL-6 have been associated with positive responses to CAR-T cell therapy in hepatocellular carcinoma (Enblad *et al.* 2018). High levels of IL-6 are associated with increased incidence of adverse events in patients receiving immunotherapy (Johnson *et al.* 2018), as well as with occurrence of cytokine release syndrome (CRS) in patients receiving CAR-T cell therapy (Neelapu *et al.* 2018), and use of anti-IL6 regimens (e.g. tocilizumab) have proven successful in mitigating the mal-effects of CRS (Maude *et al.* 2014).

Not surprisingly, elevated blood inflammatory markers have recently been reported in COVID-19 patients. In a preliminary study, NLR was reported to enable early identification of severe illness (Liu *et al.* 2020a). Similarly, elevated serum IL-6 has been associated with severe outcomes in COVID-19 patients, such as respiratory failure and acute respiratory distress syndrome (Chen *et al.* 2020, Ruan *et al.* 2020). Increased serum IL-6 and CRP levels have also been associated with increased need for mechanical ventilation (Liu *et al.* 2020b, Herold *et al.* 2020). CRS has also been reported as a complication of SARS-CoV-2 infection (Chen *et al.* 2020, Ruan *et al.* 2020, Moore & June 2020).

The corresponding trends in inflammatory markers in cancer and COVID-19 raise a number of questions. Could cancer patients be at a higher risk of severe complications of SARS-CoV-2 infection due to an already elevated baseline inflammatory marker status? Or could the inflammatory state associated with severe COViD-19 precipitate cancer progression or impair the response to therapy? Preliminary reports suggest cancer patients may experience severe complications of SARS-CoV-2 infection at a higher rate (Dai *et al.* 2020, Liang *et al.* 2020, Zhang *et al.*). For example, as of February 28, 2020, the WHO-China Joint Mission on COVID-19 reports a crude fatality ratio (CRF) of 7.6% for patients with cancer as a comorbid condition and laboratory-confirmed infection, compared to an overall CFR of 3.8% (https://www.who.int/publications-detail/report-of-the-who-china-joint-mission-on-coronavirus-disease-2019-(covid-19)). More investigation is needed to determine the etiology of the apparent elevated risk for severe COVID-19 outcomes in cancer patients and if a baseline inflammatory state may contribute to this risk. There are also supportive care

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treatment implications, and with the successful use of anti-IL6 regimens in cancer-related CRS, anti-IL6 agents are being investigated for use in COVID-19 patients with critical complications. Preliminary studies report decreased fever and reduced oxygen requirements in COVID-19 patients receiving anti-IL6 therapy (Xu *et al.* 2020). Several global clinical trials are ongoing to further investigate the potential of anti-IL6 therapy for severe SARS-CoV-2 infections (Genentech, Sanofi/Regeneron).

As with T-cell activation biomarkers, systemic inflammatory biomarkers are non-specific readouts of immunobiology, whether in the setting of cancer or COVID-19. Accordingly, careful annotation of clinical data will be necessary to enable accurate interpretation of clinical trials data collected during and following this pandemic. Additionally, knowledge of elevated inflammatory markers at baseline in a number of conditions, such as cancer, should be considered in the study of clinical data correlates in COVID-19 patients.

Clinical and research program considerations

As highlighted by the limited data currently available, patients with solid tumors are generally older, have other medical comorbidities, and may be functionally immunosuppressed from their disease or treatment or both, putting them in the highest risk category for severe outcomes with COVID-19. Patients with potentially curable malignancy should be approached differently than those with progressive metastatic disease after multiple lines of therapy and declining functional status. This has critical implications for the timing of discussions around transitioning care to palliation and/or hospice. In effort to limit further spread, some hospital systems are making the tough decision to restrict or deny any visitors, which can be heartbreaking for patients at the end of life, whether due to SARS-COV-2 or their cancer.

