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**Immune Dysfunction in
Chronic Lymphocytic Leukemia:
Translational and Epidemiological Studies
During the COVID-19 Pandemic**

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Immune Dysfunction in Chronic Lymphocytic Leukemia: Translational and Epidemiological Studies During the COVID-19 Pandemic

Thesis for Doctoral Degree (Ph.D.)

By

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To those that came before me, who made it all possible

Popular science summary of the thesis

Chronic lymphocytic leukemia (CLL) is a chronic type of blood cancer affecting white blood cells called B lymphocytes (or simply B cells). CLL is the most common type of leukemia in the Western world, with increasing prevalence. It's mainly a disease of the elderly with a median age of 72 years at diagnosis in Sweden, but around 25% of patients are <65 years of age when they are diagnosed. Around 6000 people live with CLL in Sweden today. CLL is still incurable but can be treated to suppress the disease and eliminate symptoms. Thus, CLL is not treated, but simply monitored (a so called "watch and wait" approach) until it becomes symptomatic.

B cells are an important part of the immune system, and they are the only cell type capable of producing antibodies. A patient with CLL may have high levels of B cells in the blood, but most of them are CLL cells and they are not functioning immune cells. Furthermore, all kinds of immune cells are dependent on each other to function optimally. Thus, lack of functioning B cells will also affect the presence and function of other immune cells. Another important type of immune cells are the T cells. T cells can directly kill virus infected, or otherwise sick, cells in the body, but are also important for effective antibody production by B cells. The CLL cells are able to "manipulate" other immune cells, to protect themselves from being targeted by the patient's immune system. All this results in an immune deficiency of various extent in patients with CLL. The most characteristic feature of this immune deficiency is lack of antibodies, but dysfunctional T cells is another prominent factor.

When a patient is treated for CLL this immune deficiency is further aggravated in various ways depending on the type of drug used. For the last 20 years or so the typical CLL therapy has been a combination of chemotherapy and immune therapy. However, in the last decade a gradual transition to modern targeted drugs has taken place. These new therapies, often taken orally, targets specific proteins in the CLL cell crucial for its survival and include so called BTK inhibitors and Bcl-2 inhibitors. The effects on the immune system from traditional immunochemotherapy has been quite well described, whereas the effects of modern targeted drugs on immune function are still being discovered.

The immune deficiency associated with CLL makes these patients more susceptible to infections but also limits the effect of vaccines, due to the generally reduced capacity to produce antibodies.

In early 2020 a new type of corona virus, called SARS-CoV-2, started spreading across the world. This virus causes a disease called COVID-19, a viral infection of the airways, with symptoms ranging from a common cold to severe respiratory failure or even death.

Early international studies showed high rates of hospitalization and death from COVID-19 in patients with CLL. We therefore conducted our own study on CLL patients in Stockholm, trying to determine hospitalization, intensive care and death rates due to COVID-19 among these patients (**Study I**). We also wanted to analyze whether they were able to produce antibodies or develop T-cell immunity against the SARS-CoV-2 virus after being infected. We identified 60 CLL patients in Stockholm with COVID-19 during the first year of the pandemic. Fourteen patients (23%) died from the infection, 77% were in need of hospital care for their COVID-19 symptoms, and of the hospitalized patients 24% were also admitted to an intensive care unit (ICU). This validated previous evidence of a high prevalence of severe COVID-19 and death in CLL patients early in the pandemic. However, many (82%) of the 40 patients followed up with blood samples after recovering from COVID-19 did in fact develop antibodies against the virus, and of the patients subjected to T cell analysis 82% (14/17) showed signs of T cell immunity. Many patients, 68% (19/28), also had antibodies against the virus in their saliva.

In the next study, **Study II**, we instead explored the immune responses to COVID-19 vaccine in CLL patients, as the vaccines were getting available in Sweden in early 2021, but we didn't know how effective they would be for CLL patients. We analyzed antibody levels and T cell response in 52 CLL patients and 41 healthy controls after two doses of mRNA vaccine against SARS-CoV-2. Only 60% of the CLL patients had a positive antibody test, compared to 100% of the healthy controls. Also, the antibody levels were generally much lower in CLL patients. CLL patients also had an inferior T cell response to vaccine compared to healthy controls, 29% compared to 59%. Thus, we concluded that CLL patients had an inferior effect of the vaccine compared to healthy individuals. We also found that patients with ongoing CLL treatment with BTK inhibitors (one of the modern, targeted drugs mentioned previously) had the lowest vaccine responses.

Then, in **Study III**, we analyzed immune responses in CLL patients who were first infected with SARS-CoV-2, i.e. had survived COVID-19, and were then vaccinated

with up to three doses of COVID-19 vaccine. We then saw much higher antibody levels, compared to patients in Study II who had only received the vaccine. After 2 doses of the vaccine (after previous COVID-19), 96% of the patients had positive antibody tests. Antibody levels after 2-3 vaccine doses were similar to those seen in healthy controls after 2 vaccine doses (note, with no previous infection) in Study II. T cells immunity and saliva antibodies also increased after repeated vaccination, especially after the 3rd dose. This suggests that repeated exposure to the virus, preferably by vaccination, can induce robust immune responses also in individuals with CLL.

After concluding that CLL patients seem to particularly benefit from repeated vaccination, and might need several doses to respond, we wanted to find out to what extent Swedish CLL patients had taken the COVID-19 vaccine, since there was no available data on this. In **Study IV**, we therefore used data from 6 national registries to analyze the uptake of COVID-19 vaccine in CLL patients in Sweden and explore if any subgroups of patients seemed less inclined to take the vaccine. We found that the vaccine uptake was generally high, especially for the first two doses – 94% of patients had gotten at least two doses of vaccine (by the end of the study on February 28, 2023). However, the proportion decreased for each vaccine dose, with 88% having received three doses, 78% four doses, and only 56% five doses. We also found that the vaccine uptake varied depending on age (the youngest patients had the lowest vaccine uptake), income and region of birth. The uptake was lowest in CLL patients with the lowest income and who were not born in Sweden. Among these patients 79% had received two vaccine doses, and just 24% had received five doses of COVID-19 vaccine. This is important information in continuous and future vaccination campaigns.

In **Study V**, the last study included in this thesis, we again used national registries to look back at three years of COVID-19 pandemic in Sweden and analyze the risk of severe COVID-19 and death in individuals with CLL compared to the general Swedish population during this period. We used ten different registries in this study and found that the overall risk of death during the pandemic was 1.7 times higher for individuals with CLL compared to the general population. However, the risk of death was four times higher in CLL patients younger than 65 years, compared to other Swedes <65 years. Overall, 15% of the CLL patients infected with SARS-CoV-2 died and 44% were in need of hospital care due to COVID-19, compared to 1.2% deaths and 4.8% hospitalization in the general

population. However, the increased risk was somewhat reduced later in the pandemic, and for each vaccine dose received. Even so, CLL patients still had a 50% higher risk of death or hospitalization if infected during the last Omicron variant period compared to the general population.

To summarize, the results presented in this thesis confirm that CLL patients had a high risk of severe COVID-19 and death during the pandemic and inferior effect of the SARS-CoV-2 vaccines. However, it also suggests that repeated vaccination may reduce the gap in vaccine induced immunity between CLL patients and healthy individuals, why this is especially important in this patient group. Therefore, more work needs to be done to ensure that this information reaches all CLL patients.

Abstract

Chronic lymphocytic leukemia (CLL) is a chronic malignancy of mature B lymphocytes with increasing prevalence in Sweden. CLL is associated with substantial immune deficiency, making patients more susceptible to infections and less responsive to conventional vaccines. CLL therapy may further aggravate immunosuppression. The COVID-19 pandemic therefore raised important questions about the impact of SARS-CoV-2 infection and vaccination in individuals with CLL. This thesis addresses some of these questions.

Study I investigated the clinical and immunological outcome of SARS-CoV-2 infection in CLL patients at the beginning of the pandemic. We included all known and PCR-verified COVID-19 cases among CLL patients in the Stockholm region between 1 March 2020 and 31 March 2021. Clinical data were collected from medical records, and patients who recovered were followed with repeated blood and saliva samples for up to 12 months to assess B and T cell responses. Among 60 patients, 77% required hospitalization, 24% intensive care, and approximately 30% died. Among convalescents (n=40), 82% seroconverted, 68% had detectable SARS-CoV-2-specific antibodies in saliva, and T cell immune responses was detected in 14/17 (82%). Serum antibodies remained detectable in approximately 75% at 6 and 12 months. Clinical outcomes improved during the second half of the study period.

Study II evaluated B and T cell responses after two doses of the Pfizer-BioNTech mRNA vaccine BNT162b2 in 52 CLL patients and 41 healthy controls participating in the COVAXID prospective trial. The CLL cohort included patients with untreated indolent CLL (n=14), previous immunochemotherapy including rituximab >12 months earlier (n=14), ongoing BTKi treatment (n=14), or discontinued BTKi treatment due to remission (n=10). Sixty percent of CLL patients seroconverted, with low antibody titers (median 25 U/ml), compared to 100% of controls (median 2600 U/ml). T cell responses occurred in 29% of CLL patients versus 60% of controls. Responses were more frequent after discontinuation of BTKi, whereas patients currently receiving BTKi treatment had the lowest seroconversion and T cell response rates. Fifteen patients (29%) were double-negative, and 11/15 had ongoing BTKi treatment.

Study III investigated hybrid immunity in the cohort from Study I, i.e. patients who had recovered from COVID-19 and subsequently received SARS-CoV-2

vaccination. Blood and saliva samples were collected before vaccination, after the second vaccine dose, and before and after the third dose. Serum antibody titers increased significantly after two doses, from a median of 47 U/ml to 12,500 U/ml. Titers decreased by approximately 50% between the second and third dose (median interval 5 months), to then increase even further after the third dose. SARS-CoV-2-specific T cell reactivity and salivary antibodies also increased after vaccination, but mainly after the third dose.

Study IV examined SARS-CoV-2 vaccine uptake among individuals with CLL in Sweden using six national health and sociodemographic registries. We included 6,304 individuals aged 18–90 years diagnosed with CLL up to November 30, 2022, and followed vaccination uptake from December 27, 2020 (date of first SARS-CoV-2 vaccination in Sweden), to February 28, 2023. Overall uptake was higher than age-standardized nationwide uptake: 94% received two doses, 88% three doses, 78% four doses, and 56% five doses. However, 253 individuals remained unvaccinated, and substantial disparities were observed by age, region of birth, and income. These disparities were greatest for the fourth and fifth doses; among individuals born abroad and in the lowest income quartile, uptake was only 49% and 24%, respectively, for the fourth and fifth doses.

Study V analyzed COVID-19-related mortality and hospitalization over the first three years of the pandemic in Sweden. Cross-linked data from ten national registries were used to compare individuals with CLL with the general population, stratified by age, time period/SARS-CoV-2 variant, and vaccination status. Among 8,275,839 individuals born 1930–2003, including 6,653 with CLL, 2,088,163 first SARS-CoV-2 infections were identified between February 1, 2020, and February 27, 2023, including 1,289 in individuals with CLL. The adjusted risk ratio (aRR) for 90-day all-cause mortality was 1.70 in CLL versus comparators and 4.17 among those younger than 65 years; the overall mortality rate in this age group was 3.6%. Overall mortality was highest during the Wild-type period but remained 12% during the Omicron era, with an aRR of 1.49. CLL patients receiving Bcl-2i, BTKi, PI3Ki, or corticosteroids at time of infection showed increased risk of hospitalization and death compared to CLL patients not treated with these drugs. However, aRRs for poor outcomes decreased with each vaccine dose vs comparators with the corresponding vaccination status, suggesting protection against severe COVID-19 also in individuals with CLL.

List of scientific papers

- I. **Blixt L**, Bogdanovic G, Buggert M, Gao Y, Hober S, Healy K, Johansson H, Kjellander C, Mravinacova S, Muschiol S, Nilsson P, Palma M, Pin E, Smith CIE, Stromberg O, Sällberg Chen M, Zain R, Hansson L*, Österborg A*. Covid-19 in patients with chronic lymphocytic leukemia: clinical outcome and B- and T-cell immunity during 13 months in consecutive patients. (Leukemia. 2022 Feb;36(2):476–481. doi: 10.1038/s41375-021-01424-w)
- II. **Blixt L***, Wullimann D*, Aleman S, Lundin J, Chen P, Gao Y, Cuapio A, Akber M, Lange J, Rivera-Ballesteros O, Buggert M, Ljunggren HG, Hansson L, Österborg A. T-cell immune responses following vaccination with mRNA BNT162b2 against SARS-CoV-2 in patients with chronic lymphocytic leukemia: results from a prospective open-label clinical trial. (Haematologica. 2022 Apr 1;107(4):1000–1003. doi: 10.3324/haematol.2021.280300. PMID: 35045694)
- III. **Blixt L**, Gao Y, Wullimann D, Murén Ingelman-Sundberg H, Muschiol S, Healy K, Bogdanovic G, Pin E, Nilsson P, Kjellander C, Grifoni A, Sette A, Sällberg Chen M, Ljunggren HG, Buggert M, Hansson L, Österborg A. Hybrid immunity in immunocompromised patients with CLL after SARS-CoV-2 infection followed by booster mRNA vaccination. (Blood. 2022 Dec 1;140(22):2403–2407. doi: 10.1182/blood.2022016815)
- IV. Hedberg P, **Blixt L**, Granath F, Bergman P, Carlander C, Aleman S, Hansson L; CLHIP study group. Uptake of the first to fifth doses of coronavirus disease 2019 vaccine in individuals with chronic lymphocytic leukaemia: A nationwide cohort study in Sweden. (EJHaem. 2025 Jan 6;6(1):e1077. doi: 10.1002/jha2.1077)
- V. Hedberg P, **Blixt L**, Eketorp Sylvan S, Killander Möller I, Lindahl H, Kahn FM, Nilsdotter-Augustinsson Å, Fredriksson M, Nyström S, Bergman P, arlander C, Aleman S, Naucclér P, Osterborg A, Hansson L. Outcomes of the COVID-19 pandemic in chronic lymphocytic leukemia: Focus on the very early period and Omicron era. (Blood Adv. 2025 Feb 21;bloodadvances.2024015260. doi: 10.1182/bloodadvances.2024015260)

* These authors contributed equally to the manuscript

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- II. Ingelman-Sundberg HM, **Blixt L**, Wullimann D, Wu J, Gao Y, Healy K, Muschiol S, Bogdanovic G, Åberg M, Kjellander C, Grifoni A, Sette A, Aleman S, Chen P, Blennow O, Hansson L, Ljunggren HG, Chen MS, Buggert M, Österborg A. Systemic and mucosal adaptive immunity to SARS-CoV-2 during the Omicron wave in patients with chronic lymphocytic leukemia. (Haematologica. 2024 Feb 1;109(2):646–651. doi: 10.3324/haematol.2023.282894)
- III. Killander Möller I, Hedberg P, Wagner P, Lindahl H, Nyström S, **Blixt L**, Eketorp Sylvan S, Nilsdotter-Augustinsson Å, Österborg A, Fredrikson M, Hansson L, Kahn F, Sparén P, Gisslén M, Naucélér P, Bergman P, Aleman S, Carlander C. Risk of COVID-19 hospitalisation by HIV-status and SARS-CoV-2 vaccination status during pre- and post-Omicron era in a national register-based cohort study in Sweden. Infect Dis (Lond). 2025 Feb;57(2):178–191. doi: 10.1080/23744235.2024.2405582.
- IV. Lindahl H, Kahn F, Nilsdotter-Augustinsson Å, Fredrikson M, Hedberg P, Killander Möller I, Hansson L, **Blixt L**, Eketorp Sylvan S, Österborg A, Aleman S, Carlander C, Nyström S, Bergman P. Inborn errors of immunity are associated with increased COVID-19-related hospitalization and intensive care compared to the general population. J Allergy Clin Immunol. 2025 Feb;155(2):387–397.e6. doi: 10.1016/j.jaci.2024.10.013.

* These authors contributed equally to the manuscript

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List of abbreviations

ACE2	angiotensin-converting enzyme 2
AEC	alveolar epithelial cell
AIHA	autoimmun hemolytic anemia
AIM	activation-induced marker
APC	antigen-presenting cell
ARDS	acute respiratory distress syndrome
aRR	adjusted risk ratio
Bcl-2(i)	B cell lymphoma 2 (inhibitor)
BCR	B cell receptor
BTK(i)	Bruton's tyrosine kinase (inhibitor)
CFR	case fatality rate
CI	confidence interval
CIT	chemoimmunotherapy
CLL	chronic lymphocytic leukemia
CIRS	Cumulative Illness Rating Scale
COVID-19	coronavirus disease 2019
CR	complete remission
CTL	cytotoxic T lymphocyte
CTLA-4	cytotoxic T lymphocyte-associated protein 4
DC	dendritic cell
del(11q)	deletion of chromosome 11q
del(13q)	deletion of chromosome 13q
del(17p)	deletion of chromosome 17p
ECLIA	electro-chemiluminescence immunosorbent assay
ELISA	enzyme-linked immunoassay
ELISpot	enzyme-linked immunospot assay
EMA	European Medicines Agency

FACS	fluorescence-activated cell sorting
FCR	fludarabine, cyclophosphamide, rituximab
FDR	Food and Drug Administration
FDC	follicular dendritic cell
GC	germinal centre
HFNO	high-flow nasal oxygen
ICU	intensive care unit
IFN	interferone
Ig	immunoglobulin
IGHV	immunoglobulin heavy chain variable region
IL-10	interleukin 10
INCA	information network for cancer care
IQ	interquartile
IQI	interquartile interval
ITK	IL-2-inducible T cell kinase
ITP	immune thrombocytopenia
iwCLL	International Workshop on Chronic Lymphocytic Leukemia
LAG-3	lymphocyte-activating gene 3
LISA	longitudinal integrated database for health insurance and labour market studies
LMWH	low-molecular-weight heparin
mAb	monoclonal antibody
MERS-CoV	Middle East respiratory coronavirus
MFI	median fluorescence intensity
MGR	multi-generation register
MHC	major histocompatibility complex
MSC	mesenchymal stromal cell
NFkB	nuclear factor kappa B

NIV	non-invasive ventilation
NK	natural killer
NLC	nurse-like cell
NPR	national patient register
NVR	national vaccination register
OR	Odds ratio
OS	overall survival
PBMC	peripheral blood mononuclear cells
PC	plasma cell
PCR	polymerase chain reaction
PD-1	programmed cell death protein 1
PHAS	Public Health Agency of Sweden
PI3K(i)	phosphoinositide 3 Kinase (inhibitor)
PD	progressive disease
PR	partial remission
PRCA	pure red cell aplasia
RBD	receptor-binding domain
RCC	regional cancer centers
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SCDR	Swedish cause of death register
SD	stable disease
SD	standard deviation
SFU	spot-forming units
SHM	somatic hypermutation
SIR	Swedish intensive care registry
SLL	small lymphocytic lymphoma
SLO	secondary lymphoid organs
SPDR	Swedish prescribed drug register

TCR	T cell receptor
Th	T helper
TNF	tumor necrosis factor
TP53	tumor protein 53
TPR	total population register
Tregs	regulatory T cells
WHO	World Health Organization

Introduction

Chronic lymphocytic leukemia (CLL) is a chronic malignancy of mature B lymphocytes with an increasing prevalence in the Swedish population. Clinically, CLL is a highly heterogenic disease, ranging from an essentially indolent clinical course with a normal life expectancy without treatment indication, to a severe, refractory disease of utterly poor prognosis. However, CLL of all stages is associated with substantial immune deficiency, usually increasing over time. Due to the complex interactions and crosstalk between various immune cells, this dysfunction is not limited to B cells but affects most parts of the immune system. This disease related immune deficiency may be further aggravated by CLL therapy. Apart from being more susceptible to infections, and reduced capacity to clear infections, it has also been established that CLL patients have limited effect of conventional vaccines.

