From the Department of Oncology-Pathology
Karolinska Institutet, Stockholm, Sweden

# Real-world studies on B-cell malignancies

Anna Asklid

Karolinska Institutet

# REAL-WORLD STUDIES ON B-CELL MALIGNANCIES
# THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

# Anna Asklid

*Principal Supervisor:*
Associate Professor Lotta Hansson
Karolinska Institutet
Department of Oncology-Pathology

*Co-supervisors:*
Professor Anders Österborg
Karolinska Institutet
Department of Oncology-Pathology

MD, PhD Sandra Eketorp Sylvan
Karolinska Institutet
Department of Oncology-Pathology

*Opponent:*
Associate Professor Martin Höglund
Uppsala University
Department of Medical Sciences

*Examination Board:*
Associate Professor Kourosh Lotfi
Linköping University
Department of Biomedical and Clinical Sciences

Professor Hans Hägglund
Uppsala University
Department of Medical Sciences
National Cancer Coordinator
Swedish Association of
Local Authorities and Regions

Professor Leif Stenke
Karolinska Institutet
Department of Oncology-Pathology

"Do what you can,
with what you have,
where you are."

*-Theodore Roosevelt-*

*To Nicklas*

# ABSTRACT

Randomized controlled trials remain the preferred way of evaluating new treatments. However, in studies on malignancies, trial data may not always be sufficient to address the requirements of health care providers and regulatory agencies regarding recommendations as patients are strictly selected through inclusion- and exclusion criteria. Carefully collected data from consecutive, unselected patients from a well-defined area, without missing cases, will reveal the actual results in routine medical care. These real-world results often differ from results in clinical trials and may provide important additional information to data from clinical trials and serve as control for early non-randomized clinical studies of novel drugs. It is important to find optimal ways to use new high-cost cancer drugs not just for healthcare authorities but for the wider society. The aim of this thesis was to compile reliable real-world data in certain subgroups of patients with chronic lymphocytic leukemia (CLL) and diffuse large B-cell lymphoma (DLBCL), the two most common subgroups of lymphoid malignancies.

The **first study** investigated the effectiveness and safety of $2^{nd}$ line treatment in consecutive relapsed or refractory (R/R) CLL patients treated between 2003 and 2013 in the Stockholm region. In-depth analysis of each patient file was performed retrospectively. Despite access to new therapies no significant improvement in survival over time was demonstrated. These results highlighted the need for next generation targeted therapies in this patient group and constituted a relevant context for interpretation of and comparison with data obtained in clinical trials of new drugs.

The **second study** was a nationwide study on consecutive CLL patients receiving $1^{st}$ line therapy between 2007 and 2013. Baseline characteristics, treatment, outcome and toxicity were retrospectively extracted from each patient's medical file. After a median follow-up of almost 5 years, median progression free survival (PFS) and overall survival (OS) were 24 and 58 months respectively, both significantly associated with type of treatment, del(17p), performance status, gender and age. Overall, there was no significant improvement in OS during the time period studied and importantly regional differences in outcome was observed. The study constitutes a large and unique material providing a context to evaluate the findings obtained in clinical trials of new drugs.

The **third study** was an adjusted comparison between the Bruton tyrosine kinase inhibitor ibrutinib versus previous standard of care treatments in two cohorts of patients with R/R CLL. With multivariate regression modelling to adjust for differences in baseline prognostic factors, PFS and OS were significantly longer with ibrutinib than with previous standard of care regimens. The study describes a statistical approach to provide a preliminary comparison between treatments used in clinical routine and new drugs until comparisons from randomized clinical trials are available.

In the **fourth study** outcome of $1^{st}$ line treatment in consecutive patients aged 80 years or older, diagnosed with DLBCL between 2000 and 2015 in the Stockholm region was evaluated. Retrospective data were collected from each individual patient file. Patients $\geq$ 85 years responded to and tolerated chemoimmunotherapy equally well as patients aged 80-84 years, highlighting that even very elderly patients benefit from active therapy provided that dose-adaption of chemotherapeutic drugs are performed.

# LIST OF SCIENTIFIC PAPERS

I.   **Asklid A**, Winqvist M, Eketorp Sylvan S, Mattsson A, Björgvinsson E, Søltoft F, Repits J, Diels J, Österborg A, Hansson L. Outcomes of second-line treatment in chronic lymphocytic leukemia - a population-based study from a well-defined geographical region between 2003 and 2013. *Leukemia & Lymphoma*, 58(5):1219-1223, 2017.