For patients who need to continue on treatment, multiple consensus-based guidelines are beginning to direct clinicians (https://www.dana-farber.org/uploadedFiles/Pages/COVID-19_Facts_and_Resources/gu-cancer-covid-19-guidelines.pdf; https://www.esmo.org/guidelines/cancer-patient-management-during-the-covid-19-pandemic; https://www.nccn.org/covid-19/). Overall, the general goals are to avoid, if possible, immunosuppressive steroids, minimize hospital exposure when safely feasible, and use growth-factor support in patients who are receiving systemic cytotoxic chemotherapy at risk for neutropenia. The individual tumor type, stage, and line/intent of treatment inform decision making for treatment modification if necessary or access to care. For example, kidney cancer patients with suspected significant immune-mediated side effects, large or critically located renal masses that cause symptoms, or cancer-related emergencies (such as spinal cord compression or progressive brain metastasis) need to be high priority for medical care (https://www.esmo.org/guidelines/cancer-patient-management-during-the-covid-19-pandemic/genitourinary-cancers-renal-cell-cancer-in-the-covid-19-era). However, patients with favorable risk disease, good tolerance on an established treatment (such as VEGF targeted therapy or immunotherapy) or in long-term follow-up can be safely followed by telemedicine or deferred for later in-person visits (https://www.esmo.org/guidelines/cancer-patient-management-during-the-covid-19-pandemic/genitourinary-cancers-renal-cell-cancer-in-the-covid-19-era; https://www.dana-farber.org/uploadedFiles/Pages/COVID-19_Facts_and_Resources/gu-cancer-covid-19-guidelines.pdf). For patients with resected early stage lung cancer (T2bN0) or over the age of 70 with medical comorbidities, omission or delay of adjuvant chemotherapy may be considered. In advanced non-small cell lung cancer, data for the use of single-agent immunotherapy if PD-L1 $\geq$1% may enable patients to delay cytotoxic therapy (Mok *et al.* 2019). Treatment intervals with single-agent immunotherapy may also be extended from 2 to 4 weeks with nivolumab or 3 to 6 weeks with pembrolizumab (Long *et al.* 2018, Lala *et al.* 2018, FDA). Patients on immunotherapy who have achieved complete or partial response and completed 12–18 months of therapy can consider longer delays and, given the lack of prospective data, those who have completed 2 years may move to surveillance (https://www.esmo.org/guidelines/cancer-patient-management-during-the-covid-19-pandemic/lung-cancer-in-the-covid-19-era).

In a time of such uncertainty, clinical trials, especially early stage, carry the additional burden of potentially unknown side-effect profiles and associated frequent visits for lab monitoring. This is not to say that these trials are too risky to be considered. In patients with progressive cancer and good performance status with no standard-of-care therapeutic options, the risk of dying of their cancer is a near certainty, while succumbing to SARS-CoV-2 is a possibility. Both pharmaceutical and institutional sponsors of clinical trials are making practical accommodations to limit interactions with the healthcare system while maintaining safety on studies. This has been supported by the FDA guidance document on the conduct of clinical trials during this public health emergency

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(https://www.fda.gov/regulatory-information/search-fda-guidance-documents/fda-guidance-conduct-clinical-trials-medical-products-during-covid-19-public-health-emergency). Accordingly, some sponsors are waiving certain lab-only visits or allowing patients to get blood tested locally rather than requiring central testing. When resource need – whether for invasive procedures, production of novel agents, or clinical oversight – exceeds capacity, trials are pausing enrollment of new patients to focus on patients already on treatment.

Conclusions

In conclusion, obtaining accurate, well-annotated data from both patient registries and clinical trials are now more essential than ever, and an added challenge in carefully annotating these datasets will be the varied policies and practices in SARS-CoV-2 surveillance and testing throughout the world. Clearly, clinical outcomes may be skewed by SARS-CoV-2 infection. Biomarkers of immune response may also be uninterpretable, as the robust immune response to acute viral infection could confound possible cancer-specific immune responses. Clinical and laboratory data need to be meticulously annotated with timing of suspected or confirmed SARS-CoV-2 infection so that erroneous conclusions are not drawn. Due to the possibility of asymptomatic infection, even in higher risk cancer patients, post hoc analysis of COVID-19 status will be informative. With careful and thorough analysis accounting for viral immune effects, immunotherapy research can, even amidst a global pandemic, continue to inform cancer care.

Declaration of interest

Mehmet A Bilen has acted as a paid consultant for and/or as a member of the advisory boards of Exelixis, Bayer, BMS, Eisai, Pfizer, AstraZeneca, Janssen, Genomic Health, Nektar, and Sanofi and has received grants to his institution from Xencor, Bayer, Bristol-Myers Squibb, Genentech/Roche, Seattle Genetics, Incyte, Nektar, AstraZeneca, Tricon Pharmaceuticals, Peleton Therapeutics, and Pfizer for work performed outside of the present manuscript.

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Author contribution statement

C S J, J W C, M A B, and H K conceived and composed the manuscript. C S J created the accompanying figures. All authors reviewed the manuscript.

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