

Immune Dysfunction and Infection – Interaction between CLL and Treatment: A Reflection on Current Treatment Paradigms and Unmet Needs

Ernesto Gargiulo^{a,b} Rebecca Svanberg Teglgaard^a Tereza Faitová^a
Carsten Utoft Niemann^{a,c}

^aDepartment of Hematology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark;

^bDepartment of Infectious Diseases, PERSIMUNE, Rigshospitalet, Copenhagen, Denmark; ^cDepartment of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

Keywords

Chronic lymphocytic leukemia · Immune dysfunction · Infections · Preemptive treatment

Abstract

Background: Chronic lymphocytic leukemia (CLL) is a hematological malignancy characterized by immune dysfunction, which significantly contributes to increased morbidity and mortality due to infections. **Summary:** Advancement in therapeutic strategies based on combination chemoimmunotherapy and targeted treatment have increased life expectancy for patients affected by CLL. However, mortality and morbidity due to infection showed no improvement over the last decades. Although therapy options are highly efficient in targeting leukemic cells, several studies highlighted the interactions of different treatments with the tumor microenvironment immune components, significantly impacting their clinical efficacy and fostering increased risk of infections. **Key Messages:** Given the profound immune dysfunction caused by CLL itself, treatment can thus represent a double-edged sword. Thus, it is essential to increase our understanding and awareness on how conventional therapies affect the disease-microenvironment-infection axis to ensure the best personalized strategy for each patient. This requires careful consideration of the advantages and disadvantages of efficient

treatments, whether chemoimmunotherapy or targeted combinations, leading to risk of infectious complications. To this regard, our machine learning-based algorithm CLL Treatment-Infection Model, currently implemented into the local electronic health record system for Eastern Denmark, aims at early identification of patients at high risk of serious infections (PreVent-ACall; NCT03868722). We here review strategies for management of immune dysfunction and infections in CLL.

© 2023 The Author(s).
Published by S. Karger AG, Basel

Introduction

Chronic lymphocytic leukemia (CLL) is a hematological malignancy characterized by immune dysfunction, which significantly contributes to increased morbidity and mortality due to infections [1, 2]. While advancements in therapeutic strategies have improved overall survival (OS) rates for CLL patients, the risk of infection remains a major concern. Treatment options, such as combination chemoimmunotherapy (CIT) and targeted treatments, have demonstrated efficacy in targeting leukemic cells [3]. However, these therapies can also interact with the tumor microenvironment and immune components, compromising their clinical effectiveness and increasing the

susceptibility to infections [4, 5]. Considering the inherent immune dysfunction caused by CLL itself, treatment becomes a double-edged sword. To ensure optimal personalized strategies for CLL patients, it is crucial to understand how conventional therapies impact the disease-microenvironment-infection axis. In this context, a machine learning-based algorithm called CLL Treatment-Infection Model (CLL-TIM) has been developed to identify high-risk patients for serious infections [6]. This review aims to explore management strategies for immune dysfunction and infections in CLL, highlight the foundations of infectious risk in CLL, and discuss the impact of current treatment options on infection susceptibility. By addressing these issues, we can improve patient outcomes and enhance the quality of life for individuals living with CLL.

Foundations for Infectious Risk in CLL

At the core of CLL, immune dysfunction is caused by a plethora of molecular factors, which enhance tumor cell proliferation and survival, while re-educating the surrounding tumor microenvironment into a less protective state [1, 3, 7]. In primis, CLL patients' high risk of infections is due to the decreased immune surveillance caused by intrinsic disease features [8]. The alteration of the B-cell compartment – majorly skewed toward malignant CLL B lymphocytes [9] – weakens the adaptive immunity and, thus, the functions of other immune cells like T lymphocytes. Even decades before leukemia diagnosis, monoclonal B-cell lymphocytosis, a pre-CLL stage, shows an increased monoclonal B-cell population in the peripheral blood (PB; <5,000 monoclonal B cells/mL) [9, 10]. Lymphocytosis, given the slow but progressive accumulation of CLL cells in the PB, worsens with disease progression (>5,000 monoclonal B cells/mL), leading to invasion of lymphoid organs [11], a general immune surveillance impairment (e.g., hypogammaglobulinemia [12]), ultimately increasing the risk of infections in patients affected by CLL [2, 13]. Further impact on immune response coordination is given by the considerable alteration in the antigen-presenting cell compartment, due to reduction of the complement-activating glycoprotein properdin in PB [14], binding and expression of the complement receptors 1 and 2 (CR1 and CR2) [15, 16], and further reduction of the major histocompatibility complex-II on dendritic cells [17]. Moreover, CLL cells can directly affect effector immune cells (e.g., CD8⁺ T and natural killer cells [18]) through immune checkpoint interactions [19], soluble components (including extracellular vesicles) [20], and indirectly by regulating modulatory cells, such as Treg [19, 21]. In

combination with an altered lymphoid compartment, myeloid cells, have been described to either directly support CLL cell survival, proliferation, and migration (e.g., neutrophils and monocyte-derived cells) [22–24], or having an impaired phagocytic potential [25]. A schematic summary can be found in Table 1.

Microbiome research represents an increasingly studied area with a potential to indirectly influence immune system as well as efficacy of immunotherapies in cancers and hematological malignancies [26]. Interestingly, disease-associated immune dysfunction is not the only risk factor for infections in patients affected by CLL. Indeed, gut microbiome (GMB) pathological alterations, known as dysbiosis, have been implicated in human diseases, playing a critical role by influencing host immune response, protection against pathogen overgrowth, biosynthesis, and metabolism [27]. Short-chain fatty acids, end products of anaerobic intestinal bacteria-mediated fermentation of dietary fibers, have been shown to promote naive T-cell differentiation into T helper (Th)1, Th17, or Treg [28]. Furthermore, tumor-associated chronic inflammation sustained by altered cytokine levels have been shown to be correlated with dysbiosis [29]. Finally, we recently compared the GMB's between patients with CLL and healthy control populations, observing a consistent dysbiosis in the CLL group, suggesting a link between gut dysbiosis and immune dysfunction in CLL pathogenesis [30]. Thus, given the GMB ability to influence the immune system components (e.g., macrophage, dendritic cells, and different T-cell subsets), its role in enhancing CLL-associated immune dysfunction warrants further investigation. The importance of addressing immune dysfunction in CLL is further emphasized by the increased risk of common bacterial infections like invasive pneumococci [31], COVID-19 infections [32], and even the increased need for antimicrobial prescriptions decades prior to diagnosis of CLL [33]. Despite the immune dysfunction of CLL leading to increased risk of infections, morbidity, worsened quality of life, and increased mortality, data on the interaction of CLL treatment with CLL immune dysfunction are currently lacking, while evidence for preventive measures are warranted.

Infections upon Current Treatment Options

In the last decades, CLL treatment options have improved specificity by targeting leukemic cells while reducing off-target effects. Despite these improvements, targeted treatment regimens still lead to immune suppression in CLL (Table 2) [4, 5].

Table 1. Immune dysfunctions and related consequences fostering infection risk

Disease stage	Immune dysfunction	Consequences	Infection risk
MBL (<5,000 B-cell/mL)	Progressive lymphocytosis	Progressive accumulation of premalignant B cells in PB	General increase in fungal, bacterial, and viral infections
CLL (>5,000 B-cell/mL)	Lymphocytosis	Progressive accumulation of malignant B cells in PB and lymphoid organs	Increased risk of infections (e.g., <i>Streptococcus pneumoniae</i> , <i>Haemophilus influenza</i> and viral infections)
	Hypogammaglobulinemia	Further reduction of immune surveillance impairment	
	Reduced DC functionality Reduction of functional B cells	Reduced adaptive immunity and, by consequence, cytotoxic T-cell activity	
	Reduced cytotoxic T-cell functionality and migration Reduced NK functionality	Reduced adaptive immunity and immune surveillance	
	Increased Treg amount and functionality	Reduced cytotoxic T-cell activity	
	Myeloid cell compartment	Reduced phagocytic potential. Increased supporting signals for CLL survival, proliferation, and migration	

CLL progression is associated with multiple immune dysfunctions, considerably increasing the risk of infections. A more focused overview of immune dysfunctions correlated to CLL stages (pre-CLL, "watch and wait," and during treatment) and relative infections can be found in Gargiulo et al. [4]. MBL, monoclonal B lymphocytosis; DC, dendritic cells; NK, natural killer (cells); Treg, regulatory T-cells; PB, peripheral blood.

Chemoimmunotherapy

In an era dominated by increasing use of targeted agents for CLL, CIT remains a fixed-duration treatment option for patients with mutated IGHV and no TP53 aberrations in the first-line setting, as well as the only treatment option for a number of health systems [34]. Fludarabine combined with cyclophosphamide and rituximab (FCR) are favored for patients <65 years of age due to achieving durable remissions, while bendamustine-rituximab is preferred for elderly fit patients due to lower risk of infections [35, 36]. In line with this, the phase 3 clinical trial GAIA/CLL13 (NCT02950051), assessed CIT versus targeted combinations based on venetoclax and CD20 antibodies for fit CLL patients. Superior efficacy in achieving undetectable minimal residual disease and improved progression-free survival (PFS) was demonstrated for venetoclax-obinutuzumab combinations [37, 38]. However, for mutated IGHV patients, comparable efficacy of CIT regimens was demonstrated, while

triplet combinations with venetoclax-obinutuzumab-ibrutinib resulted in the highest frequency of infections (grade ≥ 3). Despite its efficacy, fludarabine-based regimes are associated with severe and opportunistic infections like *Pneumocystis jirovecii*, *Listeria monocytogenes*, mycobacteria, and cytomegalovirus (CMV) [39], likely related to, in addition to neutropenia, significant and sustained reduction, and/or dysfunction of CD4 $^{+}$ T-cells [40].

Importantly, high-grade infections (grade ≥ 3) are more often observed in previously treated patients [41–43]. Indeed, in the CLL8 trial (NCT00281918), fungal and opportunistic infections were rare in treatment naïve (TN) patients receiving FCR despite grade 3–4 neutropenia observed in one third of patients [44], in the setting of pneumocystis prophylaxis implemented for most patients. Thus, while neutropenia and lymphopenia entail increased risk of infections, having received previous treatment, especially with an alkylating agent, significantly worsens the risk of severe

Table 2. Summary of CLL treatment-related immune dysfunction per clinical trial

Drug class	Drug	Immune dysfunction	Infections	CLL clinical trial (identification)
BTKi	I, Ac	Th-cell count ↗, B-cell functions ↗, NK, monocyte, and macrophage functions ↘	<i>Aspergillus</i> , <i>Cryptococcus</i> , and <i>Pneumocystis jirovecii</i> Urinary tract and upper respiratory system infections, pneumonia	GAIA/CLL13 (NCT02950051); GLOW (NCT03462719); ELEVATE-TN (NCT02475681); CLARITY (NCT02267590); HOVON141/VISION (NCT03226301); AVO (NCT03580928); CLL12 (NCT02863718); PreVent-ACaLL (NCT03868722); ELEVATE-RR (NCT02477696); ALPINE (NCT03734016); CLL2-GIVE (NCT02758665)
BCL-2i	V	Neutropenia	<i>Aspergillus</i> pneumonia, herpes pharyngitis, and candida esophagitis Urinary tract and upper respiratory system infections, pneumonia, febrile neutropenia	GAIA/CLL13 (NCT02950051); GLOW (NCT03462719); NCT01889186; MURANO (NCT02005471); CLL14 (NCT02242942); HOVON141/VISION (NCT03226301); AVO (NCT03580928); EVOLVE (NCT04269902); PreVent-ACaLL (NCT03868722); CLL2-GIVE (NCT02758665)
PI3Ki	Id, D	T-cell, neutrophil, and macrophage functions ↘	<i>Streptococcus</i> pneumonia Urinary tract and upper respiratory system infections	DUO (NCT02004522)
Purine analogs	F	T-cell count ↘, neutropenia, macrophage functions ↗, and hypogammaglobulinemia	<i>Pneumocystis jirovecii</i> , <i>Listeria monocytogenes</i> , <i>Mycobacteria</i> , CMV, and herpes Urinary tract and upper respiratory system infections	GAIA/CLL13 (NCT02950051); CLL8 (NCT00281918); CLL10 (NCT00769522)
Alkylating agents	C, B, Ch	T-cell count ↘ and neutropenia	Atypical mycobacteriosis Urinary tract and upper respiratory system infections	GAIA/CLL13 (NCT02950051); CLL8 (NCT00281918); NCT01871675; MURANO (NCT02005471); CLL14 (NCT02242942); ELEVATE-TN (NCT02475681); CLL10 (NCT00769522)
Monoclonal antibodies	R, O, Ob	Neutropenia, leukocytopenia, T-cell, monocyte, macrophage, and eosinophil count ↘	Sepsis, pneumonia, multifocal encephalitis, pneumonia, herpes simplex and zoster, <i>Pneumocystis jirovecii</i> , CMV, aspergillosis, and listeria meningitis. Depletion of B cells, urinary tract, and upper respiratory system infections	GAIA/CLL13 (NCT02950051); CLL8 (NCT00281918); DUO (NCT02004522); NCT01871675; MURANO (NCT02005471); CLL14 (NCT02242942); ELEVATE-TN (NCT02475681); AVO (NCT03580928); EVOLVE (NCT04269902); CLL2-GIVE (NCT02758665); CLL10 (NCT00769522)

The table is not intended to cover all CLL clinical trials, but the ones taken in exam in this manuscript. Sources: European Medicine Agency (EMA) and ClinicalTrials.org. F, fludarabine; C, cyclophosphamide; R, rituximab; O, ofatumumab; Ob, obinutuzumab; B, bendamustine; V, venetoclax; I, ibrutinib; Id, Idelalisib; D, duvelisib; Ch, chlorambucil; Ac, acalabrutinib.

and opportunistic infections upon fludarabine-based treatment. This can both be due to long-term impact of previous therapy, and due to the more severe immune dysfunction upon relapse of CLL. In line with this, risk of infections upon progression of CLL after first line therapy is emphasized by the increased mortality from infections after chlorambucil-obinutuzumab as compared to ibrutinib-venetoclax in the GLOW trial (NCT03462719), where most fatal infections occurred among patients off therapy after chlorambucil treatment [45, 46].

Worth of notice is that the CLL8 trial also demonstrated the impact of dysbiosis in posttreatment disease progression. Indeed, patients previously treated with anti-Gram-positive antibiotics achieved lower overall response rate and OS. Further analysis associated anti-Gram-positive antibiotic treatment with reduced PFS [47].

Hematological toxicities and related infections are fewer and less severe upon treatment with bendamustine and chlorambucil, hence the rationale for their use for fit, low-risk CLL patients >65, and frailer CLL patients, respectively [48]. With these regimens, common infections include *Streptococcus pneumoniae*, and *Klebsiella pneumoniae*, while fungal, viral, and opportunistic microbes are more uncommon [49]. Like fludarabine, bendamustine induces prolonged T-cell lymphopenia [50, 51]. However, the treatment-related immunosuppression is less severe. When comparing bendamustine + rituximab (BR) with FCR for fit TN CLL in the CLL10 trial (NCT00769522), BR demonstrated lower rates of high-grade neutropenia and infections compared with FCR. Importantly, infections in the FCR arm occurred primarily in patients >65, further highlighting the rationale for BR as the favored choice in this age group [48].

For CIT-combinations, it is thus important to carefully balance between a rapid and durable treatment effect (undetectable minimal residual disease) and increase in infection risk due to the chosen therapeutic regime. CIT may also impair the gut-blood barrier leading to more gut-derived blood stream infections and thus a different landscape of infections as compared to TN CLL patients with infections [52].

Targeted Therapy Alone and in Combination

Targeted therapy has gradually replaced CIT as treatment of choice over the past decade. Currently, Bruton's tyrosine kinase inhibitors (BTKi) ibrutinib, acalabrutinib, and zanubrutinib as monotherapy, B-cell lymphoma-2 inhibitor (BCL-2i) venetoclax alone or combined with anti-CD20 antibody, as well as in rare incidences (due to risk of infections and autoimmune complications), phosphoinositide 3-kinases- δ inhibitors

(PI3K δ i) idelalisib and duvelisib combined with an anti-CD20 antibody, are options in first- and later line treatment [53].

Infectious risk upon treatment with ibrutinib is proposed to be related to off-target inhibition of other TEC family tyrosine kinases such as inducible T-cell kinase as well as BTK in other immune cells such as T-cells, NK cells, and macrophages [54–56]. In particular, suppressed macrophage function has been shown to increase susceptibility to fungal infections, as seen more often on BTKi treatment [57, 58]. Infectious risk upon treatment with 2nd generation BTKi acalabrutinib and zanubrutinib is similar to the risk with ibrutinib, despite increased BTK selectivity (e.g., ELEVATE-RR, NCT02477696; ALPINE, NCT03734016) [59–62]. Thus, off-target kinase inhibition does not alone explain treatment-associated infections with BTKi. Real-world data have shown that infections, especially in the airways and urinary tract, upon ibrutinib are most frequent in the first 6 months, after which rates decline [63]. This is in accordance with Sun et al. [64], where a transient increase in immunoglobulin (Ig)M and a sustained increase in IgA could explain the post-six-month immune reconstitution in those patients. The importance of the interplay between CLL disease and treatment is emphasized by the CLL12 trial (NCT02863718) testing preemptive ibrutinib for high-risk CLL without treatment need; here, similar rates of infections are seen between the ibrutinib and placebo arm [65]. This may suggest immunosuppression correlating with dynamics of the CLL tumor burden in PB, with initial BTKi-induced lymphocytosis in the first months followed by subsequent lymphocyte decrease.

For the BCL-2i venetoclax, neutropenia constitutes a frequent adverse event, likely due to the dependency of BCL-2 in granulopoiesis [66]. However, neutropenic fever is less frequent on venetoclax treatment combined with a CD20 antibody as compared to CIT, while the infectious burden increases to the same or even higher levels than on CIT for triple combinations with venetoclax [67]. Early studies of venetoclax for patients with relapsed/refractory (RR) CLL receiving monotherapy demonstrated grade 3–4 neutropenia in approximately 40%, and reported infections were predominately upper respiratory tract infections (URTIs) and pneumonia (NCT01889186) [68, 69].

Idelalisib is a PI3K δ i primarily used in combination with the anti-CD20 antibody rituximab in RR CLL, or occasionally in TN patients with TP53 mutation or del(17)p [53]. Although demonstrating effective clinical responses, idelalisib treatment is associated

with autoimmune-related toxicities and URTIs including increased susceptibility to *Pneumococcus pneumonia* [70–73]. Furthermore, idelalisib treatment has also been associated with complications, including febrile neutropenia and neutropenia, with a 70% infection increase in the single-arm treatment [71]. Infection-related deaths in this study were due to pneumonia, sepsis, and septic shock [71]. Idelalisib-associated toxicities are likely linked to inhibition of PI3K δ in T-cell receptor signaling, impairing CD8 $^{+}$ T-cell cytotoxicity but also inhibiting Treg, resulting in impaired immune tolerance [74]. Furthermore, increased susceptibility to bacterial infections is likely related to neutropenia and impaired neutrophil function [75]. The PI3K γ/δ dual inhibitor duvelisib has been previously tested alone (DUO; NCT02004522) or in combination with anti-CD20 (NCT01871675). Generally, treatment with duvelisib was associated with neutropenia, leading to pneumonia (18%) and URTIs (16%), as well as pneumonitis and hepatitis [70, 71, 76].

Monoclonal antibodies targeting CD20 have been explored as monotherapy and in various combinations. Considerable reduction in side effects, moderate neutropenia, and infections (e.g., pneumonia) for rituximab, have been achieved with later generations (ofatumumab and obinutuzumab) due to different CD20 targeted epitopes [77]. Nevertheless, immune impairment due to anti-CD20 monotherapy persists also with later generation drugs, including myelosuppression and neutropenia in patients treated with ofatumumab [78]. In clinical practice, venetoclax is commonly combined with rituximab (Ven+R; MURANO, NCT02005471) or obinutuzumab (Ven+O; CLL14, NCT02242942) after clinical trials have demonstrated high efficacy in both TN and RR disease [79–81], although grade 3–4 neutropenia rates close to 60% have been reported. Combination therapy has never been compared to monotherapy in a trial setting; however, in a real-world study of patients with RR CLL, rates of neutropenia were much lower (34%) and similar to monotherapy (40%) [82]. Interestingly, another real-world study on venetoclax+anti-CD20 showed similar rates of neutropenia between TN and RR patients (19 and 17%, respectively), while infection rates were significantly higher in the RR group [83], thus reflecting the interplay between treatment, CLL disease and impact of previous treatment for immune dysfunction. Ven+O and Ven+R combination in the GAIA/CLL13 trial demonstrated slightly higher frequency of grade 3–4 neutropenia (45% vs. 40%) and pneumonia (5% vs. 2%) with Ven+O versus Ven+R, while the risk was significantly higher upon addition of ibrutinib as a triple combination [37]. Acalabrutinib as

monotherapy or combined with obinutuzumab in TN CLL was recently investigated in the ELEVATE-TN clinical trial (NCT02475681) [60]. Here, the combination entailed considerably higher rates of grade 3–4 neutropenia (30 vs. 10%) and respiratory tract infections (grade 3–4 pneumonia, 5 vs. 2.2%) compared to monotherapy.

Combining ibrutinib with venetoclax (Ven+Ibr) constitutes another approach aiming to deepen remissions based on drug synergy and enable fixed-duration treatment. Generally, neutropenia and infection rates with Ven+Ibr are similar in TN and RR CLL (GLOW; CLARITY, NCT02267590; HOVON141/VISION, NCT03226301) [45, 84, 85]. Grade 3–4 neutropenia is more frequent with Ven+Ibr (around 35%) [45, 84, 85] than with ibrutinib alone (10–25%) [62, 86], likely due to the additional BCL-2i impact on myelopoiesis, but less frequently than observed for venetoclax +/- anti-CD20 [68, 69]. Despite this, infection rates with Ven+Ibr [45] are similar or slightly higher than with venetoclax +/- anti-CD20, while higher than observed with chlorambucil-based CIT [37, 45, 79, 81, 87], although cross-trial comparisons should be cautiously considered with all the potential biases. The higher infection rate with Ven+Ibr may in part be due to suppressive off-target effects of ibrutinib on T cell and innate immune function, or due to differences in patient age and fitness. In line with this, infections appear to be less frequent with Ven+Ibr than what is observed for ibrutinib alone [59, 62, 86]. Here, additional mechanism could be the rapid elimination of CLL cells by venetoclax, thus eradicating CLL-mediated immune suppression faster. Triplet combination therapy with a BTKi, BCL-2i, and anti-CD20 antibody is also a strategy currently being explored. Recent data from acalabrutinib combined with venetoclax and obinutuzumab (AVO; NCT03580928) in TN CLL patients reported 37% grade 3–4 neutropenia, thus similar to other venetoclax-based regimes and acalabrutinib+obinutuzumab, while infections grade 3 or higher were observed in only 6% [88]. Meanwhile, in the GAIA/CLL13 trial, ibrutinib combined with venetoclax and obinutuzumab (IVO) in TN CLL led to grade 3–4 neutropenia in around 50% of patients, with grade 3–4 infections in 21% of patients, similar or higher rates than seen on CIT [37]. In a phase 2 study of IVO in both RR and TN CLL, grade 3–4 neutropenia was unsurprisingly higher in the RR patients (76% vs. 56%), while grade 3–4 of any reported infections were rare in both RR and TN patients [89]. Chemotherapy-free triplet combination therapy may be particularly relevant for patients with del(17)p or TP53 mutation,

Table 3. Summary of high-grade infection risk in CLL clinical trials

CLL clinical trial (identification)	Drug combinations	≥ 3 grade infection (%)
GAIA/CLL13 (NCT02950051)	FCR/BR	18.5
	RV	10.5
	ObV (GVe)	13.2
	ObIV (GIVe)	21.2
GLOW (NCT03462719)	IV	16.94
	ChOb	9.51
ELEVATE-TN (NCT02475681)	ChOb	6.5
	AcOb	25.79
	Ac	14.55
ELEVATE-RR (NCT02477696)	Ac	41.49
	I	44.47
ALPINE (NCT03734016)	Z	12.7
	I	17.9
CLARITY (NCT02267590)	IV	16.9
HOVON141/VISION (NCT03226301)	IbV	27.6
AVO (NCT03580928)	AcVOb	6
CLL12 (NCT02863718)	I	71
	Placebo	56.8
CLL2-GIVE (NCT02758665)	IVOb	5
NCT01889186	V (79 months)	55.96
	V (68 months)	58.81
MURANO (NCT02005471)	RV	26.54
	BR	27.43
CLL14 (NCT02242942)	ChOb	19.18
	ObV	24.48
DUO (NCT02004522)	D	64.52
	O	14.25
CLL8 (NCT00281918)	FC	17.56
	FRC	22.94
CLL10 (NCT00769522)	FCR	40
	BR	26

The table is not intended to cover all CLL clinical trials, but the ones taken in exam in this manuscript. Sources: ClinicalTrials.org. F, fludarabine; C, cyclophosphamide; R, rituximab; O, ofatumumab; Ob, obinutuzumab; B, bendamustine; V, venetoclax; I, ibrutinib; Id, Idelalisib; D, duvelisib; Ch, chlorambucil; Ac, acalabrutinib; Z, zanubrutinib.

given the inferior prognosis of this patient group regardless of treatment choice. The CLL2-GIVE trial (NCT02758665) investigated IVO in TN CLL patients with del(17)p and/or TP53 mutation. Here, 40% of patients had grade 3–4 neutropenia during the induction phase, which declined throughout consolidation and maintenance treatment. Additionally, grade 3–4 infections which were mainly respiratory and urinary tract infections, only occurred in 5% of

patients. It is still premature to conclude whether this treatment strategy will improve long-term depth and duration of remissions in this high-risk patient group. However, treatment-related infectious risk with triple combination therapy was, importantly, not elevated in this setting [90].

Combining targeted agents may constitute a strategy to improve efficacy, reduce toxicity, and overcome resistance. Paradoxically, while aiming for drug synergy,

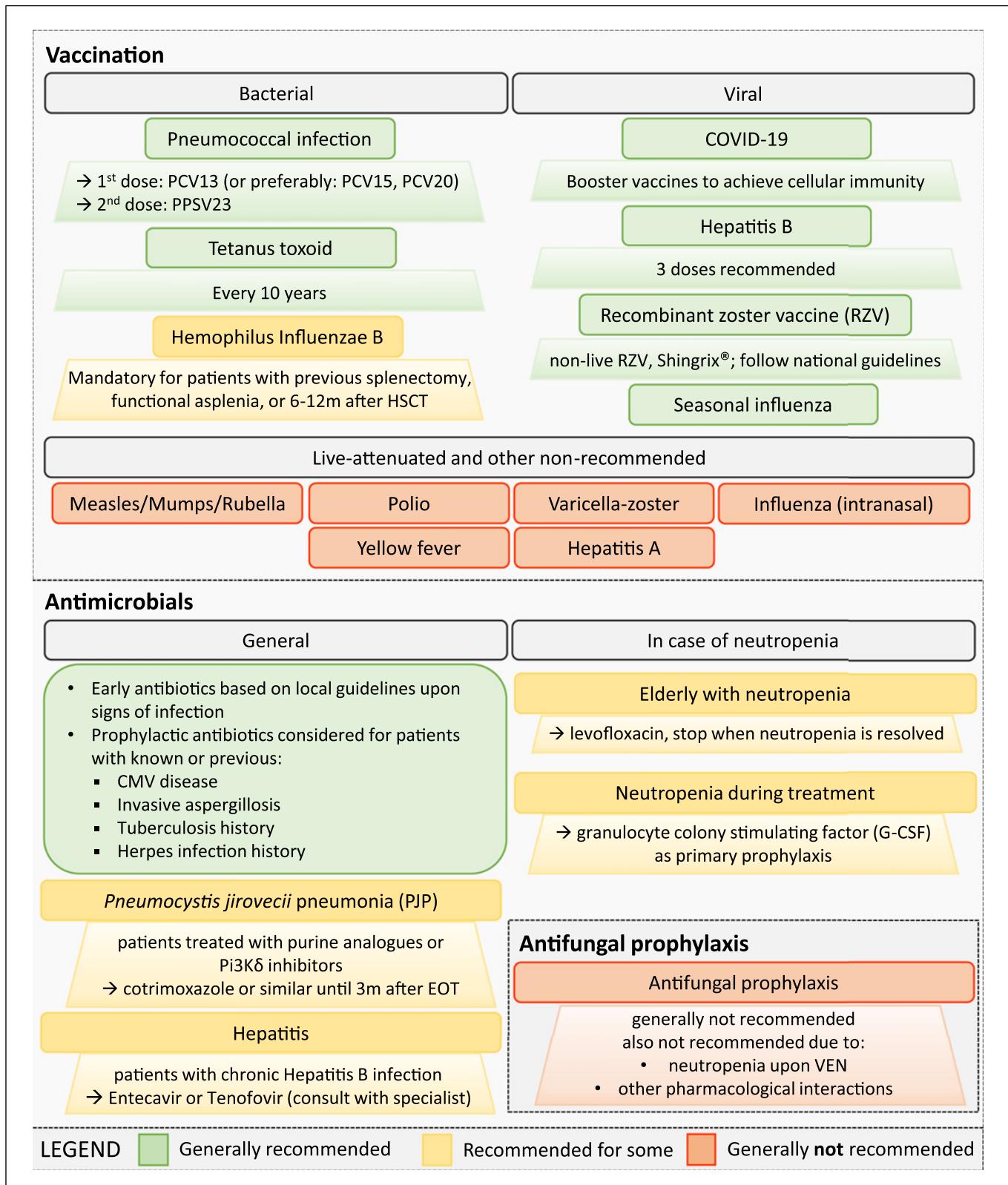


Fig. 1. Recommendations for antimicrobial prophylaxis. Green indicates generally recommended, yellow indicates indicated for some subgroups of patients, red indicates generally not recommended. 6–12 m, 6–12 months; 3 m, 3 months; HSCT, hematopoietic stem cell transplantation; CMV, cytomegalovirus; EOT, end of treatment; VEN, venetoclax.

disadvantageous immunosuppressive effects on the microenvironment may also increase along with the risk of infection (Table 3). It should still be emphasized, that differences in toxicity and risk of infections between the different treatment regimens in clinical trials are also due to differences in patient populations, treatment follow-up time, use of supportive care, and how data are reported and presented. This makes it especially important to evaluate benefits versus risks for the individual patient or patient risk group in a daily clinical setting.

Overall, the gain in treatment efficacy when choosing one regimen over another should be carefully weighed against the infectious risk associated with treatment-, CLL-, and patient-related risk factors. Here, improved strategies to identify which patients will benefit the most from a given treatment with the least risk of adverse events including infections are indeed warranted.

Conventional Preventive Strategies

Currently, vaccination and prophylaxis represent conventional strategies against infections, a topic extensively discussed in our recent review [4] and summarized in Figure 1.

Vaccination

Despite immunological impairment in CLL, vaccines can be effective and constitute an important tool in protection against infections. In particular, it is important to consider a preventing vaccination strategy before CLL requires treatment, including revaccination if there is the suspect of a reduced efficacy of previous vaccines. However, due to the immune dysfunction, it is important to be aware of which vaccines are likely to be effective versus potentially harmful, and why. Impaired humoral response is inherent to CLL [91] and is further attenuated by B-cell targeted treatments like BTKi and anti-CD20 antibodies (including cases of hepatitis B reactivation), as well as ongoing or previous chemotherapy [92–95]. As a consequence, vaccines relying solely on antibody-dependent immunization are less effective in CLL. One strategy to overcome this is the use of conjugated vaccines that, in addition to boosting the humoral response [96], also elicits a T-cell response [97] providing an additional level of immune protection. Similarly, mRNA vaccines against COVID-19 provide T-cell memory in addition to humoral response [98]. Other strategies to exacerbate the response to vaccines constitute use of adjuvants [99] as well as booster vaccines [100]. Due to the weakened immune

system, live attenuated vaccines should generally be avoided in CLL due to the risk of opportunistic infections and severe systemic reactions [101]. Finally, effective vaccination of close family and contacts could provide an additional protection for patients with CLL from infection. With this in mind, we here briefly summarize current practice recommendations for CLL.

Conjugated antipneumococcal vaccination is highly recommended, particularly with PCV13 (Prevenar 13-peptide vaccine) followed by PPSV23 (Pneumovax 23-polysaccharide vaccine) 2 months later [96]. Nevertheless, vaccine serologic response requires examination at least 4–6 weeks afterward, to evaluate the necessity of a possible revaccination [102, 103]. Preventive vaccination against varicella-zoster virus (recombinant), hepatitis B (entecavir or tenofovir) and *Clostridium tetani*, *Corynebacterium diphtheriae*, and *Bordetella pertussis* or *B. Parapertussis* (Tdap) is also highly recommended [102, 103]. Recombinant vaccines use recombinant proteins or non-replicating plasmids inserted in viral, bacterial, or mammalian vectors, inducing strong long-term cellular immune responses [104]. On the other hand, nasal vaccine and other live attenuated vaccines including measles/mumps/rubella/yellow fever viruses are discouraged for patients with CLL due to the risk of later disease severe manifestations [102–104]. Given the higher mortality associated with COVID-19 infection for CLL patients [105, 106], vaccination against COVID-19 is highly recommended. Despite the compromised seroconversion and new COVID-19 variants of concern, 2-dose scheduled vaccination (ChAdOx1, mRNA BNT162b, and mRNA1273) followed by booster vaccination shows efficacy for a third of CLL patients, with probably more patients achieving a T-cell response and increased response upon several booster vaccinations, which are recommended for patients with CLL [107, 108].

Prophylaxis

In order to justify prophylaxis in CLL, which is generally not recommended, it is important to consider infection history for each patient, as well as current or planned treatments. Indeed, knowledge of previous opportunistic infections may guide prophylactic strategies, for instance against infection caused by *Mycobacterium tuberculosis*, CMV, and herpes virus (e.g., Acyclovir 400 mg bid) [53, 109]. Environmental risk, based on regional or local microbiological recommendations (e.g., malaria and yellow fever areas), should also be considered. In these circumstances, mosquito repellents are highly recommended for CLL patients, while novel mRNA-based vaccines can be expected in the coming years. On the other hand, treatment-based prophylaxis

should also be evaluated in particular instances like prophylaxis against *Pneumocystis jirovecii pneumonia* (PJP) for purine analogs or Pi3Kδi treatment [110], while it is not recommended during BTKi treatment due to low PJP infection risk [109, 111]. Despite some prior recommendations, PJP prophylaxis is not generally recommended in R/R BTKi-treated CLL patients [109, 110]. To prevent CMV infections during idelalisib monotherapy or in combination with rituximab, seronegative patients should receive CMV-negative or purified blood products while, for confirmed presence of CMV, use of ganciclovir or valganciclovir in combination with idelalisib discontinuation is highly recommended [112, 113]. Interactions between specific antimicrobials and specific CLL treatments should be considered when prophylaxis or antimicrobial strategy is chosen [4]. Finally, granulocyte colony stimulating factor (G-CSF) should be implemented for CLL patients affected by neutropenia-associated complications [114]. Despite we currently have minimal information regarding efficacy and survival benefit, it is important to mention that Ig replacement is a viable option for CLL patient affected by recurrent infections and hypogammaglobulinemia [53, 115].

Concerning COVID-19, prophylaxis through passive immunization using tixagevimab and cilgavimab, depending on antibody availability, appears to be effective for CLL patients [116]. Beyond this, recently, secondary prophylaxis treatment efficacy using nirmatrelvir plus ritonavir was also demonstrated, suggesting how current guidelines should be now supplemented with similar preemptive treatments [117, 118].

Strategies to Prevent Infection through Early Treatment in CLL

Given vaccination and prophylaxis cannot always be applied and may not prevent a number of severe infections in CLL, and that currents treatments can result in sustained immune dysfunction, leading to increased morbidity and mortality due to infections, it is essential to define strategies aiming to identify risk factors prior to treatment to test novel interventions and inform treatment choice from the perspective of risk of immune dysfunction.

Such preventive strategies could aim at improving immune function in early-stage CLL based on preemptive treatment strategies for early disease control. Currently, this is tested in the CLL12 and EVOLVE (NCT04269902) clinical trials. With the hypothesis that an early intervention using ibrutinib would minimize toxicity and increase disease control, CLL12 demonstrated an improvement in PFS

and low toxicity. Despite this, infections of any grade were similar between ibrutinib (63.9% patients) and placebo (71% patients) groups [65]. The EVOLVE trial, currently recruiting, aims at improving OS by preemptive Ven+O combination for high-risk patients [119].

Another approach to improve immune dysfunction could be selection of treatment type and even time of treatment aiming at reducing the risk of infections. To this end, the CLL12 trial has tested whether treatment with ibrutinib monotherapy for high-risk CLL close to diagnosis, prior to iwCLL criteria for treatment [120], could improve outcome [121]. Unfortunately, early ibrutinib did not reduce risk of infection nor improved OS. In terms of selection of treatment type, the mainstay has been that CIT caused more immune dysfunction than targeted therapy. However, the GAIA/CLL13 trial demonstrated that 1st-line treatment with the triplet of obinutuzumab-venetoclax-ibrutinib led to at least as high infection rates as CIT with FCR or R-bendamustine, while obinutuzumab-venetoclax led to lower rates of infection [38]. Thus, the triplet could not be generally recommended. At time of progression after 1st-line treatment, recent data from the GLOW trial indicates that fatal infections occurred upon progression after obinutuzumab-chlorambucil treatment prior to patients meeting iwCLL criteria for next line of treatment [45]. A similar trend for increased fatal infections was not seen in the VISION/HO141 trial, testing MRD-guided reinitiation of ibrutinib-venetoclax upon molecular relapse [85, 122].

A different approach to improve outcome from preemptive treatment in CLL, would be to identify patients in the watch and wait setting with high-risk of infections, as their 30-day mortality upon the first serious infection is 10% [2]. We previously demonstrated that assessment of each CLL patient's comorbid conditions can correlate with increased infection and mortality [123]. In line with the necessity to test new strategies for identifying high-risk patients, we developed the machine learning-based algorithm CLL-TIM, identifying patients with a 70% risk of serious infection and/or CLL treatment within 2 years from diagnosis [124]. In the PreVent-ACaLL trial (NCT03868722), we selected high-risk patients based on the CLL-TIM algorithm for randomization between standard of care (watch and wait) or short term (3 cycles) treatment with acalabrutinib and venetoclax [6]. The aim of the PreVent-ACaLL study is to improve infection-free, treatment-free survival for such high-risk CLL patients, without jeopardizing their health by prolonged treatment leading to risk of infections and other adverse events. Despite studies highlighting the correlation between recurrent CLL mutations and high infection risk

[125, 126], including extensive OMICS data on top of the routine data included for the CLL-TIM algorithm did not improve predictive performance [127].

Conclusion

Current guidelines for management of CLL do not recommend general preventive strategies against infections but restrict recommendations to vaccine recommendations and very specific cases of prophylaxis. Nevertheless, infections are still the major cause of death for patients with CLL. In particular, despite novel therapeutic strategies, mortality and morbidity due to infection showed no improvement over the last decades [4, 13, 63, 128]. Thus, the lack of more general preventive strategies against infections in CLL reveals an unmet need. Cross-trial comparisons, with all the limitations of such comparisons, indicate that obinutuzumab-chlorambucil- and venetoclax- or ibrutinib-based treatment leads to similar immunosuppression, while FCR and R-bendamustine treatment causes more immunosuppression than BTK- or BCL-2 inhibitor-based treatment, except for the triplet combinations of obinutuzumab-venetoclax-ibrutinib leading to the highest infection counts. Early targeted treatment prior to iwCLL criteria for treatment initiation did not reduce risk of infections, while progression after 1st-line treatment may lead to increased risk of infections prior to meeting iwCLL criteria for next line of treatment. We encourage the research community to use these examples as an incitement to

thoroughly explore real-world data and clinical trial data to develop and test strategies to improve immune function and reduce morbidity and mortality from infections in CLL, challenging the current paradigm for management of CLL.

Conflict of Interest Statement

CUN received research funding and/or consultancy fees outside this work from AbbVie, AstraZeneca, Octapharma, Janssen, CSL Behring, BeiGene, Genmab, Eli Lilly, MSD, and Takeda. All the other authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding Sources

This work was supported by grants from the Danish National Research Foundation (DNRF; DNRF126) to E.G. R.S.T. received funding from the Danish Cancer Society. C.U.N. received funding from the Danish Cancer Society and the EU-funded ERA PerMED program for this work.

Author Contributions

E.G., R.S.T., T.F., and C.U.N. wrote the manuscript and created the figures and tables jointly. E.G. was responsible for the revision of the manuscript, the final version was approved by all authors.

References

- 1 Andersen MA, Niemann CU. Immune failure, infection and survival in chronic lymphocytic leukemia in Denmark. *Haematologica*. 2018 Jul 3;103(7):e330.
- 2 Andersen MA, Eriksen CT, Brieghel C, Bicler JL, Cunha-Bang CD, Helleberg M, et al. And predictors of infection among patients prior to treatment of chronic lymphocytic leukemia: a Danish nationwide cohort study. *Haematologica*. 2018 Jul 1; 103(7):e300–3.
- 3 Svanberg R, Janum S, Patten PEM, Ramsay AG, Niemann CU. Targeting the tumor microenvironment in chronic lymphocytic leukemia. *Haematologica*. 2021 Apr 22; 106(9):2312–24.
- 4 Gargiulo E, Ribeiro EFO, Niemann CU. SOHO state of the art updates and next questions | infections in chronic lymphocytic leukemia patients: risks and management. *Clin Lymphoma Myeloma Leuk*. 2023 Feb 10;23(5):322–32.
- 5 Rivera D, Ferrajoli A. Managing the risk of infection in chronic lymphocytic leukemia in the era of new therapies. *Curr Oncol Rep*. 2022 Aug 1;24(8):1003–14.
- 6 Da Cunha-Bang C, Agius R, Kater AP, Levin MD, Österborg A, Mattsson M, et al. PreVent-ACALL short-term combined acalabrutinib and venetoclax treatment of newly diagnosed patients with CLL at high risk of infection and/or early treatment, who do not fulfil IWCLL treatment criteria for treatment. A randomized study with extensive immune phenotyping. *Blood*. 2019 Nov 13;134(Suppl 1):4304.
- 7 Moreno C, Muñoz C, Terol MJ, Hernández-Rivas JA, Villanueva M. Restoration of the immune function as a complementary strategy to treat Chronic Lymphocytic Leukemia effectively. *J Exp Clin Cancer Res*. 2021 Dec 1;40(1):321.
- 8 Vitale C, Boccellato E, Comba L, Jones R, Perutelli F, Griggio V, et al. Impact of immune parameters and immune dysfunctions on the prognosis of patients with chronic lymphocytic leukemia. *Cancers*. 2021 Aug 1; 13(15):3856.
- 9 Landgren O, Albitar M, Ma W, Abbasi F, Hayes RB, Ghia P, et al. B-cell clones as early markers for chronic lymphocytic leukemia. *N Engl J Med*. 2009;360(7): 659–67.
- 10 Sabattini E, Bacci F, Sagramoso C, Pileri SA. WHO classification of tumours of haematopoietic and lymphoid tissues in 2008: an overview. *Pathologica*. 2010;102(3):83–7.
- 11 Gribben JG. How I treat CLL up front. *Blood*. 2010;115(2):187–97.

- 12 Andersen MA, Vojdeman FJ, Andersen MK, Brown PD N, Geisler CH, Weis Bjerrum O, et al. Hypogammaglobulinemia in newly diagnosed chronic lymphocytic leukemia is a predictor of early death. *Leuk Lymphoma*. 2016;57(7):1592–9.
- 13 da Cunha-Bang C, Simonsen J, Rostgaard K, Geisler C, Hjalgrim H, Niemann CU. Improved survival for patients diagnosed with chronic lymphocytic leukemia in the era of chemo-immunotherapy: a Danish population-based study of 10,455 patients. *Blood Cancer J*. 2016 Nov 1;6(11):e499.
- 14 Schlesinger M, Broman I, Lugassy G. The complement system is defective in chronic lymphatic leukemia patients and in their healthy relatives. *Leukemia*. 1996;10(9):1509–13.
- 15 Tooze JA, Bevan DH. Decreased expression of complement receptor type 2 (CR2) on neoplastic B cells of chronic lymphocytic leukaemia. *Clin Exp Immunol*. 1991;83(3):423–9.
- 16 Hara T, Kojima A, Fukuda H, Masaoka T, Fukumori Y, Matsumoto M, et al. Levels of complement regulatory proteins, CD35 (CR1), CD46 (MCP) and CD55 (DAF) in human haematological malignancies. *Br J Haematol*. 1992;82(2):368–73.
- 17 Orsini E, Guarini A, Chiaretti S, Mauro FR, Foa R. The circulating dendritic cell compartment in patients with chronic lymphocytic leukemia is severely defective and unable to stimulate an effective T-cell response. *Cancer Res*. 2003;63(15):4497–506.
- 18 Huergo-Zapico L, Acebes-Huerta A, Gonzalez-Rodriguez AP, Contesti J, Gonzalez-Garcia E, Payer AR, et al. Expansion of NK cells and reduction of NKG2D expression in chronic lymphocytic leukemia. Correlation with progressive disease. *PLoS One*. 2014; 9(10):e108326.
- 19 Wierz M, Pierson S, Guyonnet L, Viry E, Lequeux A, Oudin A, et al. Dual PD1/LAG3 immune checkpoint blockade limits tumor development in a murine model of chronic lymphocytic leukemia. *Blood*. 2018;131(14):1617–21.
- 20 Gargiulo E, Viry E, Morande PE, Largeot A, Gonter S, Xian F, et al. Extracellular vesicle secretion by leukemia cells in vivo promotes CLL progression by hampering antitumor T-cell responses. *Blood Cancer Discov*. 2023 Jan 6;4(1):54–77.
- 21 Burger JA, Quiroga MP, Hartmann E, Burkle A, Wierda WG, Keating MJ, et al. High-level expression of the T-cell chemokines CCL3 and CCL4 by chronic lymphocytic leukemia B cells in nurselike cell cocultures and after BCR stimulation. *Blood*. 2009;113(13):3050–8.
- 22 Podaza E, Sabbione F, Rismik D, Borge M, Almejun MB, Colado A, et al. Neutrophils from chronic lymphocytic leukemia patients exhibit an increased capacity to release extracellular traps (NETs). *Cancer Immunol Immunother*. 2017;66(1):77–89.
- 23 Gatjen M, Brand F, Grau M, Gerlach K, Kettritz R, Westermann J, et al. Splenic marginal zone granulocytes acquire an accentuated neutrophil B-cell helper phenotype in chronic lymphocytic leukemia. *Cancer Res*. 2016;76(18):5253–65.
- 24 van Attekum M, Terpstra S, Reinen E, Kater AP, Eldering E. Macrophage-mediated chronic lymphocytic leukemia cell survival is independent of APRIL signaling. *Cell Death Discov*. 2016;2:16020.
- 25 Maffei R, Bulgarelli J, Fiorcari S, Bertoncelli L, Martinelli S, Guarnotta C, et al. The monocytic population in chronic lymphocytic leukemia shows altered composition and deregulation of genes involved in phagocytosis and inflammation. *Haematologica*. 2013;98(7):1115–23.
- 26 Jain T, Sharma P, Are AC, Vickers SM, Dudeja V. New insights into the cancer–microbiome–immune axis: decrypting a decade of discoveries. *Front Immunol*. 2021 Feb 23;12:622064.
- 27 Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. *Cell Res*. 2020;30(6):492–506.
- 28 Park J, Kim M, Kang SG, Jannasch AH, Cooper B, Patterson J, et al. Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR-S6K pathway. *Mucosal Immunol*. 2015;8(1):80–93.
- 29 Francescone R, Hou V, Grivennikov SI. Microbiome, inflammation and cancer. *Cancer J*. 2014;20(3):181.
- 30 Faitova T, Jørgensen M, Svartberg R, da Cunha-Bang C, Ilett EE, MacPherson C, et al. P603: gut microbiome in chronic lymphocytic leukemia shows depletion of short-chain fatty acids producing bacteria. *HemaSphere*. 2022 Jun 23;6:502–3.
- 31 Andersen MA, Niemann CU, Rostgaard K, Dalby T, Sørrig R, Weinberger DM, et al. Differences and temporal changes in risk of invasive pneumococcal disease in adults with hematological malignancies: results from a nationwide 16-year cohort study. *Clin Infect Dis*. 2021 Feb 1;72(3):463–1.
- 32 Niemann CU, da Cunha-Bang C, Helleberg M, Ostrowski SR, Briegel C. Patients with CLL have a lower risk of death from COVID-19 in the Omicron era. *Blood*. 2022 Aug 4;140(5):445–50.
- 33 Andersen MA, Rostgaard K, Niemann CU, Hjalgrim H. Antimicrobial use before chronic lymphocytic leukemia: a retrospective cohort study. *Leukemia*. 2021;35(3):747–51.
- 34 Smolej L, Antic D, Couto E, Alexander E, Gregor M, Tran HTT, Jaksic O, et al. Differences in availability of targeted inhibitors and use of chemoimmunotherapy in the first-line therapy of chronic lymphocytic leukemia. A European survey. *Leuk Lymphoma*. 2021;62(S1):S11–3.
- 35 Thompson PA, Tam CS, O'Brien SM, Wierda WG, Stingo F, Plunkett W, et al. Fludarabine, cyclophosphamide, and rituximab treatment achieves long-term disease-free survival inIGHV-mutated chronic lymphocytic leukemia. *Blood*. 2016 Jan 21;127(3):303–9.
- 36 Fischer K, Bahlo J, Fink AM, Goede V, Herling CD, Cramer P, et al. Long-term remissions after FCR chemotherapy in previously untreated patients with CLL: updated results of the CLL8 trial. *Blood*. 2016 Jan 14;127:208–15.
- 37 Time-limited venetoclax-obinutuzumab +/- ibrutinib is superior to... EHA LIBRARY (Internet). *Eichhorst B*. 2022 Jun 12:366209. (cited 2022 Nov 12). Available from: <https://library.ehaweb.org/eha/2022/eha2022-congress/366209/barbara.eichhorst.time-limited.venetoclax-obinutuzumab.2B.-.ibrutinib.is.html>.
- 38 Eichhorst B, Niemann CU, Kater AP, Fürstenau M, von Tresckow J, Zhang C, et al. First-line venetoclax combinations in chronic lymphocytic leukemia. *N Engl J Med*. 2023 May 11;388(19):1739–54.
- 39 Byrd JC, Hargis JB, Kester KE, Hospenthal DR, Knutson SW, Diehl LF. Opportunistic pulmonary infections with fludarabine in previously treated patients with low-grade lymphoid malignancies: a role for *Pneumocystis carinii* pneumonia prophylaxis. *Am J Hematol*. 1995;49(2):135–42.
- 40 Frank DA, Mahajan S, Ritz J. Fludarabine-induced immunosuppression is associated with inhibition of STAT1 signaling. *Nat Med*. 1999 Apr;5(4):444–7.
- 41 Tavazzi E, White MK, Khalili K. Progressive multifocal leukoencephalopathy: clinical and molecular aspects. *Rev Med Virol*. 2012; 22(1):18–32.
- 42 Wierda W, O'Brien S, Wen S, Faderl S, Garcia-Manero G, Thomas D, et al. Chemoimmunotherapy with fludarabine, cyclophosphamide, and rituximab for relapsed and refractory chronic lymphocytic leukemia. *J Clin Oncol*. 2005;23(18):4070–8.
- 43 Hensel M, Kornacker M, Yammeni S, Egerer G, Ho AD. Disease activity and pretreatment, rather than hypogammaglobulinemia, are major risk factors for infectious complications in patients with chronic lymphocytic leukaemia. *Br J Haematol*. 2003 Aug; 122(4):600–6.
- 44 Hallek M, Fischer K, Fingerle-Rowson G, Fink AM, Busch R, Mayer J, et al. Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. *Lancet*. 2010 Oct 2; 376(9747):1164–74.
- 45 Kater AP, Owen C, Moreno C, Follows G, Munir T, Levin MD, et al. Fixed-duration ibrutinib-venetoclax in patients with chronic lymphocytic leukemia and comorbidities. *NEJM Evid*. 2022 May 13;1(7).

- 46 Niemann C, Munir T, Moreno C, Owen C, Follows GA, et al. Residual disease kinetics among patients with high-risk factors treated with first-line fixed-duration ibrutinib plus venetoclax (Ibr+Ven) versus chlorambucil plus obinutuzumab (Clb+O): the glow study. *Blood*. 2022;140(Suppl 1):228–30.
- 47 Pflug N, Kluth S, Vehreschild JJ, Bahlo J, Tacke D, Bieth L, et al. Efficacy of antineoplastic treatment is associated with the use of antibiotics that modulate intestinal microbiota. *Oncoimmunology*. 2016 Jun 2;5(6):e1150399.
- 48 Eichhorst B, Fink AM, Bahlo J, Busch R, Kovacs G, Maurer C, et al. First-line chemoimmunotherapy with bendamustine and rituximab versus fludarabine, cyclophosphamide, and rituximab in patients with advanced chronic lymphocytic leukaemia (CLL10): an international, open-label, randomised, phase 3, non-inferiority trial. *Lancet Oncol*. 2016 Jul 1;17(7):928–42.
- 49 Knauf WU, Lissichkov T, Aldaoud A, Liberati A, Loscertales J, Herbrecht R, et al. Phase III randomized study of bendamustine compared with chlorambucil in previously untreated patients with chronic lymphocytic leukemia. *J Clin Oncol*. 2009;27(26):4378–84.
- 50 García Muñoz R, Izquierdo-Gil A, Muñoz A, Roldan-Galiacho V, Rabasa P, Panizo C. Lymphocyte recovery is impaired in patients with chronic lymphocytic leukemia and indolent non-Hodgkin lymphomas treated with bendamustine plus rituximab. *Ann Hematol*. 2014 Nov 1; 93(11):1879–87.
- 51 Ito K, Okamoto M, Ando M, Kakumae Y, Okamoto A, Inaguma Y, et al. Influence of rituximab plus bendamustine chemotherapy on the immune system in patients with refractory or relapsed follicular lymphoma and mantle cell lymphoma. *Leuk Lymphoma*. 2015 Apr 1;56(4):1123–5.
- 52 Andersen MA, Moser CE, Lundgren J, Niemann CU. Epidemiology of bloodstream infections in patients with chronic lymphocytic leukemia: a longitudinal nationwide cohort study. *Leukemia*. 2019 Mar 1; 33(3):662–70.
- 53 Eichhorst B, Robak T, Montserrat E, Ghia P, Niemann CU, Kater AP, et al. Chronic lymphocytic leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2021 Jan 1;32(1):23–33.
- 54 Dubovsky JA, Beckwith KA, Natarajan G, Woyach JA, Jaglowski S, Zhong Y, et al. Ibrutinib is an irreversible molecular inhibitor of ITK driving a Th1-selective pressure in T lymphocytes. *Blood*. 2013; 122(15):2539–49.
- 55 Ping L, Ding N, Shi Y, Feng L, Li J, Liu Y, et al. The Bruton's tyrosine kinase inhibitor ibrutinib exerts immunomodulatory effects through regulation of tumor-infiltrating macrophages. *Oncotarget*. 2017;8(24): 39218–29.
- 56 Da Roit F, Engelberts PJ, Taylor RP, Breij ECW, Gritt G, Rambaldi A, et al. Ibrutinib interferes with the cell-mediated anti-tumor activities of therapeutic CD20 antibodies: implications for combination therapy. *Haematologica*. 2015;100(1):77–86.
- 57 Fiorcari S, Maffei R, Vallerini D, Scarfo L, Barozzi P, Maccaferri M, et al. BTK inhibition impairs the innate response against fungal infection in patients with chronic lymphocytic leukemia. *Front Immunol*. 2020;11:2158.
- 58 Ruchlemer R, Ben-Ami R, Bar-Meir M, Brown JR, Malphettes M, Mous R, et al. Ibrutinib-associated invasive fungal diseases in patients with chronic lymphocytic leukaemia and non-Hodgkin lymphoma: an observational study. *Mycoses*. 2019;62(12): 1140–7.
- 59 Burger JA, Tedeschi A, Barr PM, Robak T, Owen C, Ghia P, et al. Ibrutinib as initial therapy for patients with chronic lymphocytic leukemia. *N Engl J Med*. 2015 Dec 17; 373(25):2425–37.
- 60 Sharman JP, Egyed M, Jurczak W, Skarbnik A, Pagel JM, Flinn IW, et al. Acalabrutinib with or without obinutuzumab versus chlorambucil and obinutuzumab for treatment-naïve chronic lymphocytic leukaemia (ELEVATE TN): a randomised, controlled, phase 3 trial. *Lancet*. 2020 Apr 18;395(10232):1278–91.
- 61 Brown JR, Eichhorst B, Hillmen P, Jurczak W, Kaźmierczak M, Lamanna N, et al. Zanubrutinib or ibrutinib in relapsed or refractory chronic lymphocytic leukemia. *N Engl J Med*. 2023 Jan 26;388(4):319–32.
- 62 Byrd JC, Hillmen P, Ghia P, Kater AP, Chanhan-Khan A, Furman RR, et al. Acalabrutinib versus ibrutinib in previously treated chronic lymphocytic leukemia: results of the first randomized phase III trial. *J Clin Oncol*. 2021 Nov 1; 39(31):3441–52.
- 63 Aarup K, Rotbain EC, Enggaard L, Pedersen RS, Bergmann OJ, Thomsen RH, et al. Real-world outcomes for 205 patients with chronic lymphocytic leukemia treated with ibrutinib. *Eur J Haematol*. 2020 Nov 1; 105(5):646–54.
- 64 Sun C, Tian X, Lee YS, Gunti S, Lipsky A, Herman SEM, et al. Partial reconstitution of humoral immunity and fewer infections in patients with chronic lymphocytic leukemia treated with ibrutinib. *Blood*. 2015;126(19): 2213–9.
- 65 Langerbeins P, Zhang C, Robrecht S, Cramer P, Fürstenau M, Al-Sawaf O, et al. The CLL12 trial: ibrutinib versus placebo in treatment-naïve, early-stage chronic lymphocytic leukemia. *Blood*. 2022 Jan 13; 139(2):177–87.
- 66 Leverson JD, Phillips DC, Mitten MJ, Boghaert ER, Diaz D, Tahir SK, et al. Exploiting selective BCL-2 family inhibitors to dissect cell survival dependencies and define improved strategies for cancer therapy. *Sci Transl Med*. 2015 Mar 18; 7(279):279ra40.
- 67 Time-limited venetoclax-obinutuzumab +/- ibrutinib IS superior to... EHA Library (Internet). Eichhorst B. 2022 Jun 12: 366209. (cited 2023 Feb 12). Available from: <https://library.ehaweb.org/eha/2022/eha2022-congress/366209/barbara.eichhorst.time-limited.venetoclax-obinutuzumab.2B.-ibrutinib.is.html>.
- 68 Roberts AW, Davids MS, Pagel JM, Kahl BS, Puvvada SD, Gerecitano JF, et al. Targeting BCL2 with venetoclax in relapsed chronic LymphocyticLeukemia. *N Engl J Med*. 2016 Jan 1;374(4):311–22.
- 69 Stilgenbauer S, Eichhorst B, Schetelig J, Coutre S, Seymour JF, Munir T, et al. Venetoclax in relapsed or refractory chronic lymphocytic leukaemia with 17p deletion: a multicentre, open-label, phase 2 study. *Lancet Oncol*. 2016 Jun 1;17(6): 768–78.
- 70 Furman RR, Sharman JP, Coutre SE, Cheson BD, Pagel JM, Hillmen P, et al. Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. *N Engl J Med*. 2014; 370(11):997–1007.
- 71 Zelenetz AD, Barrientos JC, Brown JR, Coiffier B, Delgado J, Egyed M, et al. Idelalisib or placebo in combination with bendamustine and rituximab in patients with relapsed or refractory chronic lymphocytic leukaemia: interim results from a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet Oncol*. 2017;18(3): 297–311.
- 72 O'Brien SM, Lamanna N, Kipps TJ, Flinn I, Zelenetz AD, Burger JA, et al. A phase 2 study of idelalisib plus rituximab in treatment-naïve older patients with chronic lymphocytic leukemia. *Blood*. 2012;126(25):2686–94.
- 73 Maus UA, Backi M, Winter C, Srivastava M, Schwarz MK, Rucke T, et al. Importance of phosphoinositide 3-kinase gamma in the host defense against pneumococcal infection. *Am J Respir Crit Care Med*. 2007; 175(9):958–66.
- 74 Hanna BS, Roessner PM, Scheffold A, Jebara BMC, Demerdash Y, Öztürk S, et al. PI3Kδ inhibition modulates regulatory and effector T-cell differentiation and function in chronic lymphocytic leukemia. *Leukemia*. 2019 Jun 1;33(6):1427–38.
- 75 Alflen A, Stadler N, Aranda Lopez P, Teschner D, Theobald M, Heß G, et al. Idelalisib impairs TREM-1 mediated neutrophil inflammatory responses. *Sci Rep*. 2018;8(1):5558.
- 76 Flinn IW, Hillmen P, Montillo M, Nagy Z, Illes A, Etienne G, et al. The phase 3 DUO trial: duvelisib versus ofatumumab in relapsed and refractory CLL/SLL. *Blood*. 2018; 132(23):2446–55.
- 77 Maloney DG, Ogura M, Fukuhara N, Davis J, Lasher J, Izquierdo M, et al. A phase 3 randomized study (HOMER) of ofatumumab versus rituximab in iNHL relapsed after rituximab-containing therapy. *Blood Adv*. 2020;4(16):3886–93.

- 78 Wierda WG, Kipps TJ, Durig J, Griskevicius L, Stilgenbauer S, Mayer J, et al. Chemoimmunotherapy with O-FC in previously untreated patients with chronic lymphocytic leukemia. *Blood*. 2011;117(24):6450–8.
- 79 Seymour JE, Kipps TJ, Eichhorst B, Hillmen P, D’Rozario J, Assouline S, et al. Venetoclax-rituximab in relapsed or refractory chronic lymphocytic leukemia. *N Engl J Med*. 2018;378(12):1107–20.
- 80 Fischer K, Al-Sawaf O, Bahlo J, Fink AM, Tandon M, Dixon M, et al. Venetoclax and obinutuzumab in patients with CLL and coexisting conditions. *N Engl J Med*. 2019 Jun 6;380(23):2225–36.
- 81 Al-Sawaf O, Zhang C, Tandon M, Sinha A, Fink AM, Robrecht S, et al. Venetoclax plus obinutuzumab versus chlorambucil plus obinutuzumab for previously untreated chronic lymphocytic leukaemia (CLL14): follow-up results from a multicentre, open-label, randomised, phase 3 trial. *Lancet Oncol*. 2020 Sep 1;21(9):1188–200.
- 82 Mato AR, Roeker LE, Eyre TA, Nabhan C, Lamanna N, Hill BT, et al. A retrospective comparison of venetoclax alone or in combination with an anti-CD20 monoclonal antibody in R/R CLL. *Blood Adv*. 2019 May 5;3(10):1568–1573.
- 83 Herishanu Y, Goldschmidt N, Itchaki G, Levi I, Aviv A, Fineman R, et al. Real-world efficacy of venetoclax-based regimens in patients with chronic lymphocytic leukemia in Israel: a multicenter prospective study. *Blood*. 2021 Nov 23; 138(Suppl 1):3727.
- 84 Hillmen P, Rawstron AC, Brock K, Muñoz-Vicente S, Yates FJ, Bishop R, et al. Ibrutinib plus venetoclax in relapsed/refractory chronic lymphocytic leukemia: the CLARITY study. *J Clin Oncol*. 2019 Oct 20;37(30):2722–9.
- 85 Kater AP, Levin MD, Dubois J, Kersting S, Enggaard L, Veldhuis GJ, et al. Minimal residual disease-guided stop and start of venetoclax plus ibrutinib for patients with relapsed or refractory chronic lymphocytic leukaemia (HOVON141/VISION): primary analysis of an open-label, randomised, phase 2 trial. *Lancet Oncol*. 2022 Jun 1;23(6):818–28.
- 86 Neffendorf JE, Gout I, Hildebrand GD. Ibrutinib in relapsed chronic lymphocytic leukemia. *N Engl J Med*. 2013 Sep 26; 369(13):1277.
- 87 Davids MS, Hallek M, Wierda W, Roberts AW, Stilgenbauer S, Jones JA, et al. Comprehensive safety analysis of venetoclax monotherapy for patients with relapsed/refractory chronic lymphocytic leukemia. *Clin Cancer Res*. 2018;24(18):4371–9.
- 88 Ryan CE, Lampson BL, Tyekucheva S, Hackett LR, Ren Y, Shupe SJ, et al. Updated results from a multicenter, phase 2 study of acalabrutinib, venetoclax, obinutuzumab (AVO) in a population of previously untreated patients with CLL enriched for high-risk disease. *Blood*. 2022 Nov 15;140(Suppl 1):837–8.
- 89 Rogers KA, Huang Y, Ruppert AS, Abruzzo LV, Andersen BL, Awan FT, et al. Phase II study of combination obinutuzumab, ibrutinib, and venetoclax in treatment-naïve and relapsed or refractory chronic lymphocytic leukemia. *J Clin Oncol*. 2020;38(31):3626–37.
- 90 Huber H, Edenhofer S, von Tresckow J, Robrecht S, Zhang C, Tausch E, et al. Obinutuzumab (GA-101), ibrutinib, and venetoclax (GIVe) frontline treatment for high-risk chronic lymphocytic leukemia. *Blood*. 2022;139(9):1318–29.
- 91 Crassini KR, Zhang E, Balendran S, Freeman JA, Best OG, Forsyth CJ, et al. Humoral immune failure defined by immunoglobulin class and immunoglobulin G subclass deficiency is associated with shorter treatment-free and overall survival in Chronic Lymphocytic Leukaemia. *Br J Haematol*. 2018 Apr 1;181:97–101.
- 92 Herzog Tzarfaty K, Gutwein O, Apel A, Rahimi-Levene N, Sadovnik M, Harel L, et al. BNT162b2 COVID-19 vaccine is significantly less effective in patients with hematologic malignancies. *Am J Hematol*. 2021 Oct 1;96(10):1195–203.
- 93 Benjamini O, Rokach L, Itchaki G, Braester A, Shvidel L, Goldschmidt N, et al. Safety and efficacy of the BNT162b mRNA COVID-19 vaccine in patients with chronic lymphocytic leukemia. *Haematologica*. 2022 Mar 3;107(3):625–34.
- 94 Herishanu Y, Avivi I, Aharon A, Shefer G, Levi S, Bronstein Y, et al. Efficacy of the BNT162b2 mRNA COVID-19 vaccine in patients with chronic lymphocytic leukemia. *Blood*. 2021 Jun 10;137(23):3165–73.
- 95 Tsutsumi Y, Yamamoto Y, Ito S, Ohigashi H, Shiratori S, Naruse H, et al. Hepatitis B virus reactivation with a rituximab-containing regimen. *World J Hepatol*. 2015 Sep 9;7(21):2344–51.
- 96 Svensson T, Kättström M, Hammarlund Y, Roth D, Andersson PO, Svensson M, et al. Pneumococcal conjugate vaccine triggers a better immune response than pneumococcal polysaccharide vaccine in patients with chronic lymphocytic leukemia a randomized study by the Swedish CLL group. *Vaccine*. 2018 Jun 14;36(25):3701–7.
- 97 Burn OK, Farrand K, Pritchard T, Draper S, Tang CW, Mooney AH, et al. Glycolipid-peptide conjugate vaccines elicit CD8+ T-cell responses and prevent breast cancer metastasis. *Clin Transl Immunol*. 2022;11(7):e1401.
- 98 Hurme A, Jalkanen P, Heroum J, Liedes O, Vara S, Melin M, et al. Long-lasting T cell responses in BNT162b2 COVID-19 mRNA vaccinees and COVID-19 convalescent patients. *Front Immunol*. 2022 Apr 22;13:869990.
- 99 Zent CS, Brady MT, Delage C, Strawderman M, Laniowski N, Contant PN, et al. Short term results of vaccination with adjuvanted recombinant varicella zoster glycoprotein E during initial BTK inhibitor therapy for CLL or lymphoplasmacytic lymphoma. *Leukemia*. 2021 Jun 1;35(6):1788–91.
- 100 Shen Y, Freeman JA, Holland J, Naidu K, Solterbeck A, Van Bilsen N, et al. Multiple COVID-19 vaccine doses in CLL and MBL improve immune responses with progressive and high seroconversion. *Blood*. 2022 Dec 22;140(25):2709–21.
- 101 Arvas A. Vaccination in patients with immunosuppression. *Turk Arch Ped*. 2014 Sep 1;49(3):181–5.
- 102 NCCN Guideline for prevention and treatment of Cancer related infection. Version 2 (2022).
- 103 Centers for Disease Control and Prevention. Recommended Adult Immunization Schedule, United States (2022).
- 104 Nascimento IP, Leite LCC. Recombinant vaccines and the development of new vaccine strategies. *Braz J Med Biol Res*. 2012; 45(12):1102–11.
- 105 Scarfò L, Chatzikonstantinou T, Rigolin GM, Quaresmini G, Motta M, Vitale C, et al. COVID-19 severity and mortality in patients with chronic lymphocytic leukemia: a joint study by ERIC, the European Research Initiative on CLL, and CLL Campus. *Leukemia*. 2020;34(9):2354–63.
- 106 Mato AR, Roeker LE, Lamanna N, Allan JN, Leslie L, Pagel JM, et al. Outcomes of COVID-19 in patients with CLL: a multicenter international experience. *Blood*. 2020 Sep 3; 136(10):1134–43.
- 107 da Cunha-Bang C, Kirkby NS, Friis-Hansen L, Niemann CU. Serological response following vaccination with BNT162b2 mRNA in patients with chronic lymphocytic leukemia. *Leuk Lymphoma*. 2022;63(2):503–5.
- 108 Mai AS, Lee ARYB, Tay RYK, Shapiro L, Thakkar A, Halmos B, et al. Booster doses of COVID-19 vaccines for patients with haematological and solid cancer: a systematic review and individual patient data meta-analysis. *Eur J Cancer*. 2022 Sep 1;172: 65–75.
- 109 Hilal T, Gea-Banacloche JC, Leis JF. Chronic lymphocytic leukemia and infection risk in the era of targeted therapies: linking mechanisms with infections. *Blood Rev*. 2018 Sep 1;32(5):387–99.
- 110 Ryan CE, Cheng MP, Issa NC, Brown JR, Davids MS. Pneumocystis jirovecii pneumonia and institutional prophylaxis practices in CLL patients treated with BTK inhibitors. *Blood Adv*. 2020 Apr 14;4(7):1458–63.
- 111 Walewska R, Parry-Jones N, Eyre TA, Follows G, Martinez-Calle N, McCarthy H, et al. Guideline for the treatment of chronic lymphocytic leukaemia. *Br J Haematol*. 2022 Jun 1;197(5):544–57.
- 112 Maschmeyer G, De Greef J, Mellinghoff SC, Nosari A, Thiebaut-Bertrand A, Bergeron A, et al. Infections associated with immunotherapeutic and molecular targeted agents in hematology and oncology. A position paper by the European Conference on Infections in Leukemia (ECIL). *Leukemia*. 2019 Apr 1; 33(4):844–62.

- 113 Cheah CY, Fowler NH. Idelalisib in the management of lymphoma. *Blood*. 2016 Jul 21;128(3):331–6.
- 114 Laali E, Fazli J, Sadighi S, Mohammadi M, Gholami K, Jahangard-Rafsanjani Z. Appropriateness of using granulocyte colony-stimulating factor (G-CSF) for primary prophylaxis of febrile neutropenia in solid tumors. *J Oncol Pract*. 2020 Mar 1;26(2):428–33.
- 115 Raanani P, Gafter-Gvili A, Paul M, Ben-Bassat I, Leibovici L, Shpilberg O. Immunoglobulin prophylaxis in chronic lymphocytic leukemia and multiple myeloma: systematic review and meta-analysis. *Leuk Lymphoma*. 2009;50(5):764–72.
- 116 Davis JA, Granger K, Roubal K, Smith D, Gaffney KJ, McGann M, et al. Efficacy of tixagevimab-cilgavimab in preventing SARS-CoV-2 for patients with B-cell malignancies. *Blood*. 2023 Jan 12;141(2):200–3.
- 117 Tadmor T, Alapi H, Rokach L. Effectiveness of nirmatrelvir plus ritonavir treatment for patients with chronic lymphocytic leukemia during the Omicron surge. *Blood*. 2023; 141(18):2239–44.
- 118 Niemann CU. Immediate COVID-19 treatment in CLL. *Blood*. 2023 May 5; 141(18):2167–8.
- 119 Stephens DM, Moseley A, Hill BT, Pagel JM, Shadman M, Fisch MJ, et al. Randomized, phase III study of early intervention with venetoclax and obinutuzumab versus delayed therapy with venetoclax and obinutuzumab in newly diagnosed asymptomatic high-risk patients with chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL): EVOLVE CLL/SLL study (SWOG S1925, NCT#04269902). *Blood*. 2021;138(Suppl 1):2630.
- 120 Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Dohner H, et al. iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. *Blood*. 2018;131(25):2745–60.
- 121 Langerbeins P, Robrecht S, Nieper P, Cramer P, Fürstenau M, Al-Sawaf O, et al. Ibrutinib versus placebo in patients with asymptomatic, treatment-naïve early stage chronic lymphocytic leukemia (CLL): final results of the CLL12 trial. *Hematol Oncol*. 2023 Jun 9;41(S2):56–8.
- 122 Kater A, Hodkinson B, Moreno C, Munir T, Levin M-D, Niemann C, et al. Genetic alterations and outcomes with fixed-duration ibrutinib+venetoclax (ibr+ven): results from the phase 3 glow study in patients with previously untreated CLL (internet). EHA 2023 Library. 2023:620. (cited 2023 Jun 28). Available from: <https://library.ehaweb.org/eha/2023/eha2023-congress/386449/arnon.kater.genetic.alterations.and.outcomes.with.fixed.duration.html?f=list&g=listing%3D0%2Abrowseby%3D8%2Asortby%3D1%2Asearch%3Dkater>.
- 123 Rotbain EC, Niemann CU, Rostgaard K, da Cunha-Bang C, Hjalgrim H, Frederiksen H. Mapping comorbidity in chronic lymphocytic leukemia: impact of individual comorbidities on treatment, mortality, and causes of death. *Leukemia*. 2021;35(9): 2570–80.
- 124 Agius R, Brieghel C, Andersen MA, Pearson AT, Ledergerber B, Cozzi-Lepri A, et al. Machine learning can identify newly diagnosed patients with CLL at high risk of infection. *Nat Commun*. 2020; 11(1):363.
- 125 Else M, Blakemore SJ, Strefford JC, Catovsky D. The association between deaths from infection and mutations of the BRAF, FBXW7, NRAS and XPO1 genes: a report from the LRF CLL4 trial. *Leukemia*. 2021; 35(9):2563–9.
- 126 Rossi D, De Paoli L, Rossi FM, Cerri M, Deambrogi C, Rasi S, et al. Early stage chronic lymphocytic leukaemia carrying unmutatedIGHV genes is at risk of recurrent infections during watch and wait. *Br J Haematol*. 2008;141(5):734–6.
- 127 Parviz M, Brieghel C, Agius R, Niemann CU. Prediction of clinical outcome in CLL based on recurrent gene mutations, CLL-IPI variables, and (para)clinical data. *Blood Adv*. 2022 Jun 28;6(12): 3716–28.
- 128 Vainer N, Aarup K, Andersen MA, Wind-Hansen L, Nielsen T, Frederiksen H, et al. Real-world outcomes upon second-line treatment in patients with chronic lymphocytic leukaemia. *Br J Haematol*. 2023; 201(5):874–86.