As treatment of CLL has evolved drastically in the last few decades, resulting in improved prognosis, infections have become a more common cause of death in this population (1,2).

Thus, when the COVID-19 pandemic, caused by the SARS-CoV-2 virus, erupted in early 2020, around the same time as CLL treatment was undergoing a paradigm shift from immunochemotherapy (ICT) to modern targeted therapies, comprehension of the effects of this new potentially deadly virus for individuals with CLL was lacking. This thesis addresses, and tries to abate, some of these knowledge gaps.

1 Background

1.1 Epidemiology and Etiology of CLL

CLL is the most common type of leukemia in adults in the Western world. In Sweden the incidence is around 600 cases/year and the current prevalence approximately 6000 cases in the Swedish population (3,4). CLL is mainly a disease of the elderly, with a median age of 72 years at diagnosis. However, 25% of individuals are <65 years of age at diagnosis, and 10% <55 years (3). The disease is more prevalent in men than in women with a ratio of 1.7:1.0. While the incidence of CLL has been rather stable, the prevalence in Sweden has increased in the last decades, from 33,3/100.000 inhabitants in the year 2000 to 52/100.000 in 2015, coinciding with a continuous improvement in 5-year overall survival (OS) during the same time period (5). Even though only 15% of patients need treatment at diagnosis, 2/3 of patients will start treatment throughout the course of the disease.

The etiology of CLL is unknown. Most data on risk factors suggested in previous studies have been inconclusive, contradictory or hard to reproduce. However, around 5% of CLL patients have at least one relative with CLL or other lymphomas. Though the risk for first-grade relatives of CLL patients to develop CLL or other (indolent) B-cell lymphomas has been shown to be increased, the absolute risk is still low due to the rarity of the disease (6). The course of the disease, clinical features and treatment do not differ between familial or sporadic cases.

1.2 CLL pathogenesis

1.2.1 The CLL cell

Phenotypical characteristics

CLL is characterized by a progressive accumulation of clonal mature B cells in blood, bone marrow and lymphoid tissues. CLL cells present with a typical phenotype expressing the transmembrane glycoprotein CD5 (otherwise mainly associated with T cells) and the B cell antigens CD19 and CD23, combined with abnormally low levels of surface membrane immunoglobulins.

B cell maturation

B cells develop from hematopoietic stem cells via various progenitors in the bone marrow and are released to peripheral blood as mature naïve B cells, with a complete but immature and unspecific B cell receptor (BCR) of the immunoglobulin M (IgM) isotype. (The BCR will be discussed in more detail in

section 2.1.5). Upon antigen encounter, the naïve B cell then enters secondary lymphoid organs (SLO), namely lymph nodes and spleen, where it proliferates and undergoes somatic hypermutation (SHM) of the variable region of the Ig receptor in the germinal center (GC) of the lymph node. This process is dependent on signals from the GC microenvironment, including follicular dendritic cells (FDCs) and follicular helper T cells (Tfh). Adverse receptor properties are then negatively selected for apoptosis, such as affinity for self-antigen. Those few B cells where SHM results in increased affinity for the antigen are positively selected for further proliferation and differentiation including class switch, i.e. change of Ig isotype (for example IgM to IgG), and eventually become either an antibody-secreting plasma cell or memory B cell. For each step of the process the positively selected clone expands. The process of B cell maturation is illustrated in *Figure 1* (7).

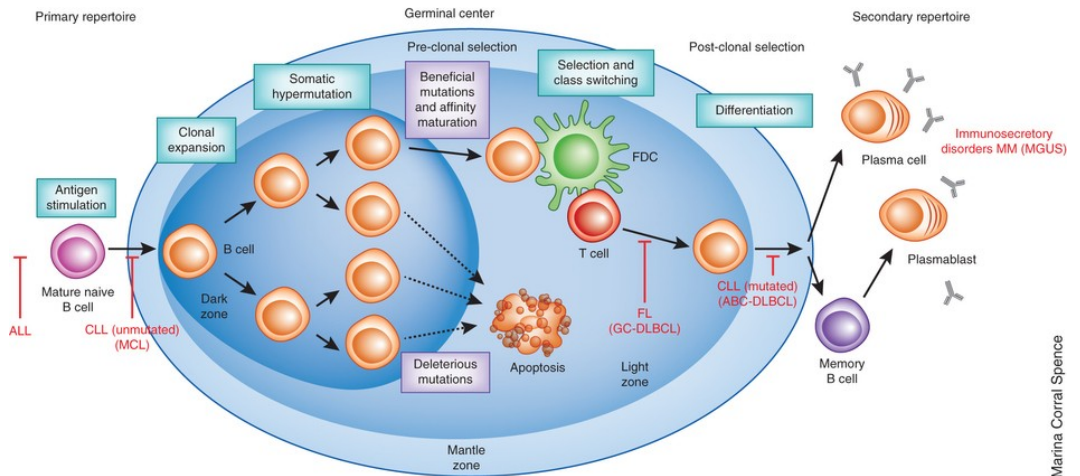


Figure 1. B cell maturation. The promise and challenge of high-throughput sequencing of the antibody repertoire (Nature Biotechnology. 2014;32(2):158–68. doi: 10.1038/nbt.2782). (Reproduced with permission from Georgiu et al)

IGHV mutational status in CLL

In CLL, around 60% of cases present with a higher degree of somatic hypermutation in the variable region of the immunoglobulin heavy chain (IGHV) genes, defined as mutated (M) CLL (3), suggesting its origin from a rather highly differentiated, post-GC, B cell. The remaining 40% display an unmutated IGHV phenotype, i.e. $\geq 98\%$ identical with the germline IGHV sequence, defined as unmutated (U) CLL of suggested pre-GC origin. Thus, CLL can be divided into two distinct entities based on the IGHV mutational status, where U-CLL is

associated with a more aggressive disease, inferior response to immunochemotherapy, and, at least before the occurrence of small molecule-based therapeutics, shorter survival (8,9). However, both mutated and unmutated CLL cells show an activated surface phenotype, including typical BCR properties, similar to antigen experienced B cells (10). Gene expression profiling also reveals only very limited differences between the two CLL subsets, and striking similarities with memory B cells (11). On the other hand, U-CLL cells often express polyreactive antibodies, which is only rarely seen in M-CLL (12).

Genetic aberrations

Approximately 80% of CLL patients present with chromosomal aberrations, typically del(13q) (50–60% of cases), trisomy 12, del(11q) and del(17p), correlating with treatment response and prognosis.

Somatic mutations of the tumor suppressing gene *TP53* may occur both in the presence and absence of del(17p) and is the main factor associated with poor prognosis. Modern methods, such as Next Generation Sequencing (NGS) techniques, have been able to identify other recurrent potential driver mutations in CLL, such as mutations in the *NOTCH1*, *SF3B1*, *ATM*, *MYD88*, and *BIRC3* genes (13,14), though yet with unclear impact on clinical decision-making. However, this has emphasized the outstanding inter- and intra-sample genetic heterogeneity of CLL (15).

The B cell receptor

B cell receptor (BCR) signaling is crucial for the development, proliferation and survival of B cells. The BCR is composed of a surface immunoglobulin (Ig) non-covalently associated with the heterodimer Ig- α /Ig- β (also known as CD79a/CD79b). Triggering of BCR signaling via external antigens from the microenvironment induces a cascade of downstream events including activation of the Bruton's tyrosine kinase (BTK), and phosphoinositide 3-kinase (PI3K), that ultimately leads to the upregulation of transcription factors such as nuclear factor kappa B (NF κ B) promoting B cell survival (16). This is illustrated in *Figure 2* (17).

CLL cells, retaining many morphological features of normal mature B lymphocytes, express functional BCRs, mainly of the IgM and IgD isotypes, and exploit the BCR for proliferation and survival (7). Many studies have implicated the crucial role of the BCR in CLL pathogenesis. Firstly, while normal B cells are dependent on external antigen activation of the BCR, CLL cells exhibit antigen-independent cell-autonomous signaling capacity (18). Secondly, the mutational status of IGHV genes highly correlates with clinical and molecular features, where

U-CLL is associated with high BCR responsiveness, poly-reactivity and autoantigen-reactivity, while M-CLL cells display an anergic BCR, i.e. low responsiveness, with higher specificity (7). Thirdly, genetic analysis of the BCR of CLL cells has found that 30% of CLL cases share identical sequences of the IGHV gene, so-called stereotyped BCRs, that might also be linked to the reactivity profile of the CLL clone as well as disease outcome (19–21). The evidence of stereotypy suggest two distinct categories of CLL – stereotyped vs heterogenous Ig – and further division into stereotyped subsets (21). Possible antigen(s) believed to trigger and drive the CLL clone is yet to be identified.

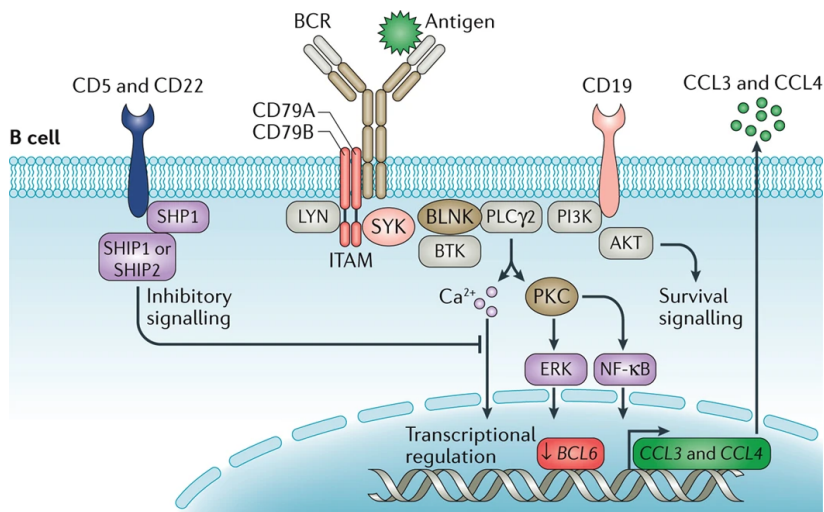


Figure 2. The B cell receptor signaling pathway. Targeting B cell receptor signaling in cancer: preclinical and clinical advances. (Nature Reviews Cancer. 2018;18:148–167. doi: 10.1038/nrc.2017.121). (Reproduced with permission from Burger JA, Wiestner A)

1.2.2 Tumor Microenvironment

The CLL cell is highly dependent on signals from the microenvironment in SLO, such as lymph nodes and spleen, as well as bone marrow, for survival and proliferation. In fact, CLL B cells cultured alone *in vitro* (unlike normal B cells) die by apoptosis, while CLL cells co-cultured with normal stromal cells do not (22–25). This was later also re-produced *in vivo* (26).

The interactions between CLL cells and surrounding cells are bidirectional, inducing changes in both cell types, and can be mediated through direct cell-to-cell interaction, via adhesion molecules, or via secretion of soluble factors including cytokines, chemokines and growth factors. Thus, the bone marrow and

SLO niches constitute a sanctuary for CLL cells providing various survival and proliferation stimuli.

CLL cells proliferate in scattered focal aggregates forming vaguely nodular areas in lymph nodes and bone marrow, called proliferation centers or pseudo-follicles (27). These proliferation centers are infiltrated with several non-leukemic cells, devoted to the proliferation and survival of CLL cells. Mesenchymal stromal cells (MSCs), known to support the growth and maturation of normal hematopoietic progenitor cells, was among the first actors in CLL microenvironment to be studied. They reside in the bone marrow where they exert secretory, immunomodulatory, differentiation and homing properties. MSCs promote homing of CLL cells to the bone marrow, where they provide protection from spontaneous or chemotherapy-induced apoptosis (28–31). Follicular dendritic cells (FDCs) play the equivalent role in the SLO niche, where they are involved in the homing, survival and proliferation of CLL cells within the lymph node germinal centers (32).

Another key player in the CLL microenvironment are Nurse-like cells (NLCs) – monocytes that in the presence of CLL cells differentiate into pro-tumoral M2-like macrophages (also called CLL-Associated Macrophages), much like Tumor Associated Macrophages (TAMs) observed in solid tumors. They secrete various cytokines and chemokines, such as B cell activating factor (BAFF), interleukin 10 (IL-10) and CXCL12, that promote proliferation, survival and tissue homing of CLL cells, as well as activation of the BCR (33–36). Our group has recently shown that NLCs may be a druggable target in CLL (37).

CLL cells also, via BCR signaling, secrete interleukins to attract additional CLL-supporting immune cells, mainly T cells and monocytes, into the tissue microenvironment.

1.3 Clinical features of CLL

Although CLL is incurable, many patients will have an indolent clinical course, and some will never need CLL-directed therapy. Others will have rapidly progressive disease, some treatment-refractory, and some may even transform into an aggressive form of non-Hodgkin lymphoma termed Richter's syndrome of utterly poor prognosis. Furthermore, CLL is associated with an inherent immune dysfunction, such as frequent and severe infections or autoimmune disorders.

1.3.1 Associated symptoms

Most patients are in an indolent, asymptomatic stage at CLL diagnosis. Often leukocytosis is found *en passant*. However, as the disease progresses several

symptoms may appear. Enlarged lymph nodes or splenomegaly, due to accumulation of CLL cells may cause discomfort or dysfunction of adjacent organs. Progressive infiltration of the bone marrow or spleen will eventually cause cytopenias. Systemic symptoms include fatigue and the triad of so-called B symptoms: fevers, night sweats and unintentional weight loss.

1.3.2 Diagnostics

The CLL diagnosis requires the presence of $\geq 5 \times 10^9/L$ monoclonal B cells in the peripheral blood (38). Flow cytometry should show the typical CLL cell phenotype (as described in section 2.1). In case CLL cells are found in lymph nodes and/or bone marrow, but monoclonal B cells in peripheral blood fall below $5 \times 10^9/L$, the disease is called small lymphocytic lymphoma (SLL), which is managed in the same way as CLL. Bone marrow aspiration is usually only needed in the presence of unexplained cytopenia. Gene mutational analyses are usually performed only when the patient becomes eligible for treatment (3). Clinical staging is defined by either the Rai staging system (Rai stage 0–IV) or the Binet staging system (Binet stage A–C), based on level of lymphocytosis, presence of any cytopenia, and extent of lymphadenopathy.

1.4 CLL therapy

1.4.1 Treatment indication

According to the 2018 international workshop on Chronic Lymphocytic Leukemia (iwCLL) guidelines, treatment is indicated if one of the following criteria is fulfilled: bone marrow failure manifested by progressive anemia and/or thrombocytopenia; massive and/or progressive and/or symptomatic lymphadenopathy or splenomegaly; autoimmune anemia and/or thrombocytopenia with poor response to standard therapy; constitutional symptoms including unintentional weight loss, prolonged fever without evidence of infection, and/or significant fatigue; progressive/accelerated lymphocytosis over a set time period ($\geq 50\%$ in 2 months or lymphocyte doubling time < 6 months) (39).

1.4.2 Treatment options

As a result of growing knowledge in CLL pathophysiology, the treatment landscape of CLL has gone through major changes in the last decade, shifting from traditional chemoimmunotherapy (CIT) to small-molecular targeted therapies, mainly BTK inhibitors (BTKi) and Bcl-2 inhibitors (Bcl-2i). When choosing treatment strategy, the physician must take into consideration the

patient's genetic profile, any previous treatment regimes, as well as age and co-morbidities.

The following regimens are a selection of the ones most used in Sweden in recent years.

Chemoimmunotherapy

For a long time, the only option to treat CLL was by chemotherapy, where purine analogues such as fludarabine and alkylating agents such as bendamustine have been mostly used in modern times, usually combined with anti-CD20 monoclonal antibodies (CD20 mAbs) such as rituximab (described in the next section). Alkylating agents were introduced as treatment for CLL already in the 1950s, and fludarabine in the 1990s. Fludarabine combined with cyclophosphamide and rituximab (FCR) was the gold standard regimen for first-line treatment of fit patients from ca 2010. Common side effects are myelosuppression, infections and gastrointestinal symptoms, and an increased long-term risk of secondary malignancies. Neutropenia is common in both regimens, though bendamustine is also known to cause lymphopenia, primarily affecting CD4+ T cells.

Anti-CD20 monoclonal antibodies

The monoclonal anti-CD20 antibody rituximab was introduced around 2010 as an add-on therapeutic to FC or bendamustine, even though it never showed a significant anti-tumor effect as a single agent in CLL. Single-agent rituximab may however be used for treating CLL-related autoimmune phenomena, such as autoimmune hemolytic anemia. Rituximab, its fully human companion mAb ofatumumab, and the type II mAb obinutuzumab, all target the surface protein CD20 expressed on cells of the B-cell lineage, including CLL cells. All three deplete B cells by binding to the B cell (via CD20) and thus activating pathways downstream of the BCR, promoting cell-cycle arrest and apoptosis. Obinutuzumab induce more direct B cell death while rituximab and ofatumumab mainly exert their B cell killing via complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADPC) (40–42). However, their B cell depleting nature cause increased risk of infections and severely impairs antibody response to novel pathogens or vaccination, for up to 12 months after treatment (43).

BTK inhibitors

Bruton's tyrosine kinase (BTK) is a cytoplasmic enzyme expressed in pre-B cells, B cells and many other hematopoietic cells, excluding T cells and plasma cells. It is activated as response to engagement of the BCR and is crucial for the

differentiation and activation of healthy B cells and correspondingly essential for the proliferation, migration and survival of CLL cells (44,45). BTK is upregulated in CLL cells compared to non-malignant B cells (44). BTK inhibitors bind to the BTK enzyme, blocking its activity, thus inhibiting BCR-dependent proliferation and survival of the CLL cell. Inactivation of BTK also prevents tissue homing of the CLL cells, which are redistributed from their protective and supportive environment in the SLO out into peripheral blood (46). Today there are three covalent BTKis approved by the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA): ibrutinib, acalabrutinib and zanubrutinib. Ibrutinib was the first-in-class, reaching FDA approval in 2014 and EMA approval in 2015. BTK inhibitors are all taken orally once or twice daily continually until disease progression or unacceptable toxicity occur. The difference between the three BTKis is the selectivity for BTK and the binding to other similar kinases, i.e. their off-target effects, as well as different clinical effectiveness (47). Well known side effects from BTKi treatment include excess bleeding and bruising, cardiovascular effects (prominently hypertension and atrial fibrillation, more often reported with ibrutinib), arthralgia, gastrointestinal symptoms, and cytopenias (48,49). It has also been shown that BTKis, to a various extent, inhibit the BTK-similar receptor interleukin-2 inducible T cell kinase (ITK) of T cells, important for the development and function of T cells through its downstream function of the T cell receptor (TCR) (50,51). In addition, the CLL cell may develop BTKi-resistance, through an acquired mutation of the BTK protein (most often C481S) or its downstream proteins (52,53). Next generation non-covalently binding BTKi (pirtobrutinib) was approved for treating CLL by EMA in 2025, and another is in ongoing phase 3 trials (nemtabrutinib). Additionally, a new way of targeting BTK, and evading BTKi-resistance, is represented by so-called BTK degraders. They bind to BTK, but instead of blocking its activity, it targets BTK for elimination through ubiquitination. Such BTK degrading agents are currently in phase 3 clinical trials.

Bcl-2 inhibitors

B cell lymphoma 2 (Bcl-2) is an anti-apoptotic protein of the intrinsic, or mitochondrial, pathway of apoptosis. Bcl-2 is universally overexpressed in CLL cells, thus preventing apoptosis. Venetoclax, at present the only clinically available Bcl-2 inhibitor (Bcl-2i), selectively antagonizes Bcl-2 and thereby induces apoptosis of CLL cells (54). Venetoclax was first approved for treatment of CLL in 2016, with extended indication in 2019. Venetoclax is taken orally once daily for 12 months (as first-line therapy) or continuously (as subsequent lines of treatment), with a five-week initial dose ramp-up to minimize the risk of tumor lysis syndrome (TLS). TLS is a potentially lethal

condition that may occur when large amounts of cancer cells are disintegrated simultaneously, causing kidney failure and electrolyte disruption. Bcl-2 therapy is usually combined with an anti-CD20 mAb (obinutuzumab or rituximab). A next-generation and in vitro more potent Bcl-2i, sonrotoclax, is currently in phase 3 clinical trials.

1.5 Immune dysregulation in CLL

Despite being a both clinically and molecularly heterogenous disease, significant perturbations of the immune system are one of the most characteristic features of CLL. Most patients show qualitative and quantitative immune defects within both innate and adaptive immune responses from the time of diagnosis, which tend to worsen over time, even in the absence of overt disease progression (55).

Apart from an increased risk of severe and recurrent infections, there is also an increased incidence of secondary malignancies in the CLL population (56), as well as blood cell-targeting autoimmune conditions (57).

Growing evidence unravels the mechanisms by which CLL cells disturb various immune cells in numerous ways, as summarized in *Figure 3*. And while traditional chemotherapy, even when successful in achieving CLL remission, has not been shown to restore antibody production, more modern immunomodulatory and targeted therapies might be able to at least partially restore immune functions in individuals with CLL (58–60).

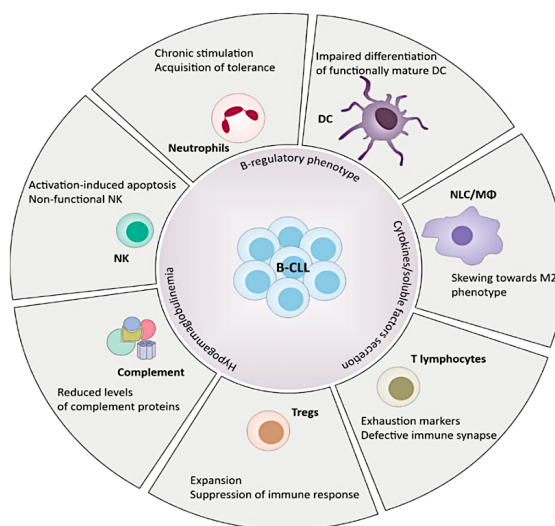


Figure 3. Immune dysregulation in CLL. Immune response dysfunction in chronic lymphocytic leukemia: dissecting molecular mechanisms and microenvironmental conditions (International Journal of Molecular Sciences. 2020, 21(5), 1825. doi: 10.3390/ijms21051825). (Reproduced with permission from Arruga et al)

1.5.1 Adaptive immunity

CLL cells influence healthy immune cells by direct cell-to-cell interactions via adhesion molecules or via secretion of inhibitory cytokines, inducing phenotypical and functional alterations in these cells. Circulating CLL cells phenotypically much resemble regulatory B cells, suppressing immune responses mainly through secretion of IL-10 (61). IL-10 is increased in CLL lymph nodes *in vivo*, and influences various immune cell responses (62).

Hypogammaglobulinemia

The serum levels of antibodies (immunoglobulins) with various specificity in the resting, non-infectious state, are produced by long-lived plasma cells, which reside mainly in the bone marrow. Overall, reduced levels of immunoglobulins in serum, i.e. hypogammaglobulinemia, is perhaps the most characteristic form of immune suppression in CLL and tends to aggravate over time. This hypogammaglobulinemia is thought to be a consequence of decreased survival and function of plasma cells. Interactions between plasma cells and CLL cells, mediated via CD95L/CD95 (a Fas-type death receptor and its ligand), lead to inhibition of immunoglobulin secretion and plasma cell apoptosis (63–65).

Hypogammaglobulinemia is observed in around 25% of CLL patients at diagnosis, and tends to subsequently develop in another 25% of patients during the course of the disease (66). There is an established correlation between low levels of IgG and the frequency and severity of bacterial infections, most commonly *Streptococcus pneumoniae* and *Haemophilus influenzae* (67). However, not all CLL patients with hypogammaglobulinemia suffer from recurrent infections, and vice versa. Thus, immunoglobulin levels alone do not seem to reflect the qualitative function of immunoglobulins and are not considered a reliable indicator of immune function.

T cells

Different subsets of T cells play distinct roles in the adaptive immune responses. All T cells display the T cell receptor (TCR) and surface protein CD3. Naïve T cells initially develop in the bone marrow, mature in the thymus, and then circulate in blood and SLO, waiting to be activated. Activation occurs when T cells encounter an antigen, presented by a major histocompatibility complex (MHC) molecule, that is recognized by their specific TCR. T cell functions also depend on stimulatory and inhibitory co-signaling receptors, also known as immune checkpoints.

Activated CD8+ cytotoxic T cells (CTLs) carry cytolytic granules and have the ability to directly kill infected cells by degranulation, after activation via MHC

class I-bound antigen on the infected cell. CD4⁺ T helper (Th) cells are activated by dedicated antigen presenting cells (APCs) via MHC class II-bound antigens and differentiate into various subsets of Th cells depending on the type of surrounding cytokines, released mainly from innate immune cells. Their general role is to enable functions of other immune cells – such as increasing phagocytic function of macrophages and neutrophils, enhancing CTL cytotoxicity, and activating antibody production by B cells.

Regulatory T cells (Tregs) is a subset of T cells that moderate the cellular immune response and are essential for maintaining tolerance and limit T cell responses to self-antigens. Apart from producing high levels of immunosuppressive cytokines such as IL-10, which downregulate inflammation and immune cell proliferation, they express inhibitory receptors such as CD25 (competitive receptor for pro-inflammatory cytokine IL-2), cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1) that compete with or block co-stimulatory receptors on effector T cells (68).

Some T cells will persist after an initial infection as long-lived memory T cells – a heterogeneous population of antigen-specific T cells including several subtypes. They are able to mount a rapid recall response upon re-exposure to the antigen that triggered their initial activation (69).

CLL is associated with major deregulations in the T cell compartment, present even in early-stage CLL. T cells are often numerically increased, but they are functionally impaired with an unbalanced CD8⁺/CD4⁺ ratio and increased numbers of Tregs (70–72). In addition, circulating T cells in CLL show an aberrant phenotype associated with increased expression of exhaustion markers such as CTLA-4, lymphocyte-activation gene 3 (LAG-3) and PD-1. However, exhausted T cells in CLL, unlike in virus-induced exhaustion, maintain the ability to secrete cytokines such as TNF- α , promoting the survival and proliferation of CLL cells (73).

The upregulation of co-inhibitory receptors in CLL patients' T cells, such as CTLA-4 and PD-1, leads to downmodulation of TCR-mediated signaling, thus limiting T-cell activation, especially of Th cells (74–76). In addition, CLL cells express higher levels of PD-1 ligand (PD-L1) than circulating lymphocytes from healthy donors, further feeding the immunosuppressing PD-1/PD-L1 axis (77). Another suggested mechanism behind the diminished number of active Th cells is the expression of CD95 ligand (CD95L) on CLL cells combined with upregulated expression of the CD95 Fas-type death receptor on patients' CD4⁺ T cells, inducing apoptosis (78).

All these deregulations contribute to the inability of T cells to effectively initiate, maintain and complete immune responses in individuals with CLL.

1.5.2 Innate immunity

CLL also alters several elements and effector cells of the innate immune system. As previously described, the presence of CLL cells *in vitro* influence monocytes to differentiate into M2-like macrophages, called Nurse-like cells, with pro-tumoral and anti-apoptotic properties (35–37). In fact, a general increase in circulating monocytes has been observed in CLL, however with an immunosuppressive phenotype (34).

Dendritic cells (DCs) are also dysfunctional in CLL and show alterations in cytokine secretion, impaired differentiation and maturation, and impaired ability to induce type 1 T cell responses (79,80).

Similar to cytotoxic T cells, natural killer (NK) cells show lack of granules (81,82) and neutrophils show impaired phagocytic and bactericidal function as well as defective migration and chemotaxis (55,83,84). In addition, as disease progresses, they may be reduced in number due to more extensive bone marrow engagement and/or previous cytotoxic treatment.

Furthermore, levels of different components of the complementary system, including C3b, have been shown to be reduced and with aberrant activation and binding, especially in advanced stages of CLL (85).

1.5.3 Treatment related immune deficiency

Classic chemotherapy with purine analogues and alkylating agents mainly contributes to increased risk of infections by general myelosuppression leading to cytopenia. This increases the risk of opportunistic infections such as herpes simplex virus, varicella-zoster virus, *Mycobacterium* spp, etc. (86). In addition, bendamustine has, as previously mentioned, a particularly T cell depleting effect. Anti-CD20 mAbs are by default a B cell depleting treatment, leading to profoundly diminished, or even obliterated, ability to mount antibody responses to (primary) infections or vaccines for up to a year after completed treatment (43,87). The immunologic (side)effects of targeted therapies are less known. However, off-target effects from BTKis, including T cell inhibiting properties, have gradually been uncovered (1,88).

1.5.4 CLL associated autoimmunity

Autoimmune complications are common in CLL, occurring in approximately 10% of the patients. Autoimmune hemolytic anemia (AIHA) is by far the most

common manifestation, followed by immune thrombocytopenia (ITP) (89). Less common manifestations are pure red cell aplasia (PRCA) and autoimmune granulocytopenia (AIG). Non-hematologic autoimmunity is rare. The pathogenesis of autoimmunity in CLL is largely unknown but is thought to be the result of multiple mechanisms associated with the immune dysregulation characteristic for CLL, such as the production of autoantibodies, aberrant antigen presentation by CLL cells resulting in autoreactive CD4+ Th cells, dysfunction of regulatory T cells (decreased tolerability), as well as alterations of the tumor microenvironment (89–92).

1.5.5 Vaccine response

The effect of vaccination depends on the ability to mobilize immunologic memory. B cell memory after the primary infection (or vaccination) is initiated by binding of antigen to the BCR, leading to B cell maturation and differentiation, where some B cells differentiate into memory B cells and long-lived plasma cells (PCs), as previously described in section 1.2.1. Long-lived PCs are subdued in the bone marrow, where they continually secrete small amounts of their specific high-affinity antibody. Memory B cells generally reside in the SLO and are able to quickly start proliferation and differentiation into short-lived antibody-producing PCs upon encountering the same pathogen anew (68,93). Memory T cells are another important part of immunologic memory. They are a small subset of antigen-specific T cells persisting long-term after an infection has cleared, enabling a faster and stronger response upon re-exposure to that antigen. They reside in the SLO but also patrol the blood and have the capacity to rapidly multiply and execute immediate neutralizing actions and/or help replenish other immune resources.

CLL patients, besides suffering from recurrent and severe infections, also have an impaired response to conventional vaccines, such as pneumococcal- and influenza vaccine (94–96). This is true even for treatment-naïve patients (97), and more pronounced than in other hematologic malignancies, such as multiple myeloma or other lymphomas (98). For patients having received CLL-directed therapy including anti-CD20 mAbs, the antibody response to vaccination will be further impaired for at least 6–9 months after treatment (99,100). In addition, the effect of targeted therapy on vaccine response has not yet been fully studied, even if there are indications that even such modern therapies may impair antibody responses to some extent (101).

1.6 SARS-CoV-2 and COVID-19

1.6.1 Epidemiology

The severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2) was first detected in Wuhan, China, in December 2019, where it caused the Coronavirus disease 2019 (COVID-19). The highly contagious SARS-CoV-2 quickly started spreading across the world and was declared a pandemic by the World Health Organization (WHO) on March 11, 2020. The first case of COVID-19 in Sweden was confirmed on January 31, 2020 (102). Compared to previous SARS-CoV outbreaks, such as the SARS and MERS, the case fatality rate (CFR) for COVID-19 was rather low, with early reports from China of CFR around 2.5%, compared to 9.6% and 34.4% for SARS and MERS, respectively (103). However, due to its contagiousness the total deaths from COVID-19 soon exceeded its predecessors. By December 1, 2020, there were already over 61.8 million confirmed cases worldwide and 1.4 million deaths according to WHO statistics (104).

In addition, through successive virus mutations throughout the pandemic, new virus variants continuously emerged – from wildtype (2020), through alpha (1st half of 2021) and delta (2nd half of 2021), to omicron variants (early 2022 and onwards).

1.6.2 Pathogenesis of SARS-CoV-2

SARS-CoV-2 is a zoonotic enveloped RNA virus of the β -Coronavirus family. It shares about 80% of its genomic identity with its precursors SARS-CoV and MERS-CoV (105,106). The virus encodes a set of structural proteins: membrane protein (M), nucleocapsid protein (N), envelope protein (E) and spike glycoprotein (S). The spike protein, consisting of two subunits – receptor binding S1 and cell membrane fusion S2 – allows the virus to bind to and enter the host cell via the host receptor angiotensin-converting enzyme 2 (ACE2) (107). SARS-CoV-2 can infect any cell expressing the ACE2 receptor (e.g. endothelial cells, vascular smooth muscle cells, macrophages, T cells, enterocytes), but primarily infects the alveolar epithelial cells (AECs) of the airways (108). The receptor-binding domain (RBD) of the S1 subunit is the most variable part of the SARS-CoV-2 (109). The N protein is involved in RNA replication, virion formation and immune evasion. The M protein is one of the most well-conserved proteins in viral structure and promotes assembly and budding of viral particles (110). The E protein is the smallest component of SARS-CoV-2 and facilitates the production, maturation and release of virions (106). The structure, viral entry and symptoms of SARS-CoV-2 are summarized in *Figure 4*.

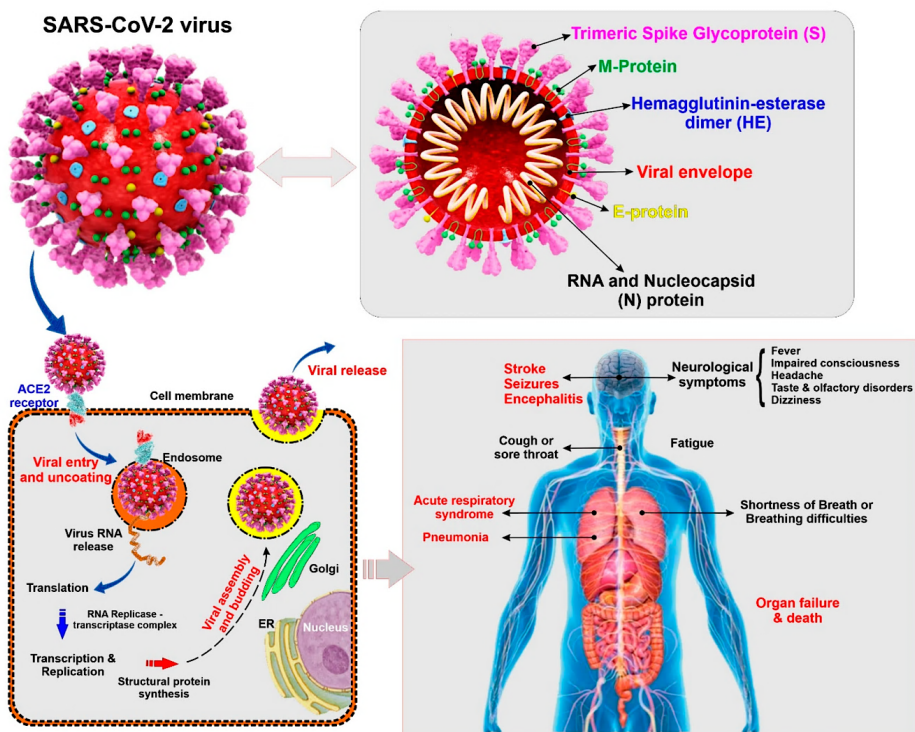


Figure 4. SARS-CoV-2 structural components and viral entry into host cells. Cerebrovascular and neurological dysfunction under the threat of COVID-19: Is there a comorbid role for smoking and vaping? (International Journal of Molecular Sciences. 2020;21(11):3916. doi: 10.3390/ijms21113916). (Reproduced from Archie SR, Cucullo L)

SARS-CoV-2 transmission occurs by respiratory droplets. For most infected patients, the infection will be restricted to the upper airways (and/or gastrointestinal tract) causing mild to moderate symptoms including fever, cough, myalgia, headaches, fatigue, and/or gastrointestinal symptoms. However, some patients will develop hyperinflammation and severe hypoxia, i.e. acute respiratory distress syndrome (ARDS), which may be lethal. The clinical course of the disease can be divided into three distinct phases: 1) Asymptomatic stage; 2) Upper airway and conducting airway response; 3) Hypoxia, ground glass infiltrates, and potential progression to ARDS (111). These three phases reflect the virus' way down the respiratory tract, from its inhaled entry through epithelial cells in the nasal cavity, down the respiratory tract along the conducting airways, and finally, if not stopped, reaching the gas exchange units of the lungs where it infects surfactant producing type II pneumocytes (112). In the lungs, the virus typically causes diffuse alveolar damage with fibrin rich hyaline membranes, oedema and a prominent increase of macrophage infiltration. This process can

be visualized by rapidly progressing changes on radiography, so called ground glass infiltrates (113,114). Early reports calculated that as many as 20% of infected patients would progress to this potentially fatal stage 3 disease, with advanced age as a significant risk factor of poor outcome (103). Additionally, COVID-19 was associated with increased frequency of thrombotic events, primarily pulmonary embolism (115,116).

When an individual is exposed to an acute injury or infection, the innate immune system responds with immediate inflammatory reactions to limit the infection and initiate the slower adaptive immune responses. If the inflammatory responses are not properly moderated and resolved after fulfilling their purposes, they may cause a hyperinflammatory reaction, which instead inhibits adaptive immune responses (e.g. through lymphopenia) and causes tissue damage. Such uncontrolled inflammation could result in a so called “cytokine storm”, manifested by unrestrained production of inflammatory cytokines such as IL-6, IL-2, IL-7, IL-10 and TNF- α , as seen in sepsis patients. Levels of such cytokines was found to be elevated also in patients with severe COVID-19, and significantly higher in intensive care unit (ICU) patients compared to non-ICU patients hospitalized for COVID-19 (117).

Thus, it was soon appreciated that neither the virus itself, nor the viral load, were solely responsible for the morbidity and mortality seen in association with COVID-19. Rather, this was a result of host damage-response initiated by the virus, as previously described for infectious diseases in general (118). Domingo et al described this in 2020 as “The four horsemen of a viral apocalypse”, causing four interplaying loops – a viral loop, an inflammatory loop, a defective non-canonical RAS loop (of the lungs), and a coagulation loop (119). Due to its genomic and structural similarity to other zoonotic β -coronaviruses, here referred to as SARS-CoVs, it was assumed that it also shared many of their mechanisms of pathogenicity and that the host response to the virus would also be comparable. Firstly, while there is a prominent innate immune response to SARS-CoV infection including acute-phase proteins, chemokines and inflammatory cytokines, there is also a noticeable lack of type 1 interferon (IFN- α and IFN- β) response. This causes a delayed and ineffective innate immune response, ultimately affecting NK-cell mediated cytotoxicity and activation of antiviral adaptive immune responses, leading to restrained clearance of the virus (109). The virus has several mechanisms of antagonizing IFN responses, and this inhibition of IFN signaling, resulting in and coinciding with free viral replication that leads to further dampening of IFN signaling and aberrant inflammatory response, constitutes the viral loop (119). Secondly, the high viral replication in alveolar epithelial cells (AECs) due to ineffective innate immune responses,

induce cytopathic effects culminating in apoptosis, as an ultimate cellular response to stop viral replication (120). This results in further increased production of proinflammatory cytokines and chemokines by AECs, leading to massive infiltration of inflammatory cells into the lungs, further enhancing the release of cytokines (121). After the initial innate immune response to infection, the adaptive immune system is meant to gradually take over, resulting in T cell responses and long-lasting antibodies against the current pathogen within 7–10 days after the onset of infection. However, while expansion and activation of CD4+ and CD8+ T cells are essential in antiviral immunity, lymphopenia is common in the acute phase of COVID-19 (107). CD4+ T cells are needed to activate T cell dependent B cells to initiate production of virus-specific antibodies, and CD8+ T cells have direct cytotoxic effect on virus-infected cells, both thus crucial for clearance of the infection. Furthermore, lack of residential T cells regulating innate immune responses, can contribute to the unleashed, hyperreactive innate immune response seen in full-blown COVID-19 (122,123). This hyperreaction affecting both innate and adaptive immune responses, resulting in immune-mediated tissue and organ damage, conforms the inflammatory loop (119). Thirdly, SARS-CoV-2 infection of AECs leads to impaired ACE2 activity in the lung, where ACE2 is vital to control inflammation by converting pro-inflammatory angiotensin-II into anti-inflammatory angiotensin 1–7 (124). Reduction of ACE2 thus lead to disturbance of the renin-angiotensin system (RAS) and increased levels of angiotensin-II resulting in enhanced inflammation and pulmonary edema. Lastly, COVID-19 is associated with coagulation disorders including increased procoagulant factors such as fibrinogen and D-dimer. Hemostasis and innate immunity are intricately intertwined, a mechanism called thrombo-inflammation. Both the intrinsic and extrinsic coagulation pathways can be activated during inflammation. The hyperinflammatory state of advanced COVID-19 induce hypercoagulation (and vice versa), which is further enhanced by hypoxia and defective counterregulatory ACE2-dependent forces – the coagulation loop (119). This intricate interface of immune responses, coagulation and tissue cells, triggered by the virus, is visualized in *Figure 5*.

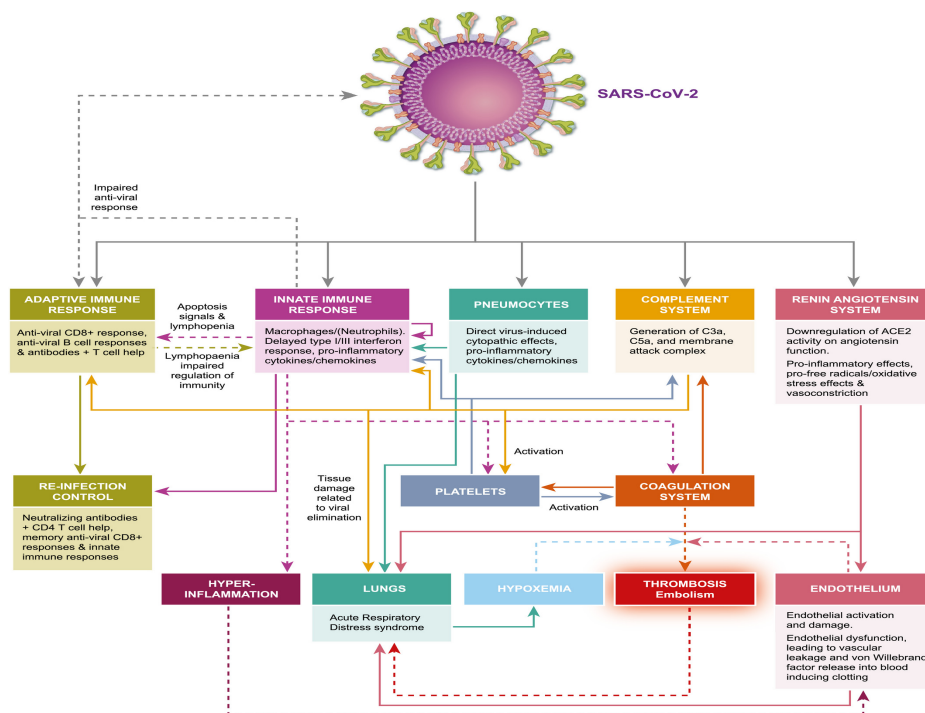


Figure 5. Pathobiology of COVID-19. COVID-19 vaccine readiness for anti-CD20-depleting therapy in autoimmune diseases. (Clinical and Experimental Immunology. 2020;202(2):149-161. Oxford Academic. doi: 10.1111/cei.13495). (Reproduced from Baker et al)

1.7 COVID-19 management

1.7.1 Supportive care and preventive measures

Initial care for patients hospitalized due to severe COVID-19 focused on supportive care, mainly supplementary oxygen, including the use of non-mechanical ventilation (NIV) or high-flow nasal oxygen (HFNO), and mechanical ventilation for patients with respiratory distress. Once recognizing the coagulopathy associated with COVID-19, thromboprophylaxis, mainly low molecular weight heparins (LMWH), were also included in routine care for hospitalized COVID-19 patients.

In addition, governments took various measures to limit spreading of the virus throughout the world. Many countries used lockdowns to various extent for this purpose. This method was however never applied in Sweden, where citizens were instead advised to take voluntary measures to prevent disease spreading, such as physical distancing in public spaces (mandatory in some settings), stay home when sick, orderly hand hygiene, etc. Face masks were never mandatory in Sweden outside of health care facilities (125–127).

1.7.2 Anti-viral therapy

The initial main strategy to treat COVID-19 was to use “repurposed” drugs, i.e. drugs that were already in use for other indications. Such a drug was Remdesivir, a broad-spectrum antiviral agent developed in 2017 as a treatment for Ebola virus, by inhibiting viral RNA production. Remdesivir had previously shown reduction of MERS-CoV viral load in lung tissue of mice and promising results in treating a patient with COVID-19 pneumonia in the United States (128,129). In February 2020, a phase III randomized trial was initiated in China, which however failed to show significant benefit of intravenous (iv) remdesivir treatment vs placebo in adults with severe COVID-19 (130). Even so, remdesivir was widely used in Sweden throughout the pandemic, for early treatment of hospitalized COVID-19 patients and subsequent studies did show a preventive effect of remdesivir in early stages of COVID-19, reducing the risk of progression to severe disease (131). Remdesivir gained EMA approval for treating COVID-19 in June 2020. In 2022, Remdesivir was however generally replaced by the new anti-SARS-CoV-2 drug Paxlovid (nirmatrelvir/ritonavir), proving to be highly effective in reducing the risk of severe COVID-19 and mortality (132–134).

In November 2021, EMA approved the first two anti-Spike monoclonal antibodies (mAbs) for treatment of COVID-19. In Sweden the most used were Evusheld (tixagevimab/cilgavimab) and Xevudy (sotrovimab). The indication was to prevent severe COVID-19 in risk patients, such as those with immune deficiency, with mild-moderate COVID-19, or as post-exposure prophylaxis. This was particularly valuable for patients who were non-responders to SARS-CoV-2 vaccination. However, there have been no effective mAbs available against the Omicron SARS-CoV-2 variants.

Hydroxychloroquine (HCQ), used to treat malaria and rheumatic disease, was another drug that was early proposed as a potential anti-viral and anti-inflammatory treatment for COVID-19. Early studies showed promising results in vitro (135) and in vivo (136). However, it never had any broad impact on COVID-19 treatment, partly due to its rather disheartening side-effects, including severe cardiac arrhythmia, hypoglycemia and skin reactions (137,138).

In addition, COVID-19 patients in hospital were often treated with antibiotics, for established or suspected bacterial co-infection (139).

1.7.3 Anti-inflammatory therapy

Many drugs were considered, and tested, for treating the inflammatory stage of COVID-19, such as statins (as a potential inducer of ACE2 receptor activity), IL-1 receptor antagonists, NSAIDs and convalescent plasma – with little or no effect

on survival or recovery from COVID-19. When the idea of using corticosteroids for treating critical COVID-19 first emerged there was some concern that this would have a negative impact on virus clearance. However, evidence was soon piling up that treating COVID-19 patients with low-dose corticosteroids significantly reduced mortality without obviously delaying virus clearance (140,141). This manifested the most significant advance yet in treating COVID-19, and since mid-2020, corticosteroids were part of routine care for patients hospitalized with COVID-19 and hypoxia. Corticosteroid treatment was usually initiated at a minimum of 7 days after onset of the infection.

By December 2021, EMA approved the use of IL-6 inhibitor tocilizumab, a humanized monoclonal antibody used for treating rheumatoid arthritis, in addition to corticosteroids for treatment of critically ill patients with COVID-19.

1.7.4 Anti-SARS-CoV-2 vaccines

Vaccination against SARS-CoV-2 started in Sweden in late December of 2020. During 2020–2022 Sweden used the mRNA vaccines provided by Pfizer BioNTech (63%) and Moderna (29%), AstraZeneca’s viral vector vaccine (4%) and the Novavax protein subunit vaccine (4%) (142). Vaccination was readily available and free of charge for all Swedish citizens, but initially according to a priority system with an “oldest first” policy, followed by certain comorbidities including immunosuppression. Booster doses were made available gradually from September 1, 2021, according to the same priority system (143).

1.8 Combining COVID-19 and CLL

When the new virus SARS-CoV-2 started spreading across the world many hematologists anticipated that patients with hematological malignancies in general, and CLL in particular, would be at great risk for severe disease. Unfortunately, early studies seemed to prove this hypothesis. With mortality rates around 35% in hospitalized/severe cases, individuals with CLL stood out, even compared to other hematological patients (144–147). Another concern was whether patients with CLL would be able to mount immunity against this novel virus, after recovering from COVID-19 or as response to the much-anticipated vaccines.

1.9 Late pandemic and current status

Since February 2022, SARS-Cov-2 Omicron sublineages have accounted for almost all cases of COVID-19 (148). Incidence of severe COVID-19 and COVID-19-related mortality generally decreased in the Omicron era. However, immunocompromised individuals, for example CLL patients, were still

overrepresented among COVID-19-related hospital/ICU admissions and deaths (149). For patients with CLL, mortality rates ranged from 2% to 10%, and hospital admission rates was estimated to 25% (150,151).

Despite initially low seroconversion rates, repeated booster vaccination has proven efficient also in individuals with CLL (152). A Swedish registry published in late 2025, presented a significant risk reduction in individuals vaccinated against SARS-CoV-2 within 180 days prior to the infection (153).

Another proposed cause of morbidity in immunocompromised individuals, is persisting (or chronic) COVID-19. Persisting COVID-19, defined as prolonged SARS-CoV-2 RNA detection (generally ≥ 30 days from first detection) is associated with lingering or rebounding symptoms and repeated re-admissions (154), and has been demonstrated in almost 14% of patients with hematological malignancies, with recent anti-CD20 mAb treatment being the most prominent risk factor (155).

On May 5, 2023, WHO declared the pandemic officially over. SARS-CoV-2 is now considered an endemic seasonal virus and the current recommendations from the Public Health Agency of Sweden (PHAS) is to take one booster vaccine dose per year for immunocompromised and elderly individuals, considered risk groups.

During the spring of 2026 the number of confirmed cases of COVID-19 in Sweden was the lowest since the pandemic outbreak, according to PHAS. Also, COVID-19-related mortality and ICU admissions have kept decreasing according to their report. The winter season (high season for viral infections in Sweden) of 2025-2026 was the first since the appearance of COVID-19 when the disease burden of influenza viruses was higher than that of SARS-CoV-2 in 65-year-old individuals. In early 2026 only 1% of performed tests were positive for SARS-CoV-2 (156).

2 Research aims

This thesis investigates the clinical and immunological outcomes of SARS-CoV-2 infection in individuals with CLL. More specifically, it analyses hospitalization and mortality rates from COVID-19 in Swedish CLL patients throughout various periods during the pandemic, and B- and T-cell immune responses after SARS-CoV-2 infection and vaccination. These questions are addressed through five consecutive and complementary studies, using both real-world data, prospective clinical trial cohorts, patient blood and saliva sampling, and national registry data. The overall aim is to understand the consequences and risks associated with COVID-19 for individuals with CLL, identify potential risk factors according to CLL status, patient characteristics and CLL-directed therapy, as well as provide information to help form protective strategies.

When **study I** was initiated, at the very beginning of the SARS-CoV-2 outbreak in Sweden, there was little knowledge about the clinical course and consequences of COVID-19, especially in immunocompromised individuals. Thus, the study aims to describe the clinical course of COVID-19 in CLL patients in terms of infectious symptoms, severity and mortality, COVID-19 management, and adaptive immune response to the virus.

Study II aims to investigate the immune responses after vaccination with the new mRNA anti-SARS-CoV-2 vaccine in CLL patients previously unexposed to SARS-CoV-2, focusing on specific T-cell reactivity. At this point, efficacy of the new vaccines had not been described for immunocompromised individuals.

Study III instead explores how immune responses evolve in CLL patients previously infected with SARS-CoV-2 (survivors of the Study I cohort) and subsequently vaccinated with the new mRNA vaccines in a real-world setting.

Study IV: After sequentially evaluating immune responses in CLL patients through repeated exposure to SARS-CoV-2 antigen, this study aims to epidemiologically evaluate the anti-SARS-CoV-2 vaccine uptake among CLL patients in Sweden.

In **study V** the aim is to use a retrospective, epidemiological approach to explore risks of severe COVID-19 and mortality in CLL patients compared to the general Swedish population on a national scale and throughout three years of the pandemic.

3 Materials and methods

3.1 Study design and study population

Study I is a regional cohort study, exploring clinical and immunological outcomes of COVID-19 in 60 consecutive CLL patients within a well-defined region (Stockholm). The study cohort was gathered by a study group of clinicians from the three hematology centers in Stockholm, based at Karolinska University Hospital Solna, Capio St. Göran Hospital and Södersjukhuset, covering >95% of all patients diagnosed with CLL in the region (as verified from the Information Network for Cancer Care (INCA) database (157). Clinicians in the study group systematically checked history of COVID-19 among CLL patients at their unit during the study period (March 1, 2020 until March 31, 2021) and reported to the study coordinator. CLL patients with a history of PCR-verified, symptomatic COVID-19 infection were included. Data sources were a combination of clinical data collected from patients' medical files and analyses of blood- and saliva samples collected from study participants. Laboratory methods to evaluate immune responses included blood cell counting, flow cytometry, multiplex-based immunoassays, fluorescence-activated cell sorting (FACS) and IFN- γ enzyme-linked immunospot (ELISpot) assay.

Study II is based on a prospective, clinical trial studying immunological responses to mRNA vaccine BNT1622b2 against SARS-CoV-2 in individuals with various immunodeficiency disorders vs healthy controls. This so-called COVAXID trial was performed in Stockholm in early 2021 and included 5 equally sized cohorts of patients with different types of immunocompromising conditions, including CLL, as well as a cohort of healthy controls. Each cohort included 90 study participants. The CLL cohort was further divided into 4 subgroups: untreated, previously treated with anti-CD20-mAbs containing chemoimmunotherapy (CIT) >6 months ago, ongoing BTKi therapy, and previous BTKi therapy (in remission, stopped BTKi since >2 months). Study participants had to be previously unexposed to SARS-CoV-2 (verified by serology and PCR test upon inclusion). Study participants received 2 doses of the vaccine, 3 weeks apart, and blood samples were collected before 1st vaccine dose, between 1st and 2nd dose (day 10), at 2nd dose (day 21) and 2 weeks after the 2nd dose (at day 35). These samples were collected at day (d) 0 (baseline, day of 1st vaccine dose), d10 (10 days after 1st dose), d21 (21 days after 1st dose, day of 2nd dose) and d35 (35 days after 1st dose). The primary aim of the COVAXID trial was to determine

seroconversion rates in the 5 different patient cohorts, compared to healthy controls. However, a predefined subgroup of each cohort (ca 50%) was subject to further analysis of blood samples, including T cell reactivity. This proportion of study participants from the CLL (n=52) cohort and corresponding healthy controls (n=41) compose the cohorts of study II, focusing on T cell response rates and seroconversion rates 2 weeks after the second vaccine dose in CLL patients vs healthy controls. Laboratory methods to evaluate immune responses included multiplex-based immunoassays and ELISpot.

Study III is a cohort study exploring immunological outcomes in 29 consecutive CLL patients previously infected with SARS-CoV-2 (March 2020–March 2021) during subsequent vaccination with up to three doses of anti-SARS-CoV-2 vaccine, based on the cohort of study I. Laboratory methods to evaluate immune responses included multiplex-based immunoassays, ELISpot and flow cytometry-based activation-induced marker (AIM) assay.

Study IV and V are national, registry-based epidemiological studies investigating anti-SARS-CoV-2 vaccine uptake and clinical outcomes in CLL patients vs the general Swedish population, respectively. Definitions of the study populations will be described in section 3.9.2.

3.2 Clinical data from medical files

In **Study I** and **III**, data collected from patients' medical files were recorded in a common pseudo-anonymized report form and included age, gender, Cumulative Illness Rating Scale (CIRS), Body Mass Index (BMI), smoking status, diabetes, CLL treatment history, blood counts and immunoglobulin (Ig) levels for baseline clinical characteristics. Furthermore, we collected data on date of COVID-19 diagnosis (date of positive PCR test), COVID-19 infectious symptoms, hospitalization and its length, intensive care unit (ICU) admittance, COVID-19 treatment and outcome (including COVID-19 related complications).

3.3 Blood and saliva sampling

In **Study I** blood tests from 40 COVID-19 survivors were collected at 3 months after the COVID-19 infectious episode, and repeated at 6 and 12 months as available, with patients' final assessment performed before start of COVID-19 vaccination. Serum samples obtained at COVID-19 diagnosis within routine health care (data from medical files) were used as baseline serologies. From 28 patients we also collected saliva at these time-points (3, 6 and 12 months post

infection). Saliva samples were collected using a self-sampling technique based on a validated protocol developed by Alkaharaan et al (158). Briefly, study participants were provided by the study-coordinator, through mail, with a self-sampling kit along with instructions and a questionnaire on COVID-19-related data and were asked to post the expectorated saliva sample in the intended container, within a pre-addressed envelope the same day.

In **Study II** we used blood samples collected within the COVAXID prospective open-label trial (159) as previously described in section 3.1.

In **Study III** blood and saliva samples were collected from 29 COVID-19 survivors at 4 timepoints: at start of vaccination (median 5.75 months after COVID-19 diagnosis), after 2 vaccine doses (median 3 weeks after 2nd dose), and before and after the 3rd dose (median 5 weeks before and 3 weeks after the 3rd vaccine dose, respectively). Saliva samples were collected using the same self-sampling technique as in study I.

3.4 Serology analyses

Serology responses have for a long time been an essential part of evaluating immunity and vaccine protection by testing sera for specific antibodies. The standard method to quantify antibody responses has long been enzyme-linked immunosorbent assay (ELISA), which uses wells covered with antibodies binding the specific antigen and creating an enzymatic reaction that produces a quantifiable signal, for example color change. Electro-chemiluminescence immunoassays (ECLIA) have since developed, a multiplex technology similar to ELISA that analyzes several different antigens in a single well.

During the pandemic serum samples were routinely analyzed for SARS-CoV-2 antibodies at the Department of Microbiology, Karolinska University Hospital, using multiplex-based immunoassays (ECLIA). However, exact assays varied early in the pandemic, affecting especially baseline and 3 months serology analyses in **Study I**. The tests routinely used at this time in Stockholm included SARS-CoV-2-IgG test iFlash 1800 YHLO (CLIA), Elecsys® Anti-SARS-CoV-2 (ECLIA, qualitative) and Elecsys® Anti-SARS-CoV-2 S (ECLIA, quantitative) (Roche Diagnostics). YHLO determines the antibodies against SARS-CoV-2 nucleocapsid (N) and spike (S) antigen, Roche qualitative against N antigen and Roche quantitative against S antigen receptor binding domain (RBD). The majority of serology samples at the 6 and 12 months evaluation of **Study I** were

analyzed by Roche quantitative antibody test, with measurement range 0.40–250 U/mL, and values <0.80 U/mL considered negative (160). According to the manufacturer, the overall clinical specificity was 99.98% (n=5991) and sensitivity was 98.8% for samples taken >14 days after positive PCR result (n=1423) (159).

In **Study II** serum samples were tested using the Roche Elecsys® quantitative anti-SARS-CoV-2 assay described above, since it measures the S RBD protein which is the target of the mRNA vaccines.

Study III used both the Roche Elecsys® qualitative and quantitative anti-SARS-CoV-2 immunoassays against S RBD (positive cutoff >0.80 U/mL) and N protein (positive cutoff >1.0 cutoff index [COI]), respectively. The assay-specific limit of detection for S-specific antibodies was 25,000 U/mL and samples with values >2,500 U/mL after 1:10 dilution were diluted at 1:100 using Diluent Universal.

3.5 Neutralizing capacity

In **Study I**, additional analysis of antibody neutralizing capacity was performed on a subset of patient blood samples by assessing the ACE2 inhibition, using a competitive bead-based binding assay. In summary, Spike RBD is immobilized to beads and exposed to antibody-containing serum, with specific antibodies binding to RBD. Then, ACE2 coupled beads with a fluorescence marker are added. Fluorescence intensity is then measured, where weak fluorescent signals suggest strong inhibiting (blocking) capacity of the RBD-binding antibodies.

In **Study I**, results on neutralizing capacity for each sample subject to this analysis were reported as +, ++ or +++ depending on fluorescence intensity.

3.6 Flow cytometry and FACS

Flow cytometry is a widely used technique to identify and quantify different immune cells in blood and various tissue samples using lasers. Modern flow cytometry often intends fluorescence-activated cell sorting (FACS). With FACS, the cells are sorted based on size and surface markers stained with a fluorochrome-conjugated antibody, and therefore commonly used for immunophenotyping cells in a sample. This technique was used in **Study I** for the quantification of T cells, and for the T cell analyzing AIM assay of **Study III** (described in the next section).

3.7 T cell analyses

The *enzyme-linked immunospot (ELISpot)* assay is a technique that is used to quantify antigen-specific B or T cell responses at the single-cell level. IFN- γ ELISpot, a technique widely used in T cell research throughout the pandemic and previously described by others (161,162), was used to assess SARS-CoV-2-specific T cell reactivity in **Studies I-III**.

Briefly, cryopreserved peripheral blood mononuclear cells (PBMCs) are stimulated in wells of a plate precoated with capture antibodies specific to IFN- γ . The number of PBMCs per well in our assays was 2.5×10^5 cells/well, as is standard. During an incubation period, immune cells are activated by the antigen of interest leading to secretion of IFN- γ , that is captured by the antibodies coating the plate. After this reaction the cells are washed away, detection antibodies are added, leading to the formation of visible spots at the site where the analyte was released. Each spot observable in the well represents an analyte-secreting cell, also referred to as a spot-forming unit (SFU). Automated ELISpot readers are often used to detect and quantify the spots. The assay includes a negative (just media) and positive control. Staphylococcal enterotoxin B (SEB) was used as positive control.

In **Study I** the peptide pool used to stimulate the cells spanned several immunogenic domains of the SARS-CoV-2 virus: spike (S), membrane (M), envelope (E) and nucleocapsid (N) proteins. Spot-forming units (SFU) were counted using an automated ELISpot reader system. Data were presented as number of spots/well, as % of CD 3 T cells (assessed by FACS) and as total numbers (IFN- γ producing cells/uL). A patient was considered positive if >20 SFUs with S and/or M+E+N pool were recorded after background subtraction (161).

Similarly, in **Study II** and **III**, cryopreserved PBMCs were stimulated with a peptide pool covering SARS-CoV-2 spike glycoprotein. SFUs were counted using the IRIS (Mabtech) automated reader system. Data points were presented as background corrected SFU/ 10^6 cells, calculated by subtracting the mean value of corresponding duplicate unstimulated wells from the mean value of duplicate spike stimulated wells. Negative values after background correction were set to 1. The threshold for positive response in study II and III was set to the average SFU/ 10^6 cells of unstimulated wells + 2 standard deviations.

In **Study III**, the more exact flow cytometry-based *activation-induced marker assay (AIM)* was used to analyze T cell responses to two different strains of SARS-CoV-2 (Wild-type Wuh1 and Omicron BA.1), in CD4+ and CD8+ T cells, respectively. Briefly, cryopreserved PBMCs were stimulated with the same peptide pool as used in the ELISpot assay and stained for CD69, CD40L and 4-1BB to identify activated antigen-specific CD4+ and CD8+ T cells. Further staining was performed for CD45RA and CCR7 to identify memory cells, CXCR5 to identify T follicular helper cells, as well as additional activation marker CXCR3. Results were shown as frequency (%) of spike-specific cells after subtraction of the paired % of AIM+ cells in unstimulated controls. Negative values after background correction were set to 0.001% to allow graphing with logarithmic scale.

3.8 Saliva antibody analyses

In **Study I** and **III**, unstimulated whole saliva was used to measure IgG reactivities to 3 SARS-CoV-2 antigens (Spike S1S2 trimer, S1, and the C-terminal part of the nucleocapsid). Saliva was self-collected by passive drooling from fasted study participants, sent in by mail overnight, and were on the day of arrival immediately processed for -80°C storage.

Prior to antibody analysis, saliva samples were thawed and centrifuged to separate any debris. Antibodies binding to the full-length spike glycoprotein in trimeric form (Spike-f) was measured using a multiplex bead-based assay, as previously described by others (158,163). The antigens were immobilized on the surface of uniquely color-coded bead identities (IDs) and the IDs pooled to generate the bead-array. Saliva samples were diluted 1:5 and incubated with the array. After cross-linking of the antibody-antigen complexes, an anti-human IgG antibody was applied for detection of IgG bound to spike. The assay readout was performed using automated readers. Each assay run included internal controls consisting of the same set of 12 pre-pandemic saliva samples (to calculate the antigen and assay-specific cutoff), and 4 positive controls of convalescent saliva. Saliva antibody reactivity was presented as median fluorescence intensity (MFI) and the assay cutoff for each antigen was determined by the mean + 6 SD of the 12 negative pre-pandemic samples.

3.9 Registry data

3.9.1 Data sources

In **Study IV**, data from six national registries were used to explore the COVID-19 vaccine uptake in adults aged 18–90 years diagnosed with CLL in Sweden:

i) National Quality Registry for CLL, ii) Total Population Register (TPR), iii) National Vaccination Register (NVR), iv) Swedish Cause of Death Register (SCDR), v) Longitudinal Integrated Database for Health Insurance and Labour Market Studies (LISA) and vi) Swedish Prescribed Drug Register (SPDR). Individuals diagnosed with CLL in Sweden are reported to the National Quality Registry for CLL (reporting coverage of 97%), which is part of the Information Network for Cancer Care (INCA) platform, maintained by the Regional Cancer Centers (RCC) in Sweden. TPR is maintained by Statistics Sweden and contains data on birth, death, emigration and immigration, sex, and country of birth for the Swedish population (164). All vaccinations within national vaccination programs and vaccinations against COVID-19 should according to Swedish law be reported to the NVR, which is governed by the Public Health Agency of Sweden (PHAS) (165). SCDR is an almost complete register of all deaths in Sweden, maintained by the National Board of Health and Welfare (166). LISA is maintained by Statistics Sweden and contains data on around 500 variables on individuals including civil status, educational status, income and allowances (167). SPDR is maintained by the National Board of Health and Welfare and contains data for all dispensed prescriptions in Sweden (168).

Study V used ten national registries: apart from the Total Population Register (TPR), the Longitudinal Integrated Database for Health Insurance and Labor Market Studies (LISA), the National Vaccination Register (NVR), Swedish Prescribed Drug Register (SPDR), the National Cause of Death Register (NCDR) and the National Quality Registry for CLL described above, data was also collected from the Multi-Generation Register (MGR), SmiNet, the National Patient Register (NPR) and the Swedish Intensive Care Registry (SIR). SmiNet is a national digital reporting system run by PHAS, where doctors and clinical laboratories are obliged to report any contagious diseases covered by the Communicable Diseases Act, including COVID-19. Data from TPR, MGR and LISA was used for sociodemographic data. SmiNet and NVR were used to identify all PCR tests positive for SARS-CoV-2 and Covid-19 vaccinations in Sweden, respectively. NPR, SPDR, NCDR and SIR were used for classification of comorbidities and outcomes of the PCR-verified SARS-CoV-2 infectious episodes (mortality,

hospitalization and intensive care unit (ICU) admission). The INCA CLL register was used to identify all individuals in Sweden diagnosed with CLL.

For the additional analysis on age- and sex-adjusted relative risk of all-cause mortality in Stockholm County on June 30 and December 31 from 2017 through 2022, data was collected from the Stockholm regional healthcare data warehouse.

3.9.2 Definition of study population

Study IV considered all individuals in Sweden born from 1930 to 2003 diagnosed with CLL any time up until the end of the study (February 28, 2023), who were alive and living in Sweden any time from February 1, 2020, to the end of the study. Among these individuals, those alive and living in Sweden on December 27, 2020 (the date of the first anti-SARS-CoV-2 vaccination in Sweden), were included. Furthermore, we only included individuals who were diagnosed with CLL up until November 30, 2022, to allow for 90 days of follow-up. Individuals diagnosed before and after the date of the first anti-SARS-CoV-2 vaccination in Sweden were analyzed separately, since knowing about the CLL might affect an individual's tendency to receive the vaccine, thus causing bias.

In **Study V**, all individuals born between 1930 and 2003 who were living in Sweden any time from February 1, 2020, to March 31, 2023, were included, and all PCR-verified SARS-CoV-2 infection episodes through February 23, 2023, were identified. An infection episode was defined as all SARS-CoV-2-positive PCR tests within 90 days (i.e. 2 consecutive positive tests >90 days apart were defined as 2 separate episodes). Since very few re-infections were identified (n=83), we only analyzed the first PCR-verified infection per individual. Among people with infection episodes, we classified the exposure of interest, CLL, using the date of CLL diagnosis. Individuals who had their CLL diagnosis up until 90 days after the date of the first SARS-CoV-2-positive PCR-test were considered exposed.

In the additional analysis comparing cumulative incidences of all-cause mortality from 2017–2022 in Stockholm, the study population consisted of all individuals with and without CLL residing in Stockholm County and ≥ 18 years old on January 1 for each of the years 2017 through 2022. Individuals with CLL should have received a CLL diagnosis any time up to 5 years before January 1 for each year 2017–2022. This last criterion was applied to have access to the same lookback period for all years investigated.

3.9.3 Defining the data

In **Study IV**, follow-up started on December 27, 2020, and finished by date of death, moving out from Sweden, or February 28, 2023, whichever occurred first. For individuals diagnosed with CLL after December 27, 2020, follow-up started at the date of diagnosis. Data on age, sex, region of birth, age-standardized income quartile, number of prescribed drug types, and CLL status was collected. Age was categorized as 18–64, 65–79 and 80–90 years. Region of birth was categorized as being born in or outside of Sweden. Income quartiles for each birth year in the entire Swedish population were first calculated, and study participants were then classified into income quartiles based on their income in relation to these population-based quartiles. The income variable was the total sum of incomes including earned income, age-related pensions, compensation from unemployment insurance, sickness allowance, etc. The number of prescribed drug types included antidiabetic and antihypertensive drugs, antithrombotic agents, drugs for obstructive airway or heart disease, immunosuppressive drugs, and lipid modifying agents prescribed from December 27, 2019, to December 27, 2020 (the last year before study start). CLL status included time since diagnosis (>5 years or ≤ 5 years) and treatment status (Treated or Untreated). Regarding CLL treatment status the following drug types were considered: anti-CD20 antibodies, BTK inhibitors, Bcl-2 inhibitors, chemotherapy, corticosteroids and PI3K inhibitors. For BTKi, Bcl-2i, PI3Ki and chemotherapy, all treatments given before December 27, 2020 (start of study), were considered. For anti-CD20 antibodies, treatments given from December 27, 2019, to December 27, 2020 (last year before study start), were considered. For corticosteroids, treatment given from September 28, 2020, to December 27, 2020 (last 3 months), was considered. Individuals who had not received any of these treatments within the given timeframe were classified as Untreated.

In **Study V**, the primary outcomes of all-cause mortality and COVID-19 related mortality were defined and identified by International Classification of Disease 10th revision (ICD-10) diagnosis U07.1 or U.07.2 as underlying cause of death in the death certificate and were presented both for the entire population (irrespective of SARS-CoV-2 infection) and for individuals with SARS-CoV-2 infection only. Mortality data was available through December 31, 2023. The secondary outcome of COVID-19 hospitalization was defined as a positive PCR test performed any time from 14 days before the date of hospital admission up until the date of hospital discharge, and if a COVID-19 diagnosis (U07.1 or U07.2)

was registered as a primary or secondary discharge code. A COVID-19 ICU admission was defined as an ICU admission that occurred during such a COVID-19 hospitalization with a COVID-19 diagnosis (U07.1 or U07.2) registered as a primary or secondary discharge code at the ICU.

Data was also collected on age, sex, region of birth, residential region in Sweden, age-standardized income quartile, prescription drug use before the infection episode, CLL treatment, time since CLL diagnosis in relation to the SARS-CoV-2 infection, COVID-19 vaccination status, and SARS-CoV-2 variant period.

Prescribed drugs were used as a surrogate for comorbidities and included drugs prescribed for conditions such as diabetes, cardiovascular disease, respiratory diseases, and conditions warranting immunosuppressive treatments. The CLL treatments considered were BTKi, Bcl-2i, PI3Ki and corticosteroids (corresponding to a daily dose of ≥ 10 mg prednisone) (159). COVID-19 vaccination status was assessed at 14 days before the positive PCR test. The variant period of the included SARS-CoV-2 infection episodes was either based on a sequenced sample or the national variant distribution (wild type: January 31, 2020–February 14, 2021; Alpha: February 15, 2021–June 27, 2021; Delta: June 28, 2021–December 26, 2021; Omicron: December 27, 2021–February 27, 2023) (169).

Regarding the additional all-cause mortality analyses of the Stockholm cohort, start of follow-up was set to January 1 for each of the years 2017 through 2022. End of follow-up was December 31, date of all-cause mortality or date of moving out from Stockholm, whichever occurred first.

3.10 Statistical analysis

3.10.1 Study I

Data was summarized using descriptive statistics such as counts, percentages, medians and range. Categorical variables were expressed as cross-tabulations and distributional differences were tested using the Chi-squared test. Univariate and multivariate modelling of the outcome death (90-day mortality) and hospitalization (yes, no) were performed using logistic regression. Variables included in the univariate analysis were sex, age (≥ 75 vs < 75 years) based on (145), BMI (≥ 25 vs < 25), diabetes (yes, no), CIRS (> 8 vs ≤ 8) based on our median, CLL treatment (never vs ever treated) as in (145), smoking status (never vs ever smoker), and hypogammaglobulinemia (yes, no). Variables without missing data were included in the multivariate analysis of both outcomes. Results from these models are reported as odds-ratios (OR) together with 95% confidence intervals

(CI) and Wald p values. The fit of the multivariate models was evaluated using Pearson goodness-of-fit test. Stata version 17 was used for the statistical analysis. A numerical comparison of clinical outcome between month 1-6 vs 7-13 of the pandemic was also performed.

3.10.2 Study II

Data was summarized using descriptive statistics such as counts, percentages, medians and range. Categorical variables are presented as cross-tabulations and distributional differences were tested using the Chi-squared test. Significance between time points with missing values was assessed using Kruskal-Wallis test with Dunn's posttest. Correlation analysis was done using non-parametric Spearman rank correlation. P -values <0.05 were considered significant. Graphs and associated statistical tests were performed in Prism v.9.

3.10.3 Study III

Similarly, **Study III** also applied descriptive statistics such as counts, percentages, medians and range. Comparisons between time points were performed using non-parametric Kruskal-Wallis test and Friedman's test in the case of no missing data points, followed by Dunn's correction for multiple comparisons. Graphs and statistical tests were performed in Prism v.9.

3.10.4 Study IV

Cumulative incidences of uptake of the first to fifth vaccine doses were used to describe the vaccine uptake among CLL patients in Sweden. First, the baseline characteristics were described both for the complete study population, as well as separately for individuals diagnosed with CLL before vs after the date of the first anti-SARS-CoV-2 vaccination in Sweden (December 27, 2020). Continuous variables were presented as median (interquartile range [IQR]) and categorical variables as frequencies (percentages).

The cumulative incidences of uptake of the first to fifth doses of anti-SARS-CoV-2 vaccine in individuals with CLL were analyzed using the Aalen and Johansen estimator, with death and emigration as competing events (170). Patients diagnosed with CLL after the start of the vaccination program (December 27, 2020) were analyzed separately to avoid bias, since having a known CLL diagnosis might impact the individual's choices regarding vaccination.

To understand if the vaccine uptake in individuals with CLL was higher than in the general population, the nationwide uptake was then standardized to the age distribution in the CLL study population. This was assessed by calculating the vaccine uptake in age groups <40, 40–49, 50–59, 60–69, 70–79 and 80–90 years using data from the National Vaccination Register (NVR), with population denominators retrieved from Statistics Sweden. The vaccine uptake in each age group was then multiplied by the proportion of CLL patients in the study population belonging to each age group and finally added together to obtain the age-standardized vaccine uptake. This was done for separately for the first to fifth doses.

The cumulative incidences (with 95% CI) of receipt of the first to fifth vaccine dose at the end of follow-up were then analyzed for each of the independent variables age, sex, region of birth, age-standardized income quartile, number of prescribed drug types, and CLL status, and presented as cumulative incidence curves, along with the corresponding *P* values, obtained from Gray's test. Statistical significance was assessed at the 0.05 alpha level. Further analysis of the combined effect of the independent variables which seemed to have the biggest impact on vaccine uptake – age group, region of birth, and income quartile – was done through two intersectional analyses. The first analysis combined region of birth (Sweden, Outside Sweden) and income quartile (Quartile 1 [lowest income], Quartile 2–Quartile 4), resulting in four intersectional strata. The cumulative incidences (with 95% CI) of receipt of the first to fifth vaccine doses at the end of follow-up were then analyzed for the four strata. The second analysis also included age groups (18–64 years, 65–79 years, and 80–90 years), thus including 12 intersectional strata. Cumulative incidence curves, along with the corresponding *P* values obtained from Gray's test, were presented for both analyses. The vaccine uptake at the end of the study in individuals diagnosed before the study period was then compared with individuals diagnosed with CLL during the study period. Analyses were conducted using R version 4.1.0.

3.10.5 Study V

In this other registry-based nationwide study, exploring COVID-19 outcomes in individuals with CLL vs the general Swedish population, we instead used regression models to obtain risk ratios (RR). Continuous variables were presented as median (interquartile interval [IQI]) and categorical variables as

frequencies (percentage). Kruskal–Wallis tests were used for continuous variables and Chi-squared tests were used for categorical variables.

The primary outcome, 90-day mortality, was presented as both crude mortality rates, and adjusted RRs with 95% CI. Modified Poisson regression models were used to obtain the RRs for individuals with CLL vs individuals without CLL. The 95% CIs were derived from a sandwich variance estimator. The fully adjusted model included age, sex, region of birth, income quartile, residential region, days since the start of the study period, SARS-CoV-2 variant period, SARS-CoV-2 vaccination status, and all investigated prescribed drugs (specified in section 3.9.3) separately. Age and time were modeled with restricted cubic splines with 4 knots set at the fifth, 35th, 65th, and 95th quantiles among individuals who experienced the investigated mortality outcome (171). The Kaplan–Meier estimates for 90-day survival and log-rank tests for individuals with and without CLL by age group (<65 years, 65–74 years, ≥75 years) were also presented.

To better understand the mortality across different populations, 18 predefined subgroups were used for the outcome analyses (<65 years, 65–74 years, ≥75 years, male sex, female sex, income quartile 1, income quartiles 2–4, born in Sweden, born abroad, unvaccinated, ≥2 vaccine doses, >3 doses, >4 doses, >5 doses, wild type period, Alpha period, Delta period, Omicron period).

Also, the secondary outcomes of COVID-19 hospitalization and ICU admission were presented as crude rates and adjusted RRs with 95% CIs. The same models and subgroup analyses were used as for mortality.

To further understand how mortality rates in individuals with CLL evolved from before the pandemic to the early pre-vaccination phase of the pandemic and onward, we also analyzed the crude cumulative incidence and age- and sex-adjusted relative risk of all-cause mortality on June 30 and December 31 for each year from 2017 through 2022. This was done for both individuals with and without CLL in Stockholm County. Cumulative incidence was estimated using the Aalen and Johansen estimator. Modified Poisson regression models adjusted for sex and age was used to obtain RRs (2019 as reference), using restricted cubic splines with 4 knots.

The characteristics of individuals with CLL who experienced vs did not experience 90-day all-cause mortality, COVID-19 hospitalization, and COVID-19 ICU admission were then compared within the nationwide cohort. Furthermore,

outcome rates, unadjusted RRs, and adjusted RRs were presented for three predefined exposures of interest: 1) treatment with BTKi, Bcl-2i or PI3Ki; 2) treatment with corticosteroids; 3) SARS-CoV-2 variant period. The COVID-19-related ICU admission rate was not included in these analyses because the number of individuals who experienced this outcome were considered too few for the adjusted analyses. BTKi/Bcl-2i/PI3Ki treatment, treatment with corticosteroids, age, residential region, anti-coagulant use, immunosuppressive drug use, number of prescribed drugs, time from CLL diagnosis to PCR test, SARS-CoV-2 vaccination status, and time since start of the pandemic were considered potential confounders and were therefore adjusted for in the adjusted analyses. SARS-CoV-2 variant period, biologic sex, region of birth, income quartile, antidiabetic drug use, antihypertensive drug use, heart disease drug use, lipid-modifier drug use, obstructive lung disease drug use, BTKi/Bcl-2i/PI3Ki use, and corticosteroid use were also adjusted for.

There were little missing data overall. Missing data was observed for region of birth (<1%), residential region (<1%), and income quartile (<1%). All other variables had complete data. An alpha level of .05 was used, and all data processing and analyses were performed using R version 4.3.1.

3.11 Ethical considerations

We obtained ethical permit for the gathering of clinical information from registers and medical files as well as for extra blood and saliva sampling prior to study start.

Patients and healthy controls participating in the translational studies included in this thesis (**Study I-III**) have received both oral and written information about the blood and saliva sampling associated with these studies, to be able to decide if they wanted to participate or not. All participants had to sign a written informed consent form before being included in these studies. The information included details about the purpose of the study, procedures and potential risks and benefits. They were also informed that they at any time-point could withdraw their participation. A study assistant (nurse) continually checked in with participants (via telephone) during the study, where they were able to ask questions, advertise any concerns or communicate their withdrawal from the study. However, it should be considered that extra blood sampling may cause pain and bruising. The amount of blood was limited to a maximum of 70 mL, and the amount was reduced and customized if we thought it could harm the patient

in any way. Another critical aspect of this, during the pandemic, was the fact that we didn't want study participants to be subjected to additional risk of infection, by having to make extra visits to the clinic for sampling. To avoid this, we tried, as often as possible, to combine the sampling with clinical controls at the hospital or with other scheduled testing.

The studies also included gathering of sensitive information from medical records. Only medical staff (doctors/nurses) involved in the patient's care and/or the study had access to medical records, and the patient's privacy was protected by using a pseudo-anonymized coding system, where only a limited number of researchers/clinicians involved in the project had access to the key.

The two register-based studies introduced a somewhat different ethical consideration. Here we handled a lot of sensitive data without giving the study subjects the chance to choose whether they wanted to participate or not, in fact, without them even knowing. However, we did this knowing that study subjects would be anonymized – due to the massive amount of data/study subjects (the entire Swedish adult population), the nature of the information collected, the handling of the data, and the fact that results were only presented on a group level – thus with a minimal risk of the study subjects being harmed in any way. Also, we believed that the results of the study would contribute to highly valuable knowledge, that would gain society, and hopefully even some of the study participants directly. In addition, it would be hard to achieve the same knowledge in any other way, and Swedish law permits such use of data from national registers without individual consent.

3.12 Ethical permits

Study I and III	Study I and III cont.	Study II
00-135	2019-05661	2021-00451
00-138	2020-01694	
2010_1478-32	2021_05579_02	Study IV-V
2013/952-31/3	2021-01107	2022-01793-01
2016_2506-32	2021-05579-02	2023-05877-02
2018_2565-32	2022-06064-02	

4 Results & Discussion

The following section will review the main findings from the five different studies (**Study I–V**), addressing 4 topics of interest regarding individuals with CLL throughout the COVID-19 pandemic: i) Clinical outcome of COVID-19; ii) Risk factors for severe COVID-19; iii) Post-infectious immune responses; and iv) Anti-SARS-CoV-2 vaccination.

4.1 Clinical outcome of COVID-19

(addressed in **Study I** and **V**)

As outlined in the background section, early international studies had shown poor COVID-19 outcome for CLL patients, at least if hospitalized (144,145). We wanted to study whether this was true also for consecutive patients in a well-defined region (Stockholm), including non-hospitalized patients with mild symptoms. In **Study I**, including 60 consecutive CLL patients infected with SARS-CoV-2 in Stockholm March 2020–March 2021, with a median age of 71 years (43–97), we show overall hospitalization rates of 77% (46/60). Of those admitted for COVID-19, 24% (11/46) were in need of intensive care, and the mortality rate was 28% (13/46) (*Table 1*). In total, there were 14 fatalities, where one patient was never admitted to hospital. One potential bias in our analyses, was that some patients in need of hospital or ICU care may never have been admitted, for example due to comorbidities, old age or poor prognosis in times of shortage of hospital beds. This could affect hospitalization and ICU admission rates in both study I and study V. Also, despite our efforts to include all SARS-CoV-2 infected CLL patients in the Stockholm region, as described in the Methods section, some patients may have gone undetected. Even though close to all CLL patients in the Stockholm region are closely managed at one of the three hematology clinics engaged in the study, we could not include patients who, for example, did not report symptoms, were asymptomatic, or never tested. The strength, however, is that even in the otherwise healthy general public of Stockholm, testing was freely and readily available, and commonly utilized, at this period (172), as recommended by WHO (155,173). Also, 5 patients in the **Study I** cohort were in fact diagnosed with CLL during hospital care for COVID-19. This reflects the hidden statistics of CLL patients not yet diagnosed, due to CLL's indolent and often overtly asymptomatic nature.

Furthermore, we saw numerical differences regarding mortality between the two time periods investigated. In the very first 6-month period, mortality rates for hospitalized patients were as high as 32% (6/19), compared to 26% (7/27) in the latter 7 months. ICU admissions were also numerically reduced, from 37% (7/19) to 15% (4/27). This improvement in clinical outcome with time could reflect a better understanding, and better management of severe COVID-19, which will be discussed in more detail in section 5.1. Of note, hospital admission rates however remained high also during month 7-13 (71% [27/38] vs 86% [19/22] month 1-6).

Table 1. Outcome and management of COVID-19 among CLL patients (n=60) in two time periods

	All patients	Admitted month 1-6 of the pandemic	Admitted month 7-13 of the pandemic
Hospital admission	77% (46/60)	86% (19/22)	71% (27/38)
ICU admission	24% (11/46)	32% (6/19) ^d	19% (5/27) ^d
Death	28% (13/46)	32% (6/19) ^c	26% (7/27) ^c
Intervention			
Supplemental oxygen	85% (39/46)	84% (16/19)	85% (23/27)
Non-invasive ventilation (incl HFNO ^a)	33% (15/46)	37% (7/19)	30% (8/27)
Mechanical ventilation	13% (6/46)	32% (6/19)	0% (0/27)
IV vasopressors	18% (8/45)	32% (6/19)	8% (2/26)
Hemodialysis	9% (4/46)	11% (2/19)	7% (2/27)

^a High flow nasal oxygen

^c overall death rate (including non-admitted patients) was 32% in month 1-6 and 18% in month 7-13 (not significant)

^d not significant (p=0.08)

(Adapted from Blixt L, et al. *Leukemia*. 2022;36:476–481. Reproduced with permission from Springer Nature)

In **Study V** we instead used a retrospective, epidemiological approach, using 10 national registries to compare the risk of hospitalization or death from COVID-19 in Swedish adults with vs without CLL. At the cost of detail and in-depth analyses, this approach provides a more comprehensive analysis of the proposed excess mortality among Swedish CLL patients during 3 years of the pandemic. From a population of 8,275,839 individuals born 1930–2003 (6,653 with a CLL diagnosis), we included 2,088,163 first SARS-CoV-2 infections (1,289 with CLL) during February 1, 2020, to February 27, 2023. The overall adjusted risk ratio (aRR) for 90-day all-cause mortality was 1.70 (1.49–1.93) in individuals with vs without CLL (*Figure 6*). The 90-day all-cause mortality rate

was 15.0% (n=193) in individuals with CLL and 1.2% (n=24,277) among individuals without CLL (Figure 7). The mortality rate for individuals with CLL was highest in the initial Wild-type period, 24.8% (vs 2.0%). In subsequent SARS-CoV-2 variant periods mortality rates were 17.2% (vs 0.5%) during Alpha, 4.1% (vs 0.6%) during Delta, and 12.6% (vs 1.0%) during Omicron period (Figure 7). Corresponding adjusted risk ratios were 2.38, 0.71, and 1.49, respectively (Figure 6). The overall aRR for hospitalization was 1.81, and for ICU admission 2.67.

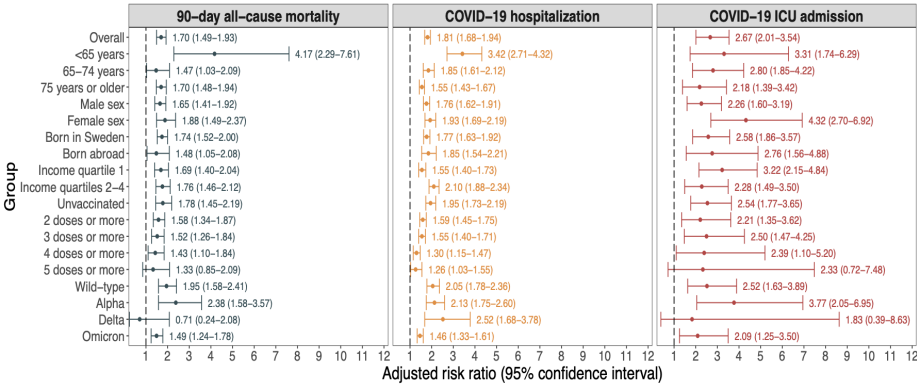


Figure 6. Forest plot of fully adjusted RRs for 90-day all-cause mortality, COVID-19 hospitalization, and COVID-19 ICU admission in individuals with CLL vs those without CLL.
(Reproduced from Hedberg et al. *Blood Advances*. 2025;9(13):3170-3181. CC BY-NC-ND 4.0)

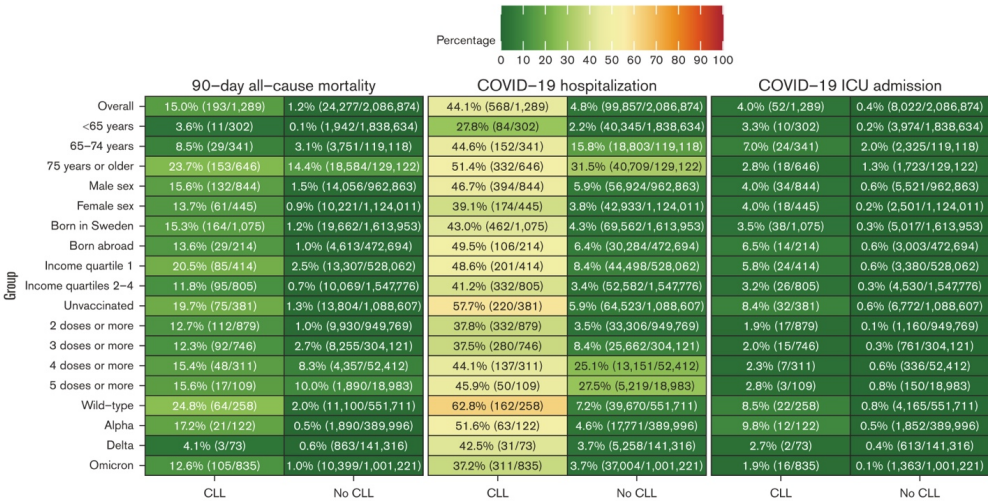


Figure 7. The 90-day all-cause mortality rates, COVID-19 hospitalization rates, and COVID-19 ICU admission rates in individuals with and those without CLL.
(Reproduced from Hedberg et al. *Blood Advances*. 2025;9(13):3170-3181. CC BY-NC-ND 4.0)

In **Study V** we also analyzed the cumulative incidence of all-cause mortality in a dataset based on individuals living in the Stockholm region (~2.4 million residents), widely affected by SARS-CoV-2 in early 2020, and for whom we had access to pre-pandemic data. In this cohort we compared all-cause mortality in individuals with vs without CLL by June 30 in the year of 2017–2022, as displayed in *Figure 8*. The cumulative incidence of all-cause mortality for individuals with CLL by June 30 was 3.02% in 2019 vs 4.67% in 2020 – an increase of 55%. The increase in mortality for the general population by June 30, 2020, compared to 2019, was 31% (0.55% vs 0.42%). The age- and sex-adjusted RR (95% CI) for all-cause mortality by June 30 in 2020 vs 2019 was 1.53 (1.09–2.15) for individuals with CLL ($p=0.02$) and 1.29 (1.25–1.33) for the general population ($p<0.001$).

By December 31, the cumulative incidence of mortality was 7.13% in 2019 and 8.04% in 2020 (+13%) for individuals with CLL and 0.82% in 2019 and 0.97% in 2020 (+18%) for the general population. Less increased cumulative incidence of mortality for individuals with CLL vs the general population by the end of 2020, as well as markedly lower mortality rate and aRR for all-cause mortality in CLL patients during the associated Alpha variant period (*Figure 6–7*), might reflect an increased risk-awareness leading to precautionary measures such as social isolation within the CLL community. However, the differences in mortality – both by aRRs, crude rates and cumulative incidence – validates a substantial excess mortality in individuals with CLL vs those without CLL during the pandemic.

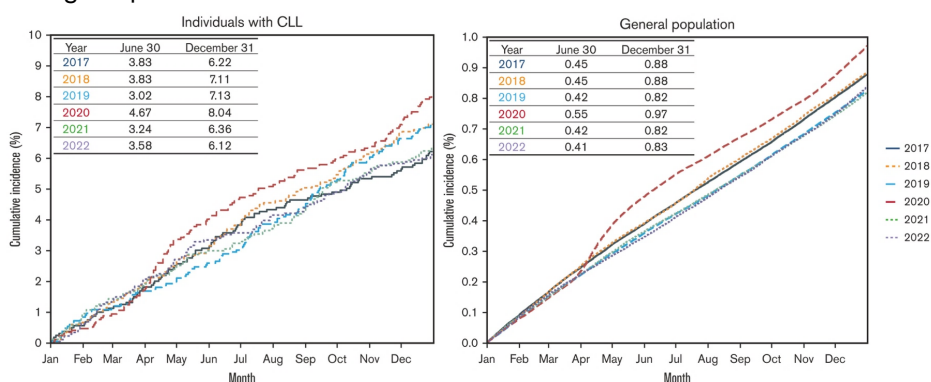


Figure 8. Cumulative incidence of all-cause mortality from 2017 through 2022 in individuals with and those without CLL in Stockholm County.

(Adapted from Hedberg et al. *Blood Advances*. 2025;9(13):3170–3181)

4.2 Risk factors for severe COVID-19

(addressed in **Study I** and **V**)

4.2.1 Age

Clinical characteristics of the **Study I** cohort (*Table 2*) show the median age of included patients was 71 years (43–97) and 65% were men, well reflecting the CLL patient group overall. Among the 14 fatalities in the cohort the median age was higher, 81 years, consistent with advanced age being a recognized risk factor for severe COVID-19 and death in the general population. Age ≥ 75 years was also one of two factors that reached statistical significance in both univariate and multivariate analysis, although statistical power was limited due to the rather small study cohort (n=60). However, the age range among fatalities was 43–97 years, thus including the youngest patient of the cohort, as well as the oldest.

Table 2. Clinical characteristics in relation to hospitalization, ICU admission and death

	All patients (n=60)	Requiring hospitalization (n=46)	ICU admission (n=11)	Death (n=14)
Median age, years (range)	71 (43-97)	71 (43-97)	71 (50-76)	81 (43-97)
≥ 75 y	32% (19/60)	35% (16/46)	18% (2/11)	57% (8/14)
Male	65% (39/60)	70% (32/46)	64% (7/11)	64% (9/14)
CIRS median (range) (n=60)	8 (4-20)	9 (4-20)	8 (4-17)	10 (4-20)
CIRS > 6	75% (45/60)	80% (37/46)	73% (8/11)	86% (12/14)
BMI, median (range)(n=49)	25 (19-42)	25 (20-42)	25 (23-30)	24 (20-30)
Diabetes	30% (18/60)	30% (14/46)	36% (4/11)	14% (2/14)
Never smoker	53% (31/59)	50% (23/46)	45% (5/11)	50% (7/14)
IgG (g/L), median (range)	7.9 (2.1-13.4)	8.3 (2.1-13.4)	8.3 (5.8-9.6)	5.4 (2.1-10.6)
CLL treatment at Covid-19 diagnosis (n=60)				
Never treated	63% (38/60)	63% (29/46)	55% (6/11)	64% (9/14)
Previously treated ^a	20% (12/60)	15% (7/46)	9% (1/11)	7% (1/14)
Ongoing therapy ^b	17% (10/60)	22% (10/46)	36% (4/11)	29% (4/14)
Current BTKi	70% (7/10)	70% (7/10)	50% (2/4)	75% (3/4)
CLL status at Covid-19 diagnosis (n=60)				
SD	63% (38/60)	69% (32/46)	64% (7/11)	64% (9/14)
CR/PR	25% (15/60)	22% (10/46)	27% (3/11)	36% (5/14)
PD	12% (7/60)	9% (4/46)	9% (1/11)	0% (0/14)

^a All >12 months ago

^b 1 patient recieved venetoclax; 1 patient recieved venetoclax + rituximab; 1 patient received bendamustin + rituximab; 7 patients received BTKi. |

(Reproduced with permission from Springer Nature. Blixt L, et al. *Leukemia*. 2022;36:476–481)

Consistently, in **Study V** the median (interquartile interval [IQR]) age among CLL patients who died within 90 days from COVID-19 diagnosis was higher than for patients who did not die within 90 days: 82 (75–86) years vs 73 (64–80) years. However, the aRR for 90-day all-cause mortality was highest in the youngest age group (<65 years) at 4.17 (*Figure 6*), probably reflecting fewer other risk factors in this age group.

4.2.2 Comorbidities

In **Study I**, Cumulative Illness Rating Scale (CIRS) was used to assess co-morbidity status, as previous studies had suggested conditions such as cardiovascular disease, kidney failure and diabetes as risk factors for severe outcomes from COVID-19 (145,173,174). Median CIRS of the **Study I** cohort was 8 (range 4–20), and patients with CIRS >6 accounted for 86% of fatalities (12/14). However, CIRS as a risk factor of death from COVID-19, is likely confounded by age, contributing to the statistically non-significant result in multivariate analysis. However, CIRS >8 vs ≤8 was significantly associated with hospitalization in both univariate and multivariate analysis.

4.2.3 CLL status

In **Study I** we tried to evaluate the impact of CLL status on COVID-19 outcomes, by applying the iwCLL response criteria on all included patients (N.B. also untreated patients). In this cohort 25% of patients were classified as being in remission (complete or partial), 63% had stable disease and only 12% (7/60) had progressive disease. Interestingly, none of the patients with progressive CLL died from COVID-19, while 64% of fatalities had stable disease and 36% were in remission. Due to the rather small and mixed cohort, reliable subgroup analyses could not be performed.

4.2.4 CLL treatment status

Within the **Study I** cohort 63% (38/60) had untreated CLL and this subgroup composed 64% (9/14) of fatalities. 20% (12/60) were previously treated, and only 17% (10/60) had ongoing CLL-directed therapy, of which 70% (7/10) were receiving a BTKi (*Table 2*). All patients with ongoing CLL-directed therapy were admitted to hospital and they accounted for 36% of CLL patients in ICU (4/11) and 29% of deaths (4/14). However, the small number of patients in each subgroup, as well as the variation of regimens received within the previously treated subgroup, did not allow for reliable subgroup analysis.

In **Study V**, we saw that patients with ongoing treatment with BTKi, Bcl-2i or PI3Ki had an increased risk of both death and hospitalization due to COVID-19 compared to CLL patients not treated with any of these drugs (aRR 1.56 [95% CI 1.03–2.37] and aRR 1.25 [95% CI 1.02–1.54], respectively) (*Table 3*). Possible causes for this difference might be associated to the immune modulating (or other) effects of the drugs themselves, or the fact that CLL patients in need of treatment can be assumed to have a more advanced stage CLL than untreated patients and thus likely more pronounced immune dysfunction, or both. Similar adjusted RRs were observed for corticosteroid treatment (1.45 [1.04–2.02] and 1.23 [1.05–1.44]). Since corticosteroids were also widely used to treat hospitalized (thus more severe) cases of COVID-19, only patients prescribed this drug prior to the time of COVID-19 diagnosis were considered.

Table 3. Hospitalization and 90-day all-cause mortality rates and RRs for individuals with CLL, by CLL treatment status and SARS-CoV-2 variant period

Variable	COVID-19 hospitalization			90-day all-cause mortality		
	Hospitalization rate % (n/N)	Unadjusted RR (95% CI)	Adjusted RR (95% CI)	Mortality rate % (n/N)	Unadjusted RR (95% CI)	Adjusted RR (95% CI)
Bcl-2i, BTKi, or PI3Ki*						
Not treated	42.6 (435/1022)	Ref	Ref	14.5 (148/1022)	Ref	Ref
Treated	49.8 (133/267)	1.17 (1.02-1.35)	1.25 (1.02-1.54)	16.9 (45/267)	1.16 (0.86-1.58)	1.56 (1.03-2.37)
Corticosteroids†						
Not treated	42.4 (488/1150)	Ref	Ref	13.8 (159/1150)	Ref	Ref
Treated	57.6 (80/139)	1.36 (1.16-1.59)	1.23 (1.05-1.44)	24.5 (34/139)	1.77 (1.28-2.45)	1.45 (1.04-2.02)
SARS-CoV-2 variant period‡,§						
Wild type	62.8 (162/258)	Ref	Ref	24.8 (64/258)	Ref	Ref
Alpha	51.6 (63/122)	0.82 (0.68-1.00)	0.85 (0.69-1.04)	17.2 (21/122)	0.69 (0.45-1.08)	0.63 (0.34-1.15)
Delta	42.5 (31/73)	0.68 (0.51-0.90)	0.86 (0.60-1.24)	4.1 (3/73)	0.17 (0.05-0.51)	0.14 (0.04-0.47)
Omicron	37.2 (311/835)	0.59 (0.52-0.67)	0.62 (0.46-0.84)	12.6 (105/835)	0.51 (0.38-0.67)	0.28 (0.13-0.61)

Ref, reference.
 *The adjusted model also included age, residential region, anticoagulant use, immunosuppressive drug use, number of prescribed drugs, time from CLL diagnosis to PCR test, COVID-19 vaccination status, and time since 29 January 2020.
 †The adjusted model also included age, residential region, anticoagulant use, immunosuppressive drug use, number of prescribed drugs, time from CLL diagnosis to PCR test, COVID-19 vaccination status, and time since 29 January 2020.
 ‡The adjusted model also included age, biologic sex, region of birth, residential region, income quartile, anticoagulant use, antidiabetic drug use, antihypertensive drug use, heart disease drug use, immunosuppressive drug use, lipid-modifier use, obstructive lung disease drug use, number of prescribed drugs, time from CLL diagnosis to PCR test, COVID-19 vaccination status, Bcl-2/BTK/PI3K inhibitor use, and corticosteroids use.
 §One individual with a SARS-CoV-2 Beta variant infection was excluded from these analyses.

(Reproduced from Hedberg et al. *Blood Advances*. 2025;9(13):3170–3181. CC BY-NC-ND 4.0)

4.3 Post-infectious immune responses

(addressed in **Study I** and **III**)

4.3.1 Seroconversion

In **Study I**, we were able to collect blood samples from 40 of the survivors of COVID-19 during the first pandemic year for immunological analyses. We found that the majority, 82%, had anti-SARS-CoV-2 antibodies above the seroconversion level at 3 months post infection. However, when we retrospectively quantified these serology samples in **Study III**, we found that

SARS-CoV-2-specific antibody levels were generally low at this point, with a median Spike-specific antibody titer of 47 U/mL (*Figure 10A*). The fact that patients who succumbed to the virus were not tested may bias the seroconversion rates. Similarly, the fact that a majority of patients had untreated CLL, whereas only a few had ongoing CLL treatment, could also induce bias.

In some patients, we were able to repeat the serology test 6 and 12 months post infection, showing lasting seropositivity in 77% (17/22) at 6 months and 73% (8/11) at 12 months of follow-up. No patients showed symptoms of re-infection with SARS-CoV-2, but considering the high transmission rates in the society at this time, boosted immunity from asymptomatic re-exposure to the virus cannot be excluded.

4.3.2 Neutralizing antibodies

Quantifying the neutralizing capacity of antibodies better reflects protectional immunity, compared to only numerical measures (175). In **Study I** we therefore applied a pseudo neutralizing assay to a subset of convalescents, demonstrating neutralizing capacity in 13/17 patient samples.

4.3.3 Antibodies in saliva

Another hypothesis is that antibodies in saliva might be a complementary, non-invasive way of measuring immunity, as well as an important element of reducing SARS-CoV-2 transmission (158,176). They might also represent a local immune response of the upper airways, that could play an important role as a first line defense when it comes to infectious microbes entering via the upper airways, such as SARS-CoV-2. Thus, as an experimental approach, we collected also saliva samples from convalescents in **Study I**. In these analyses we found that 68% (19/28) of patients were considered positive for anti-Spike saliva antibodies, one of which was not seroconverted. However, within this thesis we report only on saliva antibodies restricted to the IgG type, assumed to originate to a large extent from serum and may therefore rather reflect systemic immunity. This is also consistent with lower presence of SARS-CoV-2-specific saliva IgG in our CLL cohort compared to previous reports on immune competent individuals (158). To further investigate local mucosal immunity, we therefore applied a different strategy, measuring also Spike-specific IgA in saliva, in a subsequent study not included in this thesis (177).

4.3.4 T cell immunity

In other conditions with B cell dysfunction, such as patients with multiple sclerosis (MS) treated with anti-CD20 mAbs, the post-infectious T cell immunity was still intact (178). We wanted to investigate whether this might be the case also for CLL patients. In **Study I** we therefore performed an IFN- γ ELISpot assay to evaluate SARS-CoV-2-specific T cell reactivity. Among the 17 study participants subject to this analysis, 82% had a positive specific T cell activation, often, paralleled by seroconversion.

4.4 Anti-SARS-CoV-2 vaccination

(addressed in **Study II, III and IV**)

4.4.1 B and T cell vaccine response

In **Study II** we examined the SARS-CoV-2-specific T cell response to anti-SARS-CoV-2 mRNA vaccine BNT1622b2 at 2 weeks after the 2nd vaccine dose (day 35), in individuals with CLL vs healthy controls, and correlated this to antibody titers. Median age of the CLL cohort (n=52) was 70 years and 69% were men, compared to median age 52 years and 46% men in the control group (n=41). At day 35, 60% of CLL patients had seroconverted with median antibody titers of 24.6 U/mL (range 0.4–3,320) (*Table 4* and *Figure 9A*), compared to 100% seroconversion with median titers 2696 U/mL in healthy controls (range 766–14,269). In coherence, 29% of CLL patients had significant levels of memory T cells compared to non-exposed controls (*Table 4* and *Figure 9B*) vs 59% of healthy controls. Subgroup analysis of the CLL cohort showed highest seroconversion rates in previously CIT-treated patients (n=14) at 92%, followed by untreated patients (n=14) at 85% (*Table 4*). The lowest seroconversion rate was seen in patients with ongoing BTKi therapy (n=14) at only 14% (2/14). Patients on BTKi was also often double-negative, i.e. showed neither B nor T cell response to vaccination. Conversely, the highest frequency of T cell response was seen in patients previously treated with BTKi (n=10) where 70% had a positive T cell reactivity. Corresponding T cell response rates in the other subgroups were 29%, 14% and 14% in untreated patients, previously CIT-treated patients and patients with ongoing BTKi therapy, respectively (*Table 4* and *Figure 9C*). Although subgroup analyses are hard to interpret due to small sample sizes, this suggests a suppressing effect of BTKi on both humoral and cellular immunity during treatment, but perhaps over time a T cell restoring effect, that might not be seen after CIT treatment.

Table 4. Baseline characteristics and immunological results 2 weeks after the second dose (d35) of the mRNA BNT162b2 vaccine in relation to CLL patient subgroup

	Entire cohort n=52	Indolent untreated n=14	Previous CD20 mAb n=14	Previous ibrutinib n=10	Ongoing ibrutinib n=14
Age median (range)	70 (23 – 86)	71 (49 – 82)	71 (56 – 84)	71 (54 – 86)	70 (23 – 84)
Male sex n (%)	36 (69%)	6 (43%)	13 (93%)	6 (60%)	11 (79%)
Seroconverted d35 (n=48) n (%)	29/48 (60%)	11/13 (85%)	11/12 (92%)	5/9 (56%)	2/14 (14%)
Ab titres d35, U/ml* (n=48) median (range)	24.6 (0.4 – 3320)	81.6 (0.4 – 3320)	42.4 (0.4 – 1343)	35.5 (0.4 – 559)	0.4 (0.4 – 170)
ELISpot d35, SFU/10⁶ PBMC median (range)	10 (1 – 1096)	6 (1 – 526)	10 (1 – 490)	53 (2 – 1096)	5 (2 – 70)
Positive T cell response n (%)	12/52 (23%)	3/14 (21%)	2/14 (14%)	6/10 (60%)	1/14 (7%)

*Ab baseline = 0.4 U/ml; Seropositive >0.8 U/ml.

(Reproduced from Blixt and Wullmann, et al. *Haematologica*. 2022;107(4):1000.1003. CC BY-NC-ND 4.0)

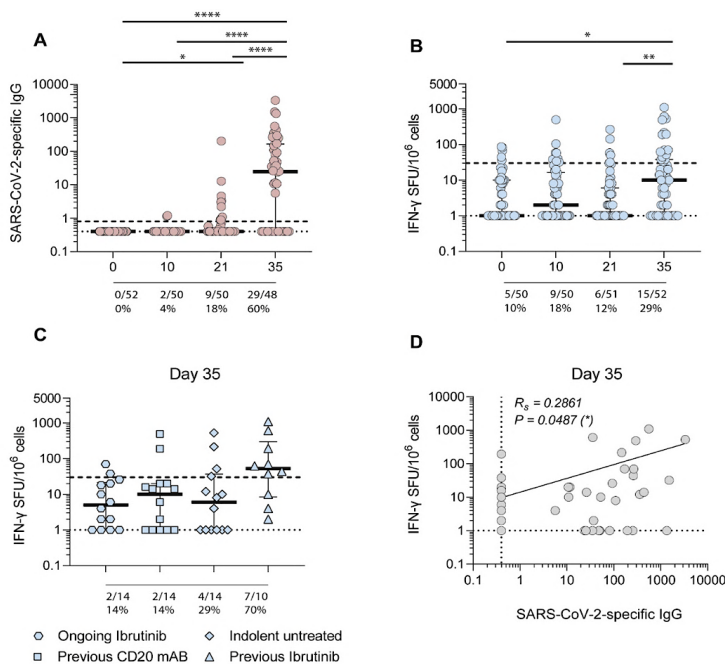


Figure 9. Humoral and cellular immune response to mRNA BNT162b2 vaccine in previously unexposed CLL patients. Longitudinal assessment (day 0, 10, 21, 35 post-vaccination) of SARS-CoV-2-specific IgG (A) and IFN-γ T cells (B) after spike glycoprotein stimulation, with summarized number and frequency of patients tested positive. Subgroup analysis of CLL patients at day 35 (C) and correlation day 35 (D). The dashed line indicates positive threshold for SARS-CoV-2-specific IgG and IFN-γ SFU/10⁶ cells, 0.8 U/ml and 30 SFU/10⁶ cells respectively. The dotted line represents the lower limit of detection of both assays. Each dot represents one patient. R_s = Spearman r value. Kruskal-Wallis test with Dunn's correction for multiple comparisons. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. (Reproduced from Blixt and Wullmann, et al. *Haematologica*. 2022;107(4):1000.1003. CC BY-NC-ND 4.0)

4.4.2 Hybrid immunity

Data from others have shown that a superior immunologic memory is achieved if post-infectious immunity is later boosted with vaccination, defined as hybrid immunity (179).

To investigate this in patients with CLL, we revisited our cohort of COVID-19 convalescents from study I, as they received anti-SARS-CoV-2 vaccination through rutin health care, as part of the Swedish SARS-CoV-2 vaccination program in spring of 2021. Out of our 40 COVID-19 survivors, 29 were eligible and willing to participate in **Study III**, in which patients who had been infected with SARS-CoV-2 March 2020 – March 2021 were sampled before vaccination start, after the 2nd vaccine dose and before and after the 3rd vaccine dose. Spike-specific and nucleocapsid-specific (representing vaccine- and virus induced immunity, respectively) serum antibodies were quantified, and T cell responses against wild-type (WuHu1) and Omicron (BA.1) SARS-CoV-2 were evaluated through flow cytometry-based activation-induced marker assay (AIM) and IFN-gamma ELISpot assay. Also, salivary IgG was analyzed at these timepoints.

Before vaccination, i.e. post-infection (0.25–3.75 months, median 3 months), median anti-Spike serum antibodies was 47 U/mL, increasing to 17,208 U/mL after the 2nd vaccine dose, decreasing to 6,825 U/mL between dose 2 and 3, and then increasing further to 24,956 U/mL after the 3rd vaccine dose (*Figure 10A*). In contrast, post-infectious anti-nucleotide antibodies remained rather stable throughout vaccination, with a decreasing trend over time. Spike-specific saliva antibodies followed a similar but slower kinetics (*Figure 10B*). A similar kinetics was also seen regarding T cell responses, with a robust CD8+ T cell activation after the 3rd vaccine dose (*Figure 10E*). To conclude, the results suggest robust, plateauing immune responses after repeated exposure including initial viral infection followed by booster vaccination, in CLL patients. However, though no patients showed symptoms of re-infection during this period, considering the high transmission rates in the society at this time, boosted immunity from re-exposure of the virus cannot be entirely ruled out. Additionally, the fact that a majority of patients had untreated CLL (72%), whereas only two had ongoing CLL treatment, may favor overall immune responses.

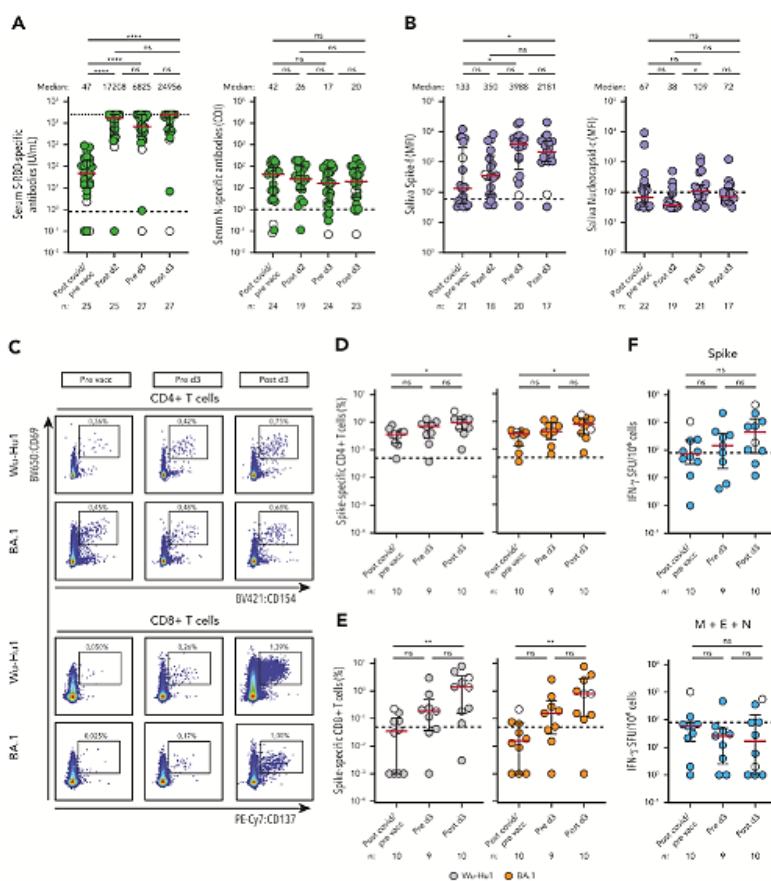


Figure 10. Time kinetics of humoral and cellular responses against spike and non-spike epitopes of SARS-CoV-2 in CLL patients after COVID-19 infection followed by 3 doses of mRNA vaccine. (Reproduced from Blixt L, et al. *Blood*. 2022;140(22):2403-2407. CC BY-NC-ND 4.0)

(A) Serum and (B) salivary levels of S (spike)-receptor binding domain (RBD) and N (nucleocapsid)-specific antibody responses in patients post-COVID or pre-vaccination, post-vaccination (dose 2), pre-vaccination (dose 3) and post-vaccination (dose 3) with indicated median values above graph. (A) Assay cut-offs are shown as dashed lines. The assay specific limit of detection for S-specific antibodies is 25.000 U/ml (small-dotted line). (C) Spike-specific CD4+ (CD69+CD154+) and CD8+ (CD69+CD137+) T cells were detected by flow cytometry (AIM assay). Spike-specific CD4+ (D) and CD8+ (E) T cell response before and after vaccination, against wildtype and Omicron. (F) ELISpot IFN- γ -specific T-cell responses to spike and membrane, envelope and nucleocapsid (M+E+N) peptide pools. Data points in (D), (E) and (F) represents peptide pool stimulated values after subtraction of paired negative controls. Thick dotted line represents positive threshold for each assay: (A) 0.8 U/ml, (B) MFI of 59 and 100 for spike and NC respectively, (D) and (E) 0,05% and (F) 80 SFU/106 cells. Error bars represent median (red line) and interquartile range. Comparisons between time points was performed by Kruskal-Wallis test with Dunn's correction for multiple comparisons.

*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. ns, not significant.

(Reproduced from Blixt L, et al. *Blood*. 2022;140(22):2403-2407. CC BY-NC-ND 4.0)

4.4.3 Vaccine uptake among Swedish CLL patients

In 2023, there were thus findings supporting improved immunity with repeated antigen exposure in CLL patients. However, data on the uptake of anti-SARS-CoV-2 vaccines in Swedish CLL patients was lacking. This gap of knowledge is addressed in **Study IV**, where 6 national registries were used to explore the uptake of COVID-19 vaccine among individuals with CLL in Sweden compared to the age-standardized nationwide uptake, as well as identify any subgroups of patients with poor vaccine uptake. In total, 6304 Swedish CLL patients were included. In individuals diagnosed with CLL before the start of the vaccination program (December 27, 2020) (n=5144), the cumulative vaccine uptake at the end of follow-up (February 28, 2023) was generally high, but declining over time. The overall uptake was 95% and 94% for dose 1 and 2, respectively, 88% for dose 3, 78% for dose 4, and 56% for dose 5 (*Figure 11*). This was higher than reported in a previous British publication, with 51% having received four doses, even though people with CLL had the highest vaccination coverage of all groups of hematologic malignancies (180). In our study, 253 patients were unvaccinated at the end of follow-up. No significant differences in vaccine uptake were observed in subgroups based on time since CLL diagnosis (>5 years vs ≤5 years before vaccination) or whether CLL had been treated or not. However, some concerning discrepancies were seen in subgroups based on origin (born in or outside of Sweden) and income quartile. These discrepancies were most pronounced when combining these two factors, where individuals born outside of Sweden in the lowest income quartile had a vaccine uptake of only 66%, 49% and 24% for the 3rd through 5th dose, respectively (*Figure 12*). This might reflect a lack of trusted and/or accessible information within this group of patients, perhaps as a result of linguistic and/or cultural barriers. A tendency to lower vaccine uptake in the youngest age group (18–64 years) was also observed (*Figure 11*), possibly due to unawareness of their exposure in the absence of the recognized risk factor of old age. Dose 4 was available from January 21, 2022, and dose 5 from August 15, 2022, but made available gradually for various subgroups of Swedish citizens and depending on time since prior vaccine doses. Considering that end of follow-up for the study was 28 February 2023, substantial additional uptake of dose 5, and even dose 4, is likely to have occurred after this date. Even so, these results indicate an overall decline in vaccine uptake over time and identify low income and non-Swedish origin as potential risk factors for inadequate vaccination.

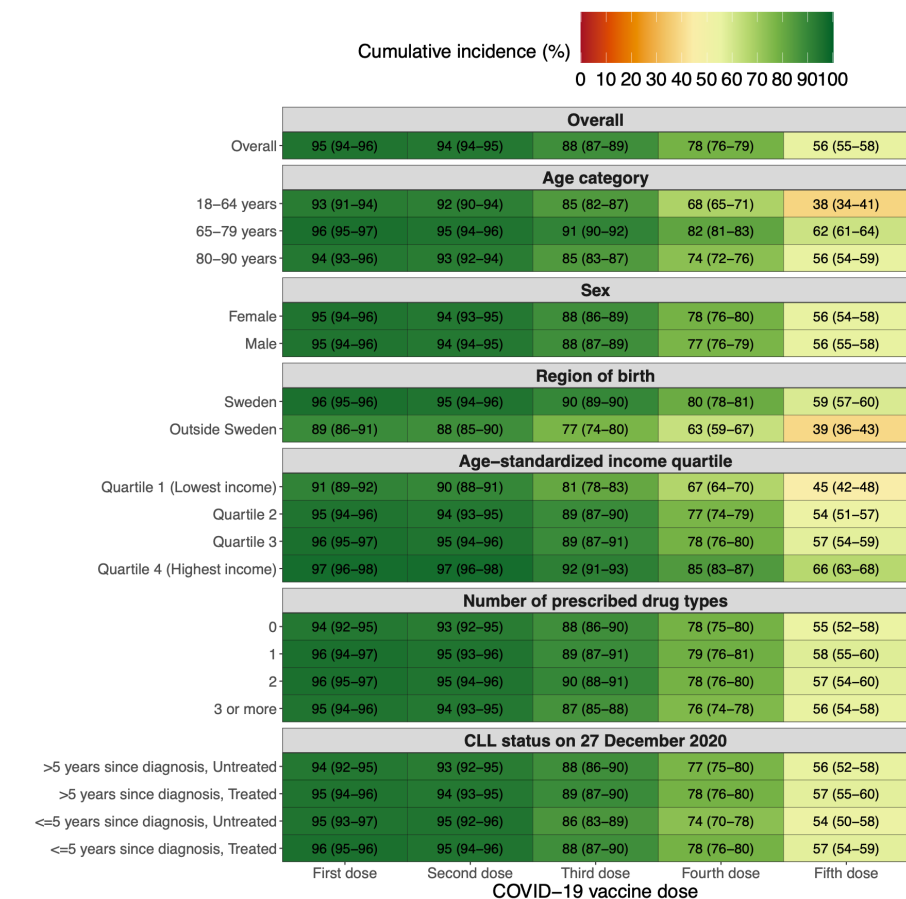


Figure 11. Cumulative incidences of uptake of first to fifth anti-SARS-CoV-2 vaccine dose at the end of follow-up by each independent variable.
(Reproduced from Hedberg et al. *eJHaem*. 2025;6:e1077. CC BY-NC-ND 4.0.)

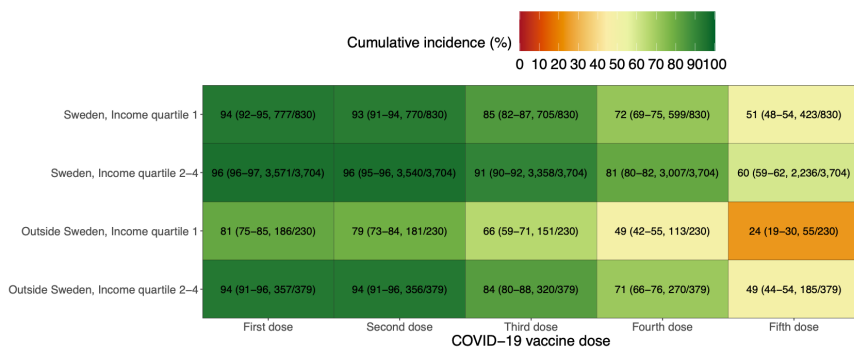


Figure 12. Cumulative incidences of first to fifth anti-SARS-CoV-2 vaccine dose at the end of follow-up in the four intersectional strata combining region of birth and income quartile.
(Reproduced from Hedberg et al. *eJHaem*. 2025;6:e1077. CC BY-NC-ND 4.0.)

5 Additional remarks and considerations

5.1 Management of severe COVID-19

In the early days of the pandemic the knowledge and experience of treating severe COVID-19 was naturally limited but evolved rapidly. This is pictured in **Study I**, where we compare treatment and outcome of hospitalized patients during the first 6 months of the pandemic in Stockholm, vs the subsequent 7 months. We saw that fatality rates for CLL patients admitted for severe COVID-19 decreased from 32% to 26%, while the use of anti-inflammatory treatment with corticosteroids increased from 47% to 78% (*Table 2*). Furthermore, antiviral therapy, initially with Remdesivir, was applied during the latter period, resulting in an increase in its use from 5% to 41% between these two time periods. It was also established that severe COVID-19 was associated with increased risk of thrombosis, and the prophylactic use of anticoagulation was widely implemented throughout Sweden. In parallel, other proposed COVID-19 directed and/or inflammation moderating therapies, such as tocilizumab and hydroxychloroquine were abandoned. This development in treating severe COVID-19 is likely to have influenced overall death rates in hospitalized patients.

5.2 CLL as a risk factor of poor outcome

As previously discussed, CLL and the immune dysregulation that comes with the disease, has in itself been demonstrated as a risk factor for severe infections and death from infections. This risk is proposed to increase with time from diagnosis and degree of disease activity (181,182). However, to identify possible confounders in this often aged, co-morbid, perhaps therapeutically immunosuppressed patient group is not an easy task. In **Study V**, we however show a sustaining increased risk ratio for severe COVID-19 and death after adjusting for many of these variables, such as age, gender, co-morbidities, etc. Correspondingly, we see the highest risk ratio in the youngest, presumably least confounded, age-group (<65 years), supporting this principle.

5.3 Implications of CLL therapy on clinical outcomes and immunity

Due to its entirely consecutive "all-inclusive" nature, and rather small number, the cohort of **Study I** was not eligible for subgroup analysis regarding patients' CLL treatment status, and poor clinical outcomes was seen in both untreated and treated patients. However, in **Study II** as well as **Study V**, ongoing treatment with BTKi stands out as a factor associated with both poorer B and T cell

response to vaccination and poorer clinical outcome from infection, respectively. Conversely, patients who had stopped BTKi therapy due to CLL remission showed superior vaccine (T cell) response in **Study II**. Even so, **Study V** does not offer a complete picture of the various CLL directed therapies' effect on clinical outcome since registry data was only available for prescribed (oral) treatments.

5.4 Impact of vaccination status and measurable immune responses on clinical outcome

There are evidence correlating serum antibody levels with clinical outcome, at least in immune competent populations (183,184). The correlation between T cell reactivity and infectious protection is however more unclear. In this thesis, there are some results that might support the clinical benefits of vaccination, even in patients with CLL – despite their generally low, or at least slow, measurable immune response to vaccination. In **Study V**, we see gradually decreasing risk ratios for hospitalization and death with each vaccine dose received, vs comparators with matched vaccination status. The aRR for 90-day all-cause mortality was 1.33 for CLL patients having received 5 doses or more of anti-SARS-CoV-2 vaccine, compared to 1.78 for unvaccinated patients.

5.5 Measuring T cell immunity

The IFN-gamma ELISpot assays mainly used to evaluate SARS-CoV-2 T cell activation within this thesis is far from a comprehensive method to understand the complex T cell activation mechanisms following infection or vaccination. For one, IFN-gamma is far from the only cytokine involved in the signaling cascade forming T cell fate, and by restricting our analysis to this one cytokine we have likely underestimated the T cell reactivity. Likewise, T cells are not the only immune cells secreting IFN-gamma – for example NK cells, as part of the early native immune response, also use IFN-gamma to activate macrophages and induce antigen presentation. A recent review on the current knowledge regarding mechanisms of T cell activation highlights the complex spatiotemporal dynamics of T cell signaling pathways and cross-regulation. Advances in imaging, single cell multi-omics approaches and mechanobiology have enabled new ways of analyzing T cell activity, underlining the insufficiency of traditional in vitro based models to effectively represent physiological circumstances. However, it also recognizes the lack of standardized frameworks and reproducibility within these methods as well as a persisting gap between experimental approaches and clinically applicable and significant methods (185).

6 Conclusions

Individuals with CLL experienced a higher risk of severe COVID-19 and increased death rates compared to the general population during the COVID-19 pandemic. This risk could be further aggravated by CLL-directed therapy, even with novel targeted therapies. Furthermore, CLL patients show lower B and T cell response to anti-SARS-CoV-2 vaccination, with slower kinetics, than seen in healthy controls. BTKi therapy appears to have a negative effect on both B and T cell immune responses during active treatment. Immune responses can however be reinforced by repeated vaccination and seem to have a positive effect on clinical outcome for these patients. However, information about the benefits of vaccination needs to be more widely spread in the community, and measures should be taken to ensure this information reaches all patient groups.

7 Points of perspective

7.1 Continued vaccination

Despite research emphasizing the importance of booster vaccination in CLL patients we currently see decreasing anti-SARS-CoV-2 vaccine uptake in the general population as well as CLL patients, both in Sweden and internationally. A Swedish report, combining questionnaires and data from medical files on 450 individuals in the north of Sweden from the risk groups defined by PHAS, recently showed that the coverage of the recommended yearly vaccination against influenza and COVID-19 was only 52% and 54%, respectively. Male sex, younger age and smoking were strong negative determinants of vaccine uptake in this study. In fact, in individuals <65 years the vaccine uptake was only 20% and 19% for influenza and COVID-19, respectively. Comparatively, the national coverage among individuals ≥65 years was 69% for influenza and 70% for COVID-19 (186). This indicates that despite national recommendations and free vaccination programs, important barriers to vaccination, such as low risk-awareness or vaccine hesitancy, persists. Additionally, an American study on longitudinal trends in vaccination among CLL patients recently reported declining uptake of several recommended vaccines from 2020 to 2025. According to this study, SARS-CoV-2 vaccination rates peaked in 2022 at 80% but had dropped to 48% in 2024 and 2025. Influenza vaccination rates were 92% in 2020 but declined to 67% in 2025 (187). At the same time, booster vaccination continue to show strengthened immune responses and protection from severe COVID-19 in CLL patients (188).

Hence, we plan another registry-based study on vaccine uptake among CLL patients in Sweden, including SARS-CoV-2, influenza and respiratory syncytial (RS) virus vaccines. The aim is to describe the rates of uptake of these vaccines, as well as the dynamics in vaccine uptake rates, from 2020 onwards. Also, we would like to associate vaccine uptake to clinical outcome and patient characteristics. By doing so we hope to be able to further improve vaccination campaigns and information accessibility for individuals with CLL.

7.2 Effects of modern targeted therapies on immune function

Modern targeted therapies are swiftly winning ground in the CLL therapeutics arsenal. Treatment with BTKi and/or Bcl-2i +/- anti-CD20 mAbs is now standard of care for CLL and upgrades of these targeting agents are already in use, such

as the covalent BTKi pirtobrutinib. An open-label, multicenter, randomized phase 3 trial published in *The Lancet* in the summer of 2026 showed superior PFS for patients receiving venetoclax (Bcl-2i)-rituximab (anti-CD20 mAbs) (VR) plus pirtobrutinib, vs patients receiving only VR (24-month PFS 87% vs 72%) (189). However, the triple combination including pirtobrutinib also showed slightly higher rates of infectious adverse events compared to the VR regime ($\geq$ grade 3 in 34% vs 23%). In parallel, a recently published prospective observational study on prolonged viral shedding in hematologic malignancies, including 31 CLL patients, showed prolonged COVID-19 (positive rRT-PCR with Ct <35 for >30 days) in 45% of patients (n=348,) with a median duration of 58 days. The proportion of CLL patients with prolonged infection was 58% (18/31). Active treatment with bispecific antibodies, mAbs, BTKi and immunosuppressive drugs for GvHD was found to be significantly associated with prolonged viral shedding, as well as absent vaccination or fewer booster vaccine doses. 56% of patients (n=156) further presented with declining and relapsing symptoms and fluctuant viral load. Furthermore, persistent COVID-19 resulted in interrupted or indefinitely suspended hematologic treatment in 55% and 20% of patients, respectively. Additionally, viral intra-host mutations across the entire viral genome, predominately within the spike region, were detected in most patients with prolonged COVID-19 (190). This highlights the importance of considering the immune modulating effects of modern therapies, as well as the many ways in which immune function may affect CLL treatment and outcome.

7.3 Mucosal immunity

Approximately two thirds of all lymphoid cells are located in the mucosal tissues, of mainly the upper respiratory tract including nose and mouth, and the intestines (191,192). Any airborne pathogen, such as SARS-CoV-2, will first encounter these mucosal immune cells and their response might prevent the virus to even enter the body, or at least restrain it to the upper airways. Local germinal centers and resident plasma cells can produce dimerized specific IgA, secreted through epithelial cells, contributing to the mucosal pathogen barrier. Local IgA production can be quantified as secretory IgA (sIgA) (193).

The interest in mucosal immunity is increasing, due to the proposed significant role for mucosal and secretory immunity in protection against and transmission of infectious diseases, as well as its potential for new diagnostic, therapeutic or prophylactic approaches (194,195).

The role of immunoglobulin deficiency in mucous membranes has not been meticulously investigated in CLL. However, some early studies indicate decreased levels of IgM antibodies in saliva as well as low levels of serum IgA in CLL patients (196). In a previous publication, not included in this thesis, we tried to describe local mucosal immunity in CLL patients in a study including 38 CLL patients of various CLL treatment status and with PCR verified SARS-CoV-2 infection during the Omicron BA.1/BA.2 time period. Most patients (79%) had received 3–4 vaccine doses. Patients showed low levels of convalescent IgA responses in both saliva and serum. Overall, salivary Spike-specific IgA was only detected in 40% (10/25) of patients and even less frequently detected in patients with ongoing BTKi or Bcl-2i therapy (2/12), and with lower magnitude, compared to early-stage untreated patients (6/9) (177).

In future studies, we wish to elaborate further on mucosal immunity in CLL patients, for example by also analyzing sIgA in patients recovering from respiratory tract infections.

7.4 Assessing immune competence

A central challenge when advising CLL patients about precautionary measures to prevent infections, is the so far limited ways to assess immune function in clinical praxis. In the future I hope we will have access to more precise, and readily available, tools to make proper, individualized risk stratifications. A systematic review suggesting such a clinical risk stratification framework for patients with secondary immune deficiency, such as hematology patients, was recently published in *Frontiers in Immunology*. This review presents three immunological pathways essential for immune deficiency in these individuals – humoral dysfunction, cellular disruption, and innate immune dysregulation – and suggests an exploratory, conceptual model for risk scoring considering immunoglobulin levels, infection history, immunosuppressive therapy and underlying host factors (such as functional antibody response). This aims to allocate the patient to a risk tier (low, moderate or high risk) and implement tier-appropriate management, for example immunoglobulin replacement therapy (197). Such risk scores, once properly validated, could be most helpful in the daily clinical work with immunocompromised individuals like CLL patients.

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9 Declaration about the use of generative AI

The AI-assisted tool ChatGPT (OpenAI, GPT-5.6) was used for language refinement purposes of the comprehensive summery, and in the Abstract section to condense the text. It was also used to generate the cover illustration.

No scientific content, data interpretation or conclusions were generated by the AI tool.

I take full responsibility for the content of the comprehensive summery of the thesis.

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