II.  Eketorp Sylvan S, **Asklid A**, Johansson H, Klintman J, Bjellvi J, Tolvgård S, Kimby E, Norin S, Andersson P-O, Karlsson C, Karlsson K, Lauri B, Mattsson M, Bergendahl Sandstedt A, Strandberg M, Österborg A, Hansson L. First-line therapy in chronic lymphocytic leukemia: a Swedish nation-wide real-world study on 1053 consecutive patients treated between 2007 and 2013. *Haematologica*, 104(4):797-804, 2019.

III. Hansson L, **Asklid A**, Diels J, Eketorp Sylvan S, Repits J, Søltoft F, Jäger U, Österborg A. Ibrutinib versus previous standard of care: an adjusted comparison in patients with relapsed/refractory chronic lymphocytic leukaemia. *Annals of Hematology*, 96(10):1681-1691, 2017.

IV.  **Asklid A**, Eketorp Sylvan S, Mattsson A, Winqvist M, Johansson H, Österborg A, Hansson L. A real-world study of first-line therapy in 280 consecutive Swedish patients ≥80 years with newly diagnosed diffuse large B-cell lymphoma: very elderly (≥85 years) do well on curative intended therapy. *Leukemia & Lymphoma*, 61(9):2136-2144, 2020.

# LIST OF ABBREVIATIONS

| | |
|---|---|
| aaIPI | age adjusted International Prognostic Index |
| ABC | Activated B-cell |
| ADCC | Antibody-Dependent Cellular Cytotoxicity |
| AE | Adverse Event |
| AKT | Anti-apoptotic protein kinase |
| B | Bendamustine |
| BCL | B-cell Lymphoma |
| BCR | B-cell Receptor |
| BR | Bendamustine, Rituximab |
| BTK | Bruton Tyrosine Kinase |
| C | Cyclophosphamide |
| CAR | Chimeric Antigen Receptor |
| CD | Cluster of Differentiation |
| CDC | Complement-Dependent Cytotoxicity |
| CGA | Comprehensive Geriatric Assessment |
| CHOP | Cyclophosphamide, Doxorubicin, Vincristine, Prednisone |
| CI | Confidence Interval |
| CIRS | Cumulative Illness Rating Scale |
| CIT | Chemoimmunotherapy |
| CLB | Chlorambucil |
| CLL | Chronic Lymphocytic Leukemia |
| CLL-IPI | Chronic Lymphocytic Leukemia International Prognostic Index |
| CNS | Central Nervous System |
| COO | Cell Of Origin |
| CR | Complete Remission |
| CRF | Case Report Form |
| CT | Computed Tomography |
| CTCAE | Common Terminology Criteria for Adverse Events |
| DA-EPOCH-R | Dose Adjusted Etoposide, Prednisone, Vincristine, Cyclophosphamide, Doxorubicin, Rituximab |
| DH | Double-Hit |
| DLBCL | Diffuse Large B-cell Lymphoma |
| DoR | Duration of Response |
| EBV | Epstein-Barr Virus |
| ECOG | Eastern Cooperative Oncology Group |
| EMA | European Medicines Agency |
| ESMO | European Society for Medical Oncology |
| EZH2 | Enhancer of Zeste Homolog 2 |
| F | Fludarabine |
| FC | Fludarabine, Cyclophosphamide |
| FCR | Fludarabine, Cyclophosphamide, Rituximab |
| FDA | Food and Drug Administration |
| FISH | Fluorescent In Situ Hybridization |
| G | Obinutuzumab (GA-101) |

| | |
|---|---|
| GCB | Germinal Center B-cell |
| G-CLB | Obinutuzumab (GA-101), Chlorambucil |
| G-CSF | Granulocyte Colony Stimulating Factor |
| GEP | Gene Expression Profiling |
| GvHD | Graft-versus-Host Disease |
| HGBL | High Grade B-cell Lymphoma |
| HLA | Human Leukocyte Antigen |
| HR | Hazard Ratio |
| I | Ibrutinib |
| Ig | Immunoglobulin |
| IGHV | Immunoglobulin Heavy Chain Variable region |
| IPI | International Prognostic Index |
| IPS-E | International Prognostic Score for Early-stage CLL |
| IR | Ibrutinib, Rituximab |
| IRF4 | Interferon Regulatory Factor 4 |
| iwCLL | International Workshop on Chronic Lymphocytic Leukemia |
| LDH | Lactate Dehydogenase |
| LON | Late Onset Neutropenia |
| mAb | Monoclonal Antibody |
| MBL | Monoclonal B-cell Lymphocytosis |
| MRD | Minimal Residual Disease |
| MUM1 | Multiple Myeloma oncogene 1 |
| MYC | Myelocytomatosis oncogene |
| MYD88 | Myeloid Differentiation primary response gene 88 |
| NCCN | National Comprehensive Cancer Network |
| NCI | National Cancer Institute |
| NF-κB | Nuclear Factor kappa-light-chain-enhancer of activated B-cells |
| NHL | Non-Hodgkin Lymphoma |
| NOS | Not Otherwise Specified |
| NOTCH1 | Notch homolog 1, translocation-associated |
| ORR | Overall Response Rate |
| OS | Overall Survival |
| PD | Progressive Disease |
| PD-1 | Programmed Cell Death 1 |
| PD-L1 | Programmed Cell Death Ligand 1 |
| PET | Positron Emission Tomography |
| PFS | Progression Free Survival |
| PI3Kδ | Phosphatidylinositol 3-kinase delta |
| PLCγ2 | Phospholipase C gamma 2 |
| PR | Partial Remission |
| PTEN | Phosphatase and Tensin Homolog |
| R | Rituximab |
| R-ACVBP | Rituximab, Doxorubicin, Cyclophosphamide, Vincristine, Bleomycin, Prednisone |
| RCC | Regional Cancer Center |
| R-CHOP | Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Prednisone |
| R-CLB | Rituximab, Chlorambucil |

| | |
|---|---|
| RCT | Randomized Controlled Trial |
| R-DHAP | Rituximab, Dexamethasone, Cytarabine, Cisplatin |
| RDI | Relative Dose Intensity |
| R-GCVP | Rituximab, Gemcitabine, Cyclophosphamide, Vincristine, Prednisolone |
| R-GDP | Rituximab, Gemcitabine, Dexamethasone, Cisplatin |
| R-GEMOX | Rituximab, Gemcitabine, Oxaliplatin |
| R-ICE | Rituximab, Ifosfamide, Carboplatin, Etoposide |
| R/R | Relapsed/Refractory |
| RT | Richter Transformation |
| RWD | Real-World Data |
| RWE | Real-World Evidence |
| RWS | Real-World Study |
| SCR | Swedish Cancer Registry |
| SCT | Stem Cell Transplantation |
| SD | Stable Disease |
| SLL | Small Lymphocytic Lymphoma |
| SLR | Swedish Lymphoma Registry |
| SOC | Standard Of Care |
| TH | Triple-Hit |
| TNT | Time to Next Therapy |
| TP53 | Tumor Protein 53 |
| Ven-R | Venetoclax, Rituximab |
| WES | Whole Exome Sequencing |
| WHO | World Health Organization |

# CONTENTS

# 1 REAL-WORLD DATA

## 1.1 DEFINITIONS

Real-world data (RWD) within the field of medicine can be described as data collected from patients in routine health care i.e. patients who do not participate in interventional clinical trials. Commonly used sources of RWD are healthcare records and patient registries. Real-world evidence (RWE) can be described as data compiled and analyzed based on the use of treatments in clinical practice. However, there is no uniform agreement on these definitions and the area of RWE is wide [1-3]. Real-world studies (RWS) often refer to non-interventional/observational studies to which the projects in this thesis belongs, but RWS do not necessarily exclude randomization and prospectively collected data. There are trial designs using randomization within clinical practice with wider eligibility criteria than randomized controlled trials (RCTs) and for example allows the presence of comorbidities, concomitant medications and a broader range of age. These studies are also referred to as pragmatic trials [3, 4].

## 1.2 IMPLEMENTATION AND CLINICAL RELEVANCE

Real-world studies aim to describe the effectiveness and safety of treatments or interventions used in routine clinical practice and RWD has many areas of application where some examples will be further described.

An essential question in decision making of health authorities is how the safety and efficacy of treatments in trials can be translated into the real-world setting. RCTs remain the preferred way of evaluating the safety and efficacy of new treatments but regarding malignancies RCTs might not always be sufficient to fully support recommendations on the optimal treatment for different subgroups of patients [5-7]. The knowledge and evidence at time of evaluation of new orphan drugs might not always be comprehensive enough for regulatory authorities and financiers as patients are strictly selected and long-term follow-up regarding overall survival (OS) and adverse events might not be provided. The literature often refers to data from centers with predominantly external referrals, where follow-up is short and representativeness is uncertain because of patient selection, entailing a risk of limited generalizability [7, 8]. The comparative arm in clinical trials is further not always optimal and consistent with standard of care [9, 10].

