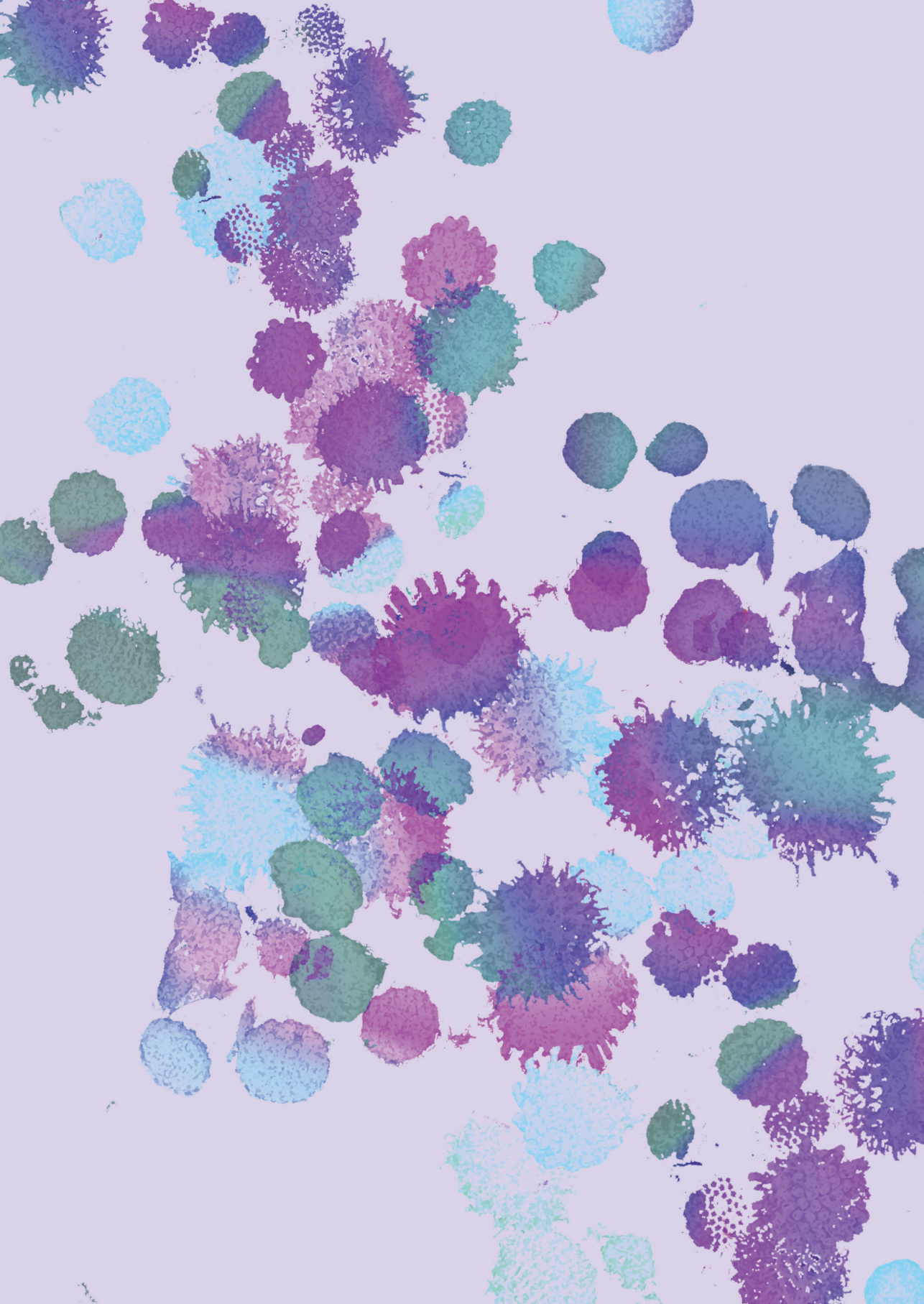
Primary T-cell responses against SARS-CoV-2 in patients with hematological disorders

Pothast, C.R.



SUMMARY

Chapter 6

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) mRNA vaccines induce effective humoral and T-cell responses in healthy individuals. Antibodies are important for preventing the entry of viral particles into cells. T cells can support other immune cells or directly lyse virus-infected cells. However, patients with hematological malignancies are often immunocompromised due to their disease or treatment. Understanding the specifics of the immunocompromised state of these patients is vital to adapting vaccination strategies or vaccine designs to improve protection against severe disease in these patients. However, vaccine-induced T-cell responses are rarely measured, as they are more laborious and complicated to interpret compared to antibodies. Therefore, in this thesis, we aimed to identify vaccine-induced SARS-CoV-2-specific T cells and antibodies in a large group of patients. The detection of vaccine-induced T cells is challenged by cross-reactive T cells since vaccine-induced T-cell responses are commonly measured using peptide-stimulation assays, which also result in the activation of cross-reactive T cells. We found that some individuals exhibit SARS-CoV-2-specific T cells even before exposure to the virus, and these T cells can originate from cytomegalovirus (CMV)-specific T cells. However, it seems that these cross-reactive T cells are not highly effective in SARS-CoV-2-infected cells, indicating that these pre-existing cells may not be able to change the course of the disease. The SARS-CoV-2 mRNA vaccines can induce antibody and T-cell responses in most individuals. Patients with aplastic anemia induced immune responses, although their cytokine production may be reduced compared to healthy individuals. In patients with hematological malignancies, it is known that the humoral response can be quite variable due to B-cell-targeting therapies that many patients receive. Despite this, most of these patients were able to develop SARS-CoV-2-specific T-cell responses. The magnitude of the CD4+ or CD8+ T-cell response and their cytokine profile did vary in some cohorts. Interestingly, poor T-cell counts or naïve T cells did not predict the vaccine-induced T-cell response. We demonstrated that, despite being immunocompromised, most patients with hematological malignancies can mount T-cell responses and therefore may have a layer of protection through these cells.

T cells can be cross-reactive against different peptide-human leukocyte antigen (HLA) complexes. By chance, individuals may have memory T cells that are reactive against a virus that they have never been exposed to. This is typically caused by sequence-homologous viruses. For this reason, most studies focused on whether pre-existing SARS-CoV-2-specific CD4+ and CD8+ T cells originate from other coronaviruses. However, in **chapter 2,** we showed that pre-existing SARS-CoV-2-reactive T cells can also originate from CMV-specific CD4+ or CD8+ T cells. Individuals who were seropositive for CMV had more measurable cross-reactive T cells. Upon isolation of these T cells, we confirmed their reactivity towards pp65, which is the most

immunogenic protein of CMV. The avidity of these cross-reactive T cells towards the CMV peptide was higher than for SARS-CoV-2 confirming that the T cells were primed and developed against CMV. The cross-reactive CD8+ T cells were detectable in multiple individuals. However, in an *in vitro* infection model, cross-reactive CD8+ T cells were less effective in limiting spread compared to vaccine-induced CD8+ T cells. Furthermore, we detected the cross-reactive CD8+ T cells in two patients with severe coronavirus disease 2019 (COVID-19) but these T cells showed a less activated phenotype compared to infection-induced T cells. This indicated that cross-reactive T cells appeared to be less functional compared to infection- or vaccine-induced T-cell responses.

Vaccination typically induces effective humoral and T-cell responses, resulting in improved viral clearance and thereby improved protection against severe disease. Vaccination is particularly important for individuals with a compromised immune system, such as patients with aplastic anemia. However, current guidelines recommend caution when it comes to vaccinating due to the potential risk of relapse of the aplastic anemia based on case reports, combined with the expectation that vaccinating might be less effective. Surprisingly, these guidelines also apply to patients who are in remission and years after immunosuppressive treatment. This is surprising considering that viral infections can trigger relapse. In **chapter 3**, we showed that this group of patients was able to mount SARS-CoV-2-specific antibodies and T-cell responses comparable to healthy individuals although cytokine production by the T cells appeared to be lower. Importantly, none of the patients showed signs of aplastic anemia relapse. In summary, the results from this chapter showed that the SARS-CoV-2 mRNA vaccine effectively induced antibody and T-cell responses in patients with aplastic anemia who were in remission and after immunosuppressive treatment, without inducing relapse.

Patients with hematological malignancies are often immunocompromised due to disease or treatment. For this reason, vaccination is prioritized for this group to minimize the severe course of COVID-19. However, the expected severity of the immunocompromised state of these patients is not clear, as immunity is often solely measured by the presence of antigen-specific antibodies. In **chapter 4**, we investigated whether patients who did not seroconvert following mRNA vaccination also lacked vaccine-induced T-cell responses. Spike-specific antibodies and CD4+ and CD8+ T cells were measured in patients with chronic lymphocytic leukemia (CLL), indolent or aggressive B-cell lymphoma, or multiple myeloma, which we stratified based on lacking (n=49) or with adequate seroconversion (n=14). We found that antibody levels can be variable among the patients and were especially low in patients with CLL.

Spike-specific CD4+ and CD8+ T cells were detected in almost all patients. Frequencies of spike-specific T cells appeared to reduce with age, as patients above 68 had lower frequencies of spike-specific CD4+ and CD8+ T cells compared to patients below 68. Interestingly, patients who did not seroconvert had higher frequencies of spike-specific CD8+ T cells. Therefore, the absence of antibodies is not a predictive measure of a lack of immunity in patients with CLL, lymphoma or multiple myeloma.

In **chapter 5**, we extended the group of patients with hematological malignancies (n=173) and stratified based on malignancy or treatment, resulting in 16 cohorts. These 16 cohorts included patients with lymphoma treated with rituximab or chemotherapy, untreated or BTK-inhibitors-treated CLL, multiple myeloma treated with chemotherapy, daratumumab or immunomodulatory drugs, acute myeloid leukemia (AML, including myelodysplastic syndrome) treated with chemotherapy or hypomethylating agents, myeloproliferative neoplasm (MPN) treated with JAK2-inhibitors, chronic myeloid leukemia (CML) treated with tyrosine-kinase-inhibitor (TKI), or patients treated with allogeneic stem cell transplantation. mRNA-induced spike-specific antibodies, CD4+ and CD8+ T cells, as well as the cytokine production and phenotype of the T cells were measured before and four weeks after each vaccine dose. Firstly, we found that in this extended cohort, most patients can generate spike-specific CD4+ and/or CD8+ T cells, also in the absence of antibodies. Combined antibody and T-cell deficiency was rare, although in patients treated with hypomethylating agents both antibodies and T cells were reduced but not absent. For patients treated with allogeneic stem cell transplantation, the antibody and T-cell responses were variable. Spike-specific CD4+ T cells showed lower frequencies in patients with multiple myeloma treated with daratumumab or immune-modulatory drugs. Cytokine production by spike-specific CD4+ T cells was reduced in patients with untreated CLL, MPN treated with JAK2 inhibitors, and allogeneic stem cell transplantation. Interestingly, lower frequencies of spike-specific T cells did not seem to be a result of the lack of availability of a large pool of naïve T cells, since T-cell counts or the percentage of naïve T cells at the start of vaccination did not correlate with vaccine-induced CD4+ or CD8+ T-cell frequencies. In summary, the immune response to mRNA vaccination was variable in the patient cohorts, but most patients developed humoral and/or T-cell responses. This shows that the SARS-CoV-2 mRNA vaccination can induce immune responses in these immunocompromised patients and thereby can protect against severe COVID-19.

GENERAL DISCUSSION

The circulation of SARS-CoV-2 and the introduction of vaccination in a previously unexposed (naïve) population, combined with bio-banking of samples, have created a unique opportunity to investigate both pre-existing immunity and primary immune responses. For this reason, our understanding of immune responses towards SARS-CoV-2 and mRNA vaccination has been widely expanded. This thesis aimed to contribute to this understanding by performing an in-depth analysis of SARS-CoV-2-specific CD4+ and CD8+ T cells in different contexts. A detailed investigation of antigen-specific T cells is often lacking due to its labor-intensive nature.[1] By employing advanced methods, this thesis aimed to expand the possible origins of pre-existing SARS-CoV-2-specific T cells and to provide novel insights into the vaccine-induced humoral and T-cell responses in immunocompromised patients. The results show the importance of in-depth analysis of antigen-specific T cells, especially considering the critical role T cells can play in protecting against severe disease.[2-6]

Detection of antigen-specific T cells

Following vaccination, each component of the adaptive immune response provides a layer of protection that is important to prevent a severe course of disease.[2] However, patients with hematological malignancies are often immunocompromised due to impaired antibody, CD4+ T-cell, and/or CD8+ T-cell responses. Assessments of vaccine-induced immune responses are usually focused on seroconversion, underreporting cellular responses.[7-11] As a result, vaccination recommendations are often based solely on antibody titers, with a lack of antibody presence being interpreted as an absence of immunity.[12-15] This may result in advice against or delayed vaccination in vulnerable individuals who are unable to seroconvert, despite evidence that T cells can provide protection against severe disease and are more effective against variants of concern compared to antibodies.[2-6,12] This issue of underreported cellular responses and vaccination recommendations solely based on antibody titers is not specific for SARS-CoV-2 vaccination but also extends to other vaccinations.[16] The main cause of the limited attention on T-cell responses is the lack of high-throughput and standardized detection methods, underscoring an urgent need for improved detection of antigen-specific T cells.[6]

The current most standardized methods for measuring antigen-specific T cells are the interferon gamma release assay (IGRA) and the enzyme-linked immunosorbent spot (ELIspot) assay. These techniques were commonly used during the SARS-CoV-2 pandemic to measure T-cell responses in patients with hematological malignancies.[1,6] In these assays, whole blood or peripheral blood mononuclear cells (PBMCs)

are co-incubated with peptides, and the release of IFN-γ is measured either as a concentration in the supernatant (IGRA) or as spot-forming units captured directly upon cytokine release from individual cells (ELIspot). These spots are then counted as a measurement of the number of interferon-γ (IFN-γ)-producing cells. Although these assays can quickly provide insights into the number of T cells per unit of PBMCs or whole blood, these assays appear to be variable between studies and generally underestimate the true magnitude of the T-cell response.[17-19] This issue is probably related to differences in peptide selection, culturing conditions, analysis methods, IFN-γ-exclusive measurement, and inconsistent PBMC input across samples. This issue is further complicated in patients with hematological malignancies, where due to lymphopenia or a high burden of malignant cells, abnormal PBMC composition can distort the results. Because these assays do not correct for the actual amount of T cells present in the samples, the chance of missing antigen-specific T cells is high when T-cell frequencies fall outside the normal range.[20] Therefore, while ELISA-based assays are highly valuable for large-scale measurement of T-cell responses in healthy individuals, they may be less optimal for patients with hematological malignancies. In such cases, flow cytometry would be a more accurate and informative approach. This is because flow cytometry gives more insight into the frequencies of antigen-specific T cells, independent of the amount of other immune cells, and can also provide more insight into cytokine-producing cell subsets. However, flow cytometry is more labor-intensive and less standardized. Potential solutions to improve feasibility could be circumventing permeabilization steps using optimized cytokine-capture-based assays or simplifying analysis using artificial intelligence.[21-23] Accordingly, there is a need to optimize and standardize flow cytometry protocols for high-throughput detection of antigen-specific T cells.[1]

As mentioned before, flow cytometry allows for a more accurate and specific detection of antigen-specific T cells. However, elaborate studies on antigen-specific T-cell responses following SARS-CoV-2 vaccination using flow cytometry are scarce and mainly focused on CD4+ T cells. This is partly caused by the challenges that come with the detection of antigen-specific CD8+ T cells, which are present at lower frequencies in the circulation, and because the assays are usually optimized for the detection of CD4+ T cells.[1,24,25] As a result, uncertainty may occur about infection- or vaccine-induced CD8+ T-cell responses, whether the (lack of) detected frequencies are biologically accurate or caused by a technical artefact.[6,26,27] In **chapter 5**, the spike-specific CD8+ T-cell frequencies before and during vaccination detected through AIM assay were more variable compared to the CD4+ T cell frequencies, highlighting the need to optimize antigen-specific CD8+ T cell detection methods. In **chapter 4,** we combined the AIM assay with ICS and defined the antigen-specific CD8+ T cells

based on activation markers combined with cytokine production. This resulted in frequency kinetics that were more similar to spike-specific CD4+ T cells. However, spike-specific CD8+ T cells which are unable to produce cytokines might still be important, potentially underestimating the full spectrum of the CD8+ T cell response. Other studies used detection methods for antigen-specific CD8+ T cells that were more complicated than those typically used for CD4+ T cells. They include the use of more activation markers to reduce background noise, stimulating with HLA class I (predicted) epitopes, or the incorporation of a T-cell expansion step to enhance detectable frequencies.[28-31] The fact that each study measures antigen-specific CD8+ T cells differently underlines that a consensus on an optimal detection method is currently lacking. This is a remaining issue, particularly since CD8+ T cells are a key component in the clearance of virus-infected cells and the strong association between SARS-CoV-2-specific T cell responses and the protection against severe disease.[2,4-6]

Apart from the detection method, epitope selection is key to an accurate detection of antigen-specific T cells and affects results obtained through IGRA, ELIspot, and flow cytometry. In the case of the emergence of a new pathogen, this is more challenging since the epitope immunogenicity is unknown. Peptide pools are therefore initially based on covering an immunogenic protein by overlapping peptides, or peptides are selected based on *in silico* predictions. Previous studies have shown that commercialized kits that measure antigen-specific T cells may use suboptimal peptide pools and therefore result in an underestimation of T-cell responses and variable outcomes depending on the kit used.[17,32] In **chapters 4** and **5**, peptide-stimulation assays using 15-mer peptides were complemented using peptide-HLA tetramers for the detection of spike-specific CD8+ T cells. To generate the peptide-HLA tetramers, a selection was made of 23 predicted immunogenic peptide-HLA antigens. The frequencies detected through peptide-HLA tetramers correlated well with the frequencies obtained by AIM assay. Furthermore, peptide-HLA tetramer staining resulted in frequency kinetics that were similar to the frequencies of the antigen-specific CD4+ T cells obtained by AIM assay. Although this indicates that peptide-HLA tetramers could be a preferred method over AIM assays, the frequencies of spike-specific CD8+ T cells as measured by peptide-HLA tetramer frequencies were lower and detected in fewer individuals. This is most likely due to the restricted peptide-HLA combinations present in the tetramer pool, which do not cover the full range of HLA alleles in the population. This exposes the challenge of peptide-HLA tetramers: the ability to include as many immunogenic peptide-HLA complexes as possible. Fortunately, the large focus on measuring T-cell responses during the pandemic has resulted in a fast discovery of T-cell epitopes and therefore improves the accuracy of detection of antigen-specific T cells.[18,28]

To summarize, the measurement of antigen-specific T cells has some challenges to overcome. These challenges are mostly related to the detection method used, limited understanding of how to reliably detect antigen-specific CD8+ T cells, and the complexity of peptide selection. Currently available detection methods are either high throughput but less accurate or more precise yet labor-intensive. Simplifying the measurement of antigen-specific T cells using flow cytometry could pave the way for more accurate measurement of adaptive immune responses to vaccination. The widespread circulation of SARS-CoV-2 has resulted in a boost in optimized detection of antigen-specific T cells. These developments must continue to develop accurate and large-scale detection methods of antigen-specific T cells that can also be applied to other vaccination strategies.

Detection of cross-reactive T cells

T-cell cross-reactivity refers to the ability of a single T-cell receptor (TCR) to recognize different peptide-HLA complexes. This can range from a single amino acid change in the peptide to a completely different peptide and HLA complex. T-cell cross-reactivity is widely studied in different contexts. It is commonly described as beneficial since it can protect against a wider range of pathogens, but it can also be detrimental when it causes autoimmunity. T cell cross-reactivity depends on multiple factors, including sequence similarity, molecular mimicry, hotspot binding, and the structural plasticity of the peptide-HLA complex and/or TCR. In **chapter 2**, we identified CMV-specific CD4+ and CD8+ T cells that were also reactive towards SARS-CoV-2, highlighting an example of such cross-reactivity. Understanding the origin and function of cross-reactive T cells is challenging, but it is vital to expand our understanding of T-cell immunity.

Many research groups have reported the presence of cross-reactive T cells targeting SARS-CoV-2 in samples from SARS-CoV-2-unexposed individuals.[33-37] Since most commonly used assays for detecting antigen-specific T cells have limited sensitivity, the cross-reactivities that are identified tend to be biased toward T cells that are already present at relatively high frequencies in the circulation. In **chapter 2**, we detected and characterized CMV pp65-specific HLA-B*35:01-restricted T cells from multiple SARS-CoV-2 unexposed individuals that were cross-reactive towards a peptide derived from the spike protein of SARS-CoV-2, also presented in the context of HLA-B*35:01. These T cells were likely to have been picked up since CMV-specific T cells are known to be present at high frequencies in a significant portion of the population. Detection methods also vary in their sensitivity to TCR affinity. For instance, peptide-HLA tetramer staining can detect even low-affinity TCR interactions, since the assay relies solely on the physical binding of peptide-HLA complex and the TCR. However, such binding does not always correlate with functional avidity and the ability to

induce a functional T-cell response.[38] Similarly, in peptide-stimulation assays, usually high peptide concentrations are used that also induce activation of low-avidity T cells.[39] This phenomenon can likely have been observed in **chapter 5**, where CD8+ T cell responses after *in vitro* exposure to spike peptide pools were commonly observed before the patients were vaccinated and exposed to SARS-CoV-2. After the detection of cross-reactive T cells, researchers tried to identify their original target peptide-HLA complex. This was commonly only tested against a restricted pool of peptides, based on the assumption that cross-reactivity results from minor peptide sequence changes. However, our data in **chapter 2** demonstrates that a single TCR can be cross-reactive towards peptides that differ in 5 out of 9 amino acids. Therefore, identifying the original antigen of cross-reactive T cells is especially challenging when it involves the recognition of two dissimilar peptides, since the original peptide could originate from any pathogen. Therefore, mapping the antigenic specificity of cross-reactive T cells requires large peptide libraries that cover multiple different pathogens or unbiased peptide discovery approaches. As described in **chapter 2,** applying the combinatorial peptide library enabled an unbiased identification of the original pathogen by testing the T cells against a library of peptides that identify the key amino acids for T cell recognition, independent of known epitopes.[40,41] This expands our understanding of the mechanisms behind T-cell cross-reactivity and their potential implications.

Numerous studies have detected pre-existing SARS-CoV-2-specific CD4+ and CD8+ T cells, but it is currently unclear whether they can play a pivotal role in prevention of severe disease.[28,33,35-37,42-51] Current studies mostly lean towards an important role of cross-reactive T cells during SARS-CoV-2 infection. If these cross-reactive T cells indeed play an pivotal role, this could have implications for the pathogenesis of SARS-CoV-2 infections and could aid in the development of a pan-coronavirus vaccine.[34,36,52-55] However, we propose that the cross-reactive T-cell responses towards SARS-CoV-2 have a high likelihood of being of too low avidity. Pre-existing SARS-CoV-2-reactive T cells are, in most cases, reported to be primed by related common human coronaviruses (229E, NL63, HKU1, and OC43).[56] Despite the relatively high sequence homology with SARS-CoV-2 (65-69%), the sequence similarity is scattered over the genome, resulting in a maximum protein similarity of ~35% and a low amount of predicted shared epitopes.[53,57,58] This is confirmed by the observation that, to our knowledge, none of the identified epitopes of cross-reactive T cells share 100% sequence homology.[33,35-37,42-50] As a result, a limited pool of T cells that are reactive towards the common coronaviruses can recognize SARS-CoV-2, and their avidity appears to be lower due to amino acid changes in the peptide sequence.[37,45,50,53,59] Furthermore, these low-avidity T cells may appear functional in *in vitro* peptide-stimulation assays that use a T-cell optimal environment, but this is likely not

representative of *in vivo* functionality. The data presented in **chapter 2** show that the cross-reactive T cells were SARS-CoV-2-reactive in peptide-stimulation assays, but in a SARS-CoV-2 live virus infection assay, cross-reactive CD8+ T cells were unable to effectively inhibit virus spread. Apart from optimal T-cell conditions, most assays also fail to test if the targeted peptides are effectively processed and presented during infection. This reduces the chances that cross-reactive T cells can form a polyclonal T cell response that plays a major role in protection against severe disease and the ability to develop an effective pan coronavirus vaccine that covers a diverse range of HLA types.

Functionality of T cells in disease and therapy

Vaccination-induced immune responses are commonly measured by the presence of antigen-specific antibodies only, since antibody measurements are standardized and high-throughput. As a result, vaccination guidelines are commonly based on seroconversion. However, not all individuals develop a (measurable) antibody response, which is especially the case for immunocompromised individuals. As illustrated in **chapters 3-5**, it became clear that the absence of antigen-specific antibodies does not correlate with the absence of cellular immune responses and that most immunocompromised patients were able to develop vaccine-induced T-cell responses. This indicates that vaccination should be recommended also in patients where an inability to seroconvert is expected, to increase T-cell-mediated protection against severe disease.

As briefly mentioned, **chapters 4** and **5** show that an absence of a humoral response does not necessarily correlate with an absence of T cell responses: a combined humoral and T-cell response deficiency was rarely observed. This shows that an absence of humoral immunity does not directly indicate a hampered overall immune response. Patients who often have reduced or absent humoral responses are patients with B-cell lymphoma. These patients are usually treated with B-cell-targeting treatments, which explain their variable antibody levels. In contrast to vaccine-induced antibody responses, T cell responses in this patient group were similar to those of healthy individuals, as shown in this thesis in **chapters 4** and **5**, and by others.[60-64] Vaccination schedules should therefore not be based solely on the likelihood of seroconversion. Instead, patients should be vaccinated regardless of treatment status to protect this patient population through T cell immunity.

Patients with CLL are more sensitive to a severe course of infections due to the hampered immune system caused by the disease.[65] This has been particularly well studied for the effect of CLL cells on T cells.[66-68] **Chapters 4-5** of this thesis showed

that while the frequencies of vaccine-induced T-cell responses were similar to healthy controls, the ability to produce cytokines was reduced. Interestingly, when CLL cells were removed from the T-cell stimulation assays, T cells were able to produce normal levels of TNF-α and IL-2. This suggests that CLL cells can hamper cytokine production by T cells when they are in close proximity. Whether this suppression of TNF-α and IL-2 also occurs *in vivo* is unknown and may depend on the local concentration of CLL cells within tissues.[69] After removal of CLL cells, IFN-γ production was still hampered, indicating an intrinsically reduced production of IFN-γ by T cells in patients with untreated CLL. This was likely caused by the presence of CLL cells, since upon inhibition of CLL cells by BTK inhibitors, the cytokine production by *de novo* induced T-cell responses was restored. Although it appears that BTK inhibitors restore T cell immunity, they can negatively affect B cell responses, as presented in **chapters 4-5** and the current literature.[70,71] Therefore, based on the data presented in this thesis and given the risk of viral complications in this patient population, vaccination should be recommended in this patient cohort, independent of their current treatment.[72]

In **chapter 5,** we demonstrate that patients treated with hypomethylating agents (HMA) for AML/MDS had reduced spike-specific antibody concentrations, as well as lower spike-specific CD4+ and CD8+ T-cell frequencies.[73,74] The observation that patients with AML treated with high-dose chemotherapy developed humoral and T-cell responses similar to healthy individuals indicates that the HMA treatment could be the underlying cause of the hampered immune responses. A likely explanation is that HMA, which targets proliferating cells, may impair the expansion of activated, spike-specific T and B cells following vaccination.[73-76] This is supported by the observation that the frequencies of T cells with other specificities (CMV, EBV, flu, and more; CEFX) that are usually at rest during therapy were not reduced. It is important to note that the reduced T-cell frequencies could be attributed to the higher age of patients in the HMA cohort.[62,77,78] Current literature indicates that patients treated with hypomethylating agents have an increased risk of severe infections, independent of age.[79-81] This suggests that the observed reduced immune responses could indeed be caused by hypomethylating agents, urging the need for more elaborate investigation into whether hypomethylating agents indeed can affect vaccine-induced immunity. Investigating the vaccine-induced B- and T-cell responses in a heterogeneous group of patients with different hypomethylating treatment regimens and ages would provide insight into this matter. Until then, the reduced immune function observed in these patients and the continued circulation of SARS-CoV-2 in the population support the use of the SARS-CoV-2 mRNA vaccination in this cohort to provide some protection against severe disease. Since HMA is typically given for a long period, vaccination should not be delayed until after therapy. To protect these patients during therapy,

the vaccination could be scheduled two weeks before the next HMA cycle to allow T cell responses to develop with minimal effect of the HMA. Furthermore, additional booster vaccinations might be necessary to fully protect these patients against a severe course of SARS-CoV-2 infection.

Patient cohorts with low T-cell counts and/or low frequencies of naïve T cells were able to generate vaccine-induced spike-specific CD4+ and CD8+ T cells similar to healthy donors, both based on frequencies and absolute numbers. This includes patients treated with allogeneic stem cell transplantation (SCT) and chimeric antigen receptor (CAR) T cell therapy. Although counterintuitive, the lack of correlation between absolute T-cell counts and vaccine-induced T-cell responses has been reported before.[78,82] It has previously been proposed that T-cell counts in the context of allogeneic SCT could be a better predictor of vaccine immunogenicity than time since transplantation.[12] However, **chapter 5** showed that low T-cell counts and/or percentage of naïve T cells were not indicative of proper T-cell activation and thus should not function as a predictor for adequate *de novo* T-cell responses. Therefore, vaccination should not be delayed based on low lymphocyte counts in circulation, as is often observed shortly after allogeneic SCT and CAR T cell therapy.

The observation that patients with hematological malignancies generated vaccine-induced antibody and/or T-cell responses similarly to healthy individuals supports the use of mRNA vaccination to protect these patients. Compared to other SARS-CoV-2 vaccine modalities, mRNA vaccines appear more effective and can generate stronger humoral and T-cell responses in healthy individuals.[83,84] Of the two mRNA vaccines, the mRNA-1273 (Moderna) induced stronger immune responses than BNT162b2 (Pfizer/BionTech), potentially related to the differences in vaccination dosage, components, or timing between dosages.[84-86] Therefore, mRNA-1273 may be preferred over BNT162b2 for immunocompromised patients. This could change in the near future since BioNTech developed a new SARS-CoV-2 vaccine (BNT162b4) which includes antigens of the membrane, nucleocapsid, and open reading frame 1ab (ORF1ab) genes.[87] This vaccine could further enhance T-cell responses compared to the original spike vaccination only and could thereby outperform mRNA-1273 regarding T-cell responses. At the beginning of the pandemic, major concerns were raised about the waning antibodies after mRNA vaccination. Fortunately, a large study showed that antibody levels quickly decline at first but then stabilized.[88] This supports the use of mRNA vaccines for patients, independent of whether the B cells or T cells may be hampered.

The data presented in this study may have implications for other vaccines as well. Viral complications are one of the major concerns for patients with hematological malignancies, and for this reason, vaccinations against influenza, pneumococcal infection, and herpes zoster virus (VZV) are commonly recommended for patients who are treated with immunosuppressive drugs or stem cell transplantation.[12,89] The vaccination guidelines are based on several factors, including expected vaccine efficacy based on treatment, B- and T-counts in circulation, but also vaccine type.[13-15] One of the issues is that live vaccines are preferably not administered in immunocompromised patients due to the risk of severe side effects.[13,90] Inactivated vaccines are a safer alternative, but they are usually less effective or not yet available.[15] The data presented in this thesis show that mRNA vaccines can induce effective humoral and cellular immune responses in these patients. Furthermore, previous research indicates that mRNA vaccines are more effective compared to other vaccine modalities, supporting their potential as a safe and effective vaccine modality.[84,91] Currently, mRNA vaccines are being developed against multiple infectious diseases, including influenza and VZV.[92] The ability of such vaccines to induce robust humoral and cellular responses will highly depend on the antigen selection and potentially other vaccine modifications.[92]

Concluding remarks

To summarize, in-depth analyses of antigen-specific T cells can provide valuable information to understand protection against severe disease beyond antibody titers. The immune response to SARS-CoV-2 mRNA vaccination was heterogeneous in patients with hematological malignancies. Fortunately, we detected vaccine-specific T-cell responses in most patients, also in the absence of a humoral response. Current vaccination guidelines for immunocompromised patients are frequently based on T-cell counts and/or the predicted ability to develop humoral responses. However, this thesis highlights that this approach is likely unreliable. Importantly, even when vaccine-induced immune responses are diminished compared with those in healthy individuals, immunocompromised patients can still benefit from vaccination, as partial protection is preferable to none. This thesis underscores the importance of investigating the vaccine-induced T-cell responses in patients with hematological malignancies and emphasizes the need for future vaccine development to induce both strong humoral and T-cell immunity to protect vulnerable individuals.

REFERENCES

1. Binayke, A. *et al*. A quest for universal anti-SARS-CoV-2 T cell assay: systematic review, meta-analysis, and experimental validation. *NPJ Vaccines* **9**, 3, doi:10.1038/s41541-023-00794-9 (2024).
2. Sette, A. & Crotty, S. Adaptive immunity to SARS-CoV-2 and COVID-19. *Cell* **184**, 861-880, doi:10.1016/j.cell.2021.01.007 (2021).
3. Tarke, A. *et al*. Early and Polyantigenic CD4 T Cell Responses Correlate with Mild Disease in Acute COVID-19 Donors. *Int J Mol Sci* **23**, doi:10.3390/ijms23137155 (2022).
4. Rydyznski Moderbacher, C. *et al*. Antigen-Specific Adaptive Immunity to SARS-CoV-2 in Acute COVID-19 and Associations with Age and Disease Severity. *Cell* **183**, 996-1012 e1019, doi:10.1016/j.cell.2020.09.038 (2020).
5. Tan, A. T. *et al*. Early induction of functional SARS-CoV-2-specific T cells associates with rapid viral clearance and mild disease in COVID-19 patients. *Cell Rep* **34**, 108728, doi:10.1016/j.celrep.2021.108728 (2021).
6. Sette, A., Sidney, J. & Crotty, S. T Cell Responses to SARS-CoV-2. *Annu Rev Immunol* **41**, 343-373, doi:10.1146/annurev-immunol-101721-061120 (2023).
7. Piechotta, V. *et al*. Effectiveness, immunogenicity, and safety of COVID-19 vaccines for individuals with hematological malignancies: a systematic review. *Blood Cancer J* **12**, 86, doi:10.1038/s41408-022-00684-8 (2022).
8. Wang, K. Y. *et al*. Vaccination efficacy in patients with chronic lymphocytic leukemia. *Leuk Lymphoma* **64**, 42-56, doi:10.1080/10428194.2022.2133538 (2023).
9. Chong, P. P. & Avery, R. K. A Comprehensive Review of Immunization Practices in Solid Organ Transplant and Hematopoietic Stem Cell Transplant Recipients. *Clin Ther* **39**, 1581-1598, doi:10.1016/j.clinthera.2017.07.005 (2017).
10. Kamboj, M. & Shah, M. K. Vaccination of the Stem Cell Transplant Recipient and the Hematologic Malignancy Patient. *Infect Dis Clin North Am* **33**, 593-609, doi:10.1016/j.idc.2019.02.007 (2019).
11. Kim, C., Fang, F., Weyand, C. M. & Goronzy, J. J. The life cycle of a T cell after vaccination - where does immune ageing strike? *Clin Exp Immunol* **187**, 71-81, doi:10.1111/cei.12829 (2017).
12. Tsigrelis, C. & Ljungman, P. Vaccinations in patients with hematological malignancies. *Blood Rev* **30**, 139-147, doi:10.1016/j.blre.2015.10.001 (2016).
13. Lopez, A. *et al*. Vaccination recommendations for the adult immunosuppressed patient: A systematic review and comprehensive field synopsis. *J Autoimmun* **80**, 10-27, doi:10.1016/j.jaut.2017.03.011 (2017).
14. Reynolds, G., Hall, V. G. & Teh, B. W. Vaccine schedule recommendations and updates for patients with hematologic malignancy post-hematopoietic cell transplant or CAR T-cell therapy. *Transpl Infect Dis* **25 Suppl 1**, e14109, doi:10.1111/tid.14109 (2023).
15. Pollard, A. J. & Bijker, E. M. A guide to vaccinology: from basic principles to new developments. *Nat Rev Immunol* **21**, 83-100, doi:10.1038/s41577-020-00479-7 (2021).

16. Groeneweg, L., Loeffen, Y. G. T., Versluys, A. B. & Wolfs, T. F. W. Safety and efficacy of early vaccination with live attenuated measles vaccine for hematopoietic stem cell transplant recipients and solid organ transplant recipients. *Vaccine* **39**, 3338-3345, doi:10.1016/j.vaccine.2021.04.049 (2021).

17. Aiello, A. *et al*. Accuracy of QuantiFERON SARS-CoV-2 research use only assay and characterization of the CD4(+) and CD8(+) T cell-SARS-CoV-2 response: comparison with a homemade interferon-gamma release assay. *Int J Infect Dis* **122**, 841-849, doi:10.1016/j.ijid.2022.07.049 (2022).

18. Yu, E. D. *et al*. Development of a T cell-based immunodiagnostic system to effectively distinguish SARS-CoV-2 infection and COVID-19 vaccination status. *Cell Host Microbe* **30**, 388-399 e383, doi:10.1016/j.chom.2022.02.003 (2022).

19. Mak, W. A., Visser, W., Koeleman, J. G. M. & Ong, D. S. Y. SARS-CoV-2-specific T cell responses: a comparative analysis between QuantiFERON SARS-CoV-2, T-SPOT.COVID, and an in-house Omicron ELISpot. *J Virol Methods* **327**, 114949, doi:10.1016/j.jviromet.2024.114949 (2024).

20. de Vries, R. D. *et al*. Difference in sensitivity between SARS-CoV-2-specific T cell assays in patients with underlying conditions. *J Clin Invest* **131**, doi:10.1172/JCI155499 (2021).

21. Assenmacher, M., Lohning, M. & Radbruch, A. Detection and isolation of cytokine secreting cells using the cytometric cytokine secretion assay. *Curr Protoc Immunol* **Chapter 6**, 6 27 21-26 27 10, doi:10.1002/0471142735.im0627s46 (2002).

22. Ng, D. P. *et al*. Recommendations for using artificial intelligence in clinical flow cytometry. *Cytometry B Clin Cytom* **106**, 228-238, doi:10.1002/cyto.b.22166 (2024).

23. Zandvliet, M. L. *et al*. Detailed analysis of IFNg response upon activation permits efficient isolation of cytomegalovirus-specific CD8+ T cells for adoptive immunotherapy. *J Immunother* **32**, 513-523, doi:10.1097/CJI.0b013e3181a2712c (2009).

24. Kiecker, F. *et al*. Analysis of antigen-specific T-cell responses with synthetic peptides--what kind of peptide for which purpose? *Hum Immunol* **65**, 523-536, doi:10.1016/j.humimm.2004.02.017 (2004).

25. Zhang, W., Moldovan, I., Targoni, O. S., Subbramanian, R. A. & Lehmann, P. V. How much of virus-specific CD8 T cell reactivity is detected with a peptide pool when compared to individual peptides? *Viruses* **4**, 2636-2649, doi:10.3390/v4112636 (2012).

26. Huang, L. *et al*. Herpes zoster mRNA vaccine induces superior vaccine immunity over licensed vaccine in mice and rhesus macaques. *Emerg Microbes Infect* **13**, 2309985, doi:10.1080/22221751.2024.2309985 (2024).

27. Sette, A. & Crotty, S. Immunological memory to SARS-CoV-2 infection and COVID-19 vaccines. *Immunol Rev*, doi:10.1111/imr.13089 (2022).

28. Grifoni, A. *et al*. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. *Cell* **181**, 1489-1501 e1415, doi:10.1016/j.cell.2020.05.015 (2020).

29. Painter, M. M. *et al*. Rapid induction of antigen-specific CD4(+) T cells is associated with coordinated humoral and cellular immunity to SARS-CoV-2 mRNA vaccination. *Immunity* **54**, 2133-2142 e2133, doi:10.1016/j.immuni.2021.08.001 (2021).

30. Oberhardt, V. *et al.* Rapid and stable mobilization of CD8(+) T cells by SARS-CoV-2 mRNA vaccine. *Nature* **597**, 268-273, doi:10.1038/s41586-021-03841-4 (2021).

31. Schnatbaum, K. *et al.* An Overview of Peptides and Peptide Pools for Antigen-Specific Stimulation in T-Cell Assays. *Methods Mol Biol* **2768**, 29-50, doi:10.1007/978-1-0716-3690-9_3 (2024).

32. Phillips, E. *et al.* Comparison of two T-cell assays to evaluate T-cell responses to SARS-CoV-2 following vaccination in naive and convalescent healthcare workers. *Clin Exp Immunol* **209**, 90-98, doi:10.1093/cei/uxac042 (2022).

33. Johansson, A. M. *et al.* Cross-reactive and mono-reactive SARS-CoV-2 CD4+ T cells in prepandemic and COVID-19 convalescent individuals. *PLoS Pathog* **17**, e1010203, doi:10.1371/journal.ppat.1010203 (2021).

34. Kundu, R. *et al.* Cross-reactive memory T cells associate with protection against SARS-CoV-2 infection in COVID-19 contacts. *Nat Commun* **13**, 80, doi:10.1038/s41467-021-27674-x (2022).

35. Le Bert, N. *et al.* SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. *Nature* **584**, 457-462, doi:10.1038/s41586-020-2550-z (2020).

36. Loyal, L. *et al.* Cross-reactive CD4(+) T cells enhance SARS-CoV-2 immune responses upon infection and vaccination. *Science* **374**, eabh1823, doi:10.1126/science.abh1823 (2021).

37. Mateus, J. *et al.* Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans. *Science* **370**, 89-94, doi:10.1126/science.abd3871 (2020).

38. Hombrink, P. *et al.* Mixed functional characteristics correlating with TCR-ligand koff -rate of MHC-tetramer reactive T cells within the naive T-cell repertoire. *Eur J Immunol* **43**, 3038-3050, doi:10.1002/eji.201343397 (2013).

39. Betts, M. R. *et al.* The functional profile of primary human antiviral CD8+ T cell effector activity is dictated by cognate peptide concentration. *J Immunol* **172**, 6407-6417, doi:10.4049/jimmunol.172.10.6407 (2004).

40. Wooldridge, L. *et al.* CD8 controls T cell cross-reactivity. *J Immunol* **185**, 4625-4632, doi:10.4049/jimmunol.1001480 (2010).

41. Bijen, H. M. *et al.* Preclinical Strategies to Identify Off-Target Toxicity of High-Affinity TCRs. *Mol Ther* **26**, 1206-1214, doi:10.1016/j.ymthe.2018.02.017 (2018).

42. Florian, D. M. *et al.* Enhanced and long-lasting SARS-CoV-2 immune memory in individuals with common cold coronavirus cross-reactive T cell immunity. *Front Immunol* **16**, 1501704, doi:10.3389/fimmu.2025.1501704 (2025).

43. Low, J. S. *et al.* Clonal analysis of immunodominance and cross-reactivity of the CD4 T cell response to SARS-CoV-2. *Science* **372**, 1336-1341, doi:10.1126/science.abg8985 (2021).

44. Nelde, A. *et al.* SARS-CoV-2-derived peptides define heterologous and COVID-19-induced T cell recognition. *Nat Immunol* **22**, 74-85, doi:10.1038/s41590-020-00808-x (2021).

45. Dykema, A. G. *et al.* Functional characterization of CD4+ T cell receptors crossreactive for SARS-CoV-2 and endemic coronaviruses. *J Clin Invest* **131**, doi:10.1172/JCI146922 (2021).

46. Stoddard, C. I. *et al.* Epitope profiling reveals binding signatures of SARS-CoV-2 immune response in natural infection and cross-reactivity with endemic human CoVs. *Cell Rep* **35**, 109164, doi:10.1016/j.celrep.2021.109164 (2021).

47. Schulien, I. *et al.* Characterization of pre-existing and induced SARS-CoV-2-specific CD8(+) T cells. *Nat Med* **27**, 78-85, doi:10.1038/s41591-020-01143-2 (2021).

48. Francis, J. M. *et al*. Allelic variation in class I HLA determines CD8(+) T cell repertoire shape and cross-reactive memory responses to SARS-CoV-2. *Sci Immunol* **7**, eabk3070, doi:10.1126/sciimmunol.abk3070 (2022).

49. Jing, L. *et al*. T cell response to intact SARS-CoV-2 includes coronavirus cross-reactive and variant-specific components. *JCI Insight* **7**, doi:10.1172/jci.insight.158126 (2022).

50. Lineburg, K. E. *et al*. CD8(+) T cells specific for an immunodominant SARS-CoV-2 nucleocapsid epitope cross-react with selective seasonal coronaviruses. *Immunity* **54**, 1055-1065 e1055, doi:10.1016/j.immuni.2021.04.006 (2021).

51. Weiskopf, D. *et al*. Phenotype and kinetics of SARS-CoV-2-specific T cells in COVID-19 patients with acute respiratory distress syndrome. *Sci Immunol* **5**, doi:10.1126/sciimmunol.abd2071 (2020).

52. Humbert, M. *et al*. Functional SARS-CoV-2 cross-reactive CD4(+) T cells established in early childhood decline with age. *Proc Natl Acad Sci U S A* **120**, e2220320120, doi:10.1073/pnas.2220320120 (2023).

53. Murray, S. M. *et al*. The impact of pre-existing cross-reactive immunity on SARS-CoV-2 infection and vaccine responses. *Nat Rev Immunol* **23**, 304-316, doi:10.1038/s41577-022-00809-x (2023).

54. Moss, P. The T cell immune response against SARS-CoV-2. *Nat Immunol* **23**, 186-193, doi:10.1038/s41590-021-01122-w (2022).

55. Sagar, M. *et al*. Recent endemic coronavirus infection is associated with less-severe COVID-19. *J Clin Invest* **131**, doi:10.1172/JCI143380 (2021).

56. Vojdani, A., Yaqinuddin, A., Beretta, A. & Reche, P. A. Editorial: Cross-reactive immunity and COVID-19. *Front Immunol* **15**, 1509379, doi:10.3389/fimmu.2024.1509379 (2024).

57. Kaur, N. *et al*. Genetic comparison among various coronavirus strains for the identification of potential vaccine targets of SARS-CoV2. *Infect Genet Evol* **89**, 104490, doi:10.1016/j.meegid.2020.104490 (2021).

58. Lee, C. H. *et al*. Potential CD8+ T Cell Cross-Reactivity Against SARS-CoV-2 Conferred by Other Coronavirus Strains. *Front Immunol* **11**, 579480, doi:10.3389/fimmu.2020.579480 (2020).

59. Bacher, P. *et al*. Low-Avidity CD4(+) T Cell Responses to SARS-CoV-2 in Unexposed Individuals and Humans with Severe COVID-19. *Immunity* **53**, 1258-1271 e1255, doi:10.1016/j.immuni.2020.11.016 (2020).

60. Boerenkamp, L. S. *et al*. Increased CD8 T-cell immunity after COVID-19 vaccination in lymphoid malignancy patients lacking adequate humoral response: An immune compensation mechanism? *Am J Hematol*, doi:10.1002/ajh.26729 (2022).

61. Riise, J. *et al*. Rituximab-treated patients with lymphoma develop strong CD8 T-cell responses following COVID-19 vaccination. *Br J Haematol* **197**, 697-708, doi:10.1111/bjh.18149 (2022).

62. Bitoun, S. *et al*. Rituximab Impairs B Cell Response But Not T Cell Response to COVID-19 Vaccine in Autoimmune Diseases. *Arthritis Rheumatol* **74**, 927-933, doi:10.1002/art.42058 (2022).

63. Keppler-Hafkemeyer, A. *et al*. Potent high-avidity neutralizing antibodies and T cell responses after COVID-19 vaccination in individuals with B cell lymphoma and multiple myeloma. *Nat Cancer* **4**, 81-95, doi:10.1038/s43018-022-00502-x (2023).

64. Zonozi, R. *et al*. T cell responses to SARS-CoV-2 infection and vaccination are elevated in B cell deficiency and reduce risk of severe COVID-19. *Sci Transl Med* **15**, eadh4529, doi:10.1126/scitranslmed.adh4529 (2023).

65. Forconi, F. & Moss, P. Perturbation of the normal immune system in patients with CLL. *Blood* **126**, 573-581, doi:10.1182/blood-2015-03-567388 (2015).

66. van Bruggen, J. A. C. *et al.* Depletion of CLL cells by venetoclax treatment reverses oxidative stress and impaired glycolysis in CD4 T cells. *Blood Adv* **6**, 4185-4195, doi:10.1182/bloodadvances.2022007034 (2022).

67. Riches, J. C. *et al.* T cells from CLL patients exhibit features of T-cell exhaustion but retain capacity for cytokine production. *Blood* **121**, 1612-1621, doi:10.1182/blood-2012-09-457531 (2013).

68. Palma, M. *et al.* T cells in chronic lymphocytic leukemia display dysregulated expression of immune checkpoints and activation markers. *Haematologica* **102**, 562-572, doi:10.3324/haematol.2016.151100 (2017).

69. Camerini, E., Amsen, D., Kater, A. P. & Peters, F. S. The complexities of T-cell dysfunction in chronic lymphocytic leukemia. *Semin Hematol* **61**, 163-171, doi:10.1053/j.seminhematol.2024.04.001 (2024).

70. Solman, I. G. *et al.* Impact of long-term ibrutinib treatment on circulating immune cells in previously untreated chronic lymphocytic leukemia. *Leuk Res* **102**, 106520, doi:10.1016/j.leukres.2021.106520 (2021).

71. Einhaus, J. *et al.* Inhibition of effector B cells by ibrutinib in systemic sclerosis. *Arthritis Res Ther* **22**, 66, doi:10.1186/s13075-020-02153-8 (2020).

72. Morrison, V. A. Infectious complications in patients with chronic lymphocytic leukemia: pathogenesis, spectrum of infection, and approaches to prophylaxis. *Clin Lymphoma Myeloma* **9**, 365-370, doi:10.3816/CLM.2009.n.071 (2009).

73. Loke, J. *et al.* Defective T-cell response to COVID-19 vaccination in acute myeloid leukaemia and myelodysplastic syndromes. *Br J Haematol* **202**, 498-503, doi:10.1111/bjh.18894 (2023).

74. Costantini, B. *et al.* The effects of 5-azacytidine on the function and number of regulatory T cells and T-effectors in myelodysplastic syndrome. *Haematologica* **98**, 1196-1205, doi:10.3324/haematol.2012.074823 (2013).

75. Sato, T., Issa, J. J. & Kropf, P. DNA Hypomethylating Drugs in Cancer Therapy. *Cold Spring Harb Perspect Med* **7**, doi:10.1101/cshperspect.a026948 (2017).

76. Sanchez-Abarca, L. I. *et al.* Immunomodulatory effect of 5-azacytidine (5-azaC): potential role in the transplantation setting. *Blood* **115**, 107-121, doi:10.1182/blood-2009-03-210393 (2010).

77. Vitalle, J. *et al.* Immune defects associated with lower SARS-CoV-2 BNT162b2 mRNA vaccine response in aged people. *JCI Insight* **7**, doi:10.1172/jci.insight.161045 (2022).

78. Demaret, J. *et al.* Impaired Functional T-Cell Response to SARS-CoV-2 After Two Doses of BNT162b2 mRNA Vaccine in Older People. *Front Immunol* **12**, 778679, doi:10.3389/fimmu.2021.778679 (2021).

79. Kirkizlar, T. A. *et al.* Incidence and predisposing factors of infection in patients treated with hypomethylating agents. *Leuk Res* **127**, 107043, doi:10.1016/j.leukres.2023.107043 (2023).

80. Shargian-Alon, L., Gurion, R., Raanani, P., Yahav, D. & Gafter-Gvili, A. Hypomethylating Agents-associated Infections-Systematic Review and Meta-analysis of Randomized Controlled Trials. *Clin Lymphoma Myeloma Leuk* **18**, 603-610 e601, doi:10.1016/j.clml.2018.05.017 (2018).

81. Vilorio-Marques, L. *et al*. Relevance of infections on the outcomes of patients with myelodysplastic syndromes, chronic myelomonocytic leukemia, and acute myeloid leukemia treated with hypomethylating agents: a cohort study from the GESMD. *Ther Adv Hematol* **13**, 20406207221127547, doi:10.1177/20406207221127547 (2022).

82. Janssen, M. J. M. *et al*. Predictive factors for vaccine failure to guide vaccination in allogeneic hematopoietic stem cell transplant recipients. *Bone Marrow Transplant* **56**, 2922-2928, doi:10.1038/s41409-021-01437-0 (2021).

83. Fiolet, T., Kherabi, Y., MacDonald, C. J., Ghosn, J. & Peiffer-Smadja, N. Comparing COVID-19 vaccines for their characteristics, efficacy and effectiveness against SARS-CoV-2 and variants of concern: a narrative review. *Clin Microbiol Infect* **28**, 202-221, doi:10.1016/j.cmi.2021.10.005 (2022).

84. Zhang, Z. *et al*. Humoral and cellular immune memory to four COVID-19 vaccines. *Cell* **185**, 2434-2451 e2417, doi:10.1016/j.cell.2022.05.022 (2022).

85. Greenberger, L. M. *et al*. Anti-spike T-cell and Antibody Responses to SARS-CoV-2 mRNA Vaccines in Patients with Hematologic Malignancies. *Blood Cancer Discov* **3**, 481-489, doi:10.1158/2643-3230.BCD-22-0077 (2022).

86. Heinz, F. X. & Stiasny, K. Distinguishing features of current COVID-19 vaccines: knowns and unknowns of antigen presentation and modes of action. *NPJ Vaccines* **6**, 104, doi:10.1038/s41541-021-00369-6 (2021).

87. Arieta, C. M. *et al*. The T-cell-directed vaccine BNT162b4 encoding conserved non-spike antigens protects animals from severe SARS-CoV-2 infection. *Cell* **186**, 2392-2409 e2321, doi:10.1016/j.cell.2023.04.007 (2023).

88. Srivastava, K. *et al*. SARS-CoV-2-infection- and vaccine-induced antibody responses are long lasting with an initial waning phase followed by a stabilization phase. *Immunity* **57**, 587-599 e584, doi:10.1016/j.immuni.2024.01.017 (2024).

89. Rieger, C. T. *et al*. Anti-infective vaccination strategies in patients with hematologic malignancies or solid tumors-Guideline of the Infectious Diseases Working Party (AGIHO) of the German Society for Hematology and Medical Oncology (DGHO). *Ann Oncol* **29**, 1354-1365, doi:10.1093/annonc/mdy117 (2018).

90. Righi, E. *et al*. A Review of Vaccinations in Adult Patients with Secondary Immunodeficiency. *Infect Dis Ther* **10**, 637-661, doi:10.1007/s40121-021-00404-y (2021).

91. Monslow, M. A. *et al*. Immunogenicity generated by mRNA vaccine encoding VZV gE antigen is comparable to adjuvanted subunit vaccine and better than live attenuated vaccine in nonhuman primates. *Vaccine* **38**, 5793-5802, doi:10.1016/j.vaccine.2020.06.062 (2020).

92. Pardi, N. & Krammer, F. mRNA vaccines for infectious diseases - advances, challenges and opportunities. *Nat Rev Drug Discov* **23**, 838-861, doi:10.1038/s41573-024-01042-y (2024).