For many malignancies there is an urgent need for more efficient treatments. Thus, authorizations are sometimes based on phase 2 studies via accelerated approval programs and in cases where RCTs are not feasible or ethical, RWD can play an important part as an external or historical control group supplementing data from early phase trials to support this process and moreover add information on long-term outcome and safety post authorization [6]. The interest of RWD in addition to results from RCTs is growing and becoming increasingly important in health care decisions by regulatory authorities when approving and evaluating new, often costly treatment regimens [11, 12].

Moreover, RWD can aid treating physicians to interpret results from trials into expectations regarding patients in certain subgroups where data are scarce and thus may facilitate informed decisions in daily clinical care [6]. Elderly and fragile patients are often

more prone to worse side-effects which will not be captured in studies where such subgroups are excluded. Further, quality of life might sometimes be preferred over prolonged survival. Even though quality of life is not captured in retrospective analyzes, comprehensive data on adverse events from patients treated within routine care might aid in the risk-benefit discussion [13, 14]. Besides long-term data on survival and toxicity at the time of approval other questions can remain such as optimal dosing regimen and outcome in different subgroups, where RWD can provide additional information [14].

Chronic lymphocytic leukemia (CLL) and diffuse large B-cell lymphoma (DLBCL) are the two most common subgroups of lymphoid malignancies and the two diseases studied within this thesis. Regarding CLL numerous efficient treatment options both in frontline and as salvage therapy has entered the market. Moreover, several new drugs and combination strategies are currently being explored moving towards individualized medicine. Thus, it will probably become even harder to capture OS benefits within the relatively short follow-up of clinical trials [6, 15].

In DLBCL many patients are cured but there are still relapses where most patients and especially certain subgroups remain difficult to treat. Intensive research has been conducted over the years and potential agents are on the market or in pipeline [16].

Short term surrogate endpoints will not necessarily correlate to an actual OS benefit at long-term follow-up. A report on approvals of cancer drugs by the Food and Drug Administration (FDA) during 2008-2012 showed that 67% of drug approvals (e.g. ofatumumab, bendamustine and rituximab in CLL) was made on the basis on surrogate markers such as response rates and progression-free survival (PFS). Response rate was the primary outcome measure in 53% and PFS in 47% of these approvals. After a median follow-up of 4.4 years, 14% of the drugs demonstrated an OS benefit within randomized trials while 50 % did not. In the remaining 36% the effect on OS was still unknown [17].

In a retrospective report on cancer therapies approved by the European Medicines Agency (EMA) between 2009-2013, 35% of the treatments demonstrated a significant survival benefit at marketing and an additional 7% showed improved OS during the follow-up period ranging from 3.3-8.1 years. Approximately one out of ten approvals on treatment indications was based on studies without a comparator arm [18].

RWD might help to support decision making of regulatory agencies as a complement to clinical trials in assessing the effectiveness and safety of currently used treatment strategies in routine health care as well as provide data on the relative effectiveness and safety of new high-cost therapies. Several studies have pointed out the enormous cost of some new treatments and moreover questioned the balance in cost-effectiveness in some indications. Optimized use and evaluation of new therapies from a health-economic perspective is further an area where RWD might provide useful information [19-21].

## 1.3   CHALLENGES AND OPPORTUNITIES

Real-world results often differ from results in clinical trials where patients usually are younger and have less comorbidities [7, 8]. This was also demonstrated in a previous report on CLL patients by our research group [22] where the real-world cohort presented promising outcome with a new agent (ibrutinib) in spite of heavier comorbidity burden

and worse performance status which would have excluded half of them from the pivotal trial [23].

Swedish quality registries in combination with our civil registration system and equal access to the predominantly public health care, provide a great opportunity with the possibility of identifying nearly all patients diagnosed with a specific disease and further obtain high-quality RWD from patients within a well-defined geographical area with a very low rate of external referrals and almost complete follow-up. This generates reliable, representative data on groups of patients that can serve as a basis for comparison with data obtained in clinical trials of orphan drugs. Reliable RWD may be difficult and time consuming to achieve, but outcome research projects with carefully collected data from consecutive, unselected patients from a well-defined region, without missing cases, reflects the actual results in routine medical care.

It is important to be aware of the inherent limitations and risks with RWS of retrospective nature affecting its internal validity. Potential unmeasured confounders with non-randomized setting, information bias due to measurement uncertainties and differences in individual assessments with risk of misclassification as well as possible reporting bias in registries employed to identify patients or when collecting data. Further, missing data due to deficient or incorrect information in patient records are all examples of potential pitfalls. The quality of the information and how the data is gathered must be considered and conclusions of RWS interpreted with caution [24, 25].

Moreover, it is important to emphasize that RWS cannot take the place of RCTs and should not be seen as competitive to the robust methodology and evidence gained from these trials, but rather as a complement to reach deeper insight into the effectiveness and long-term outcomes applicable to a broader population [7]. At last, RWD can constitute an opportunity to generate hypotheses, identify potential differences between regions or levels of health care facilities and also identify certain subgroups of patients that appear to have a worse outcome which may justify in-depth analysis of potential prognostic and predictive markers [7].

# 2    CHRONIC LYMPHOCYTIC LEUKEMIA

## 2.1    EPIDEMIOLOGY AND ETIOLOGY

The B-cell malignancy chronic lymphocytic leukemia (CLL) constitutes around 40% of all leukemias in adults and is thereby the most common type of leukemia in western countries [26]. The median age is 72 years at diagnosis and in Sweden the incidence is around 500 people per year and increases with age [27]. In 2015 the prevalence of CLL was 52/100.000 inhabitants and has increased over the last decades due to more efficient treatments yielding increased survival and the prevalence is estimated to rise further [28].

For unknown reasons, men have an almost twice as high incidence of CLL than women [29]. CLL is also more common among people of European origin than people of Asian origin. It is unclear why, but genetic rather than environmental factors seem to be the main explanation [30].

The etiology of CLL is incompletely understood but there are some potential risk factors. The most important risk factor is advanced age with a possible explanation being accumulation of genotoxic substances in lymphoid tissues [31]. Having a first-degree relative with CLL is further associated with an up to 8.5-fold increased risk of developing CLL in comparison to the general population. Family history of any hematological malignancy has also been found to be a risk factor associated with CLL [32]. Genetic predisposition in combination with exposure to certain environmental factors such as pesticides, agricultural agents and petroleum may partly explain some cases but the etiology of CLL remain unsure to a large extent [31].

## 2.2    PATHOGENESIS AND GENETIC FEATURES

CLL is characterized by a clonal expansion of mature CD5 positive B-lymphocytes accumulating in blood, bone marrow and other lymphoid tissues. Evidence have amassed indicating the pluripotent hematopoietic stem cell as the cell of origin to CLL but controversy remains regarding the exact phenotype of the B-cell that clonally expands leading to CLL [33].

### 2.2.1    Monoclonal B-cell lymphocytosis

Monoclonal B-cell lymphocytosis (MBL) is considered an essential precursor state of CLL. It is defined as a peripheral blood count of $< 5x10^9$/L monoclonal B-lymphocytes in the absence of other signs and symptoms of CLL. MBL is divided into low- and high-count MBL, based on whether the number of clonal B-cells are below or above $0.5x10^9$/L [34]. Low-count MBL is at very low risk of progressing into CLL and specific follow-up is not necessary in contrast to high-count MBL where the yearly risk of progression is 1-2% [35].

### 2.2.2    The B-cell receptor and signaling pathways

The maturation process of B-cells begins in the bone marrow whereupon they express immunoglobulin (Ig) M and IgD isotype receptors on their surface. After exposure to

antigens the development then proceeds in lymph nodes and spleen where B-cell clonal expansion and somatic hypermutation of the variable region of the B-cell receptor (BCR) occur in structures called germinal centers [36].

Based on the mutational status of the immunoglobulin heavy chain variable (*IGHV*) gene, CLL can be divided into two subtypes referred to as IGHV-mutated (IGHV-M) and IGHV-unmutated (IGHV-UM) CLL. IGHV-M status is defined as less than 98% homology with the germline nucleotide sequence and is associated with a superior prognosis compared to IGHV-UM status featured by 98-100% homology and usually a more aggressive clinical behavior [37, 38]. This may partly reflect disparities in genetic aberrations, signaling pathways activated and maybe also cell of origin. The IGHV-M subtype is proposed to originate from a post-germinal center (GC) B-cell that has been exposed to a T-cell dependent antigen succeeded by somatic hypermutation. The IGHV-UM subtype probably derives from a naïve pre-GC B-cell, expressing unmutated immunoglobulins. Additional genetic lesions, BCR stimulation and interactions in the microenvironment may subsequently lead to progenitor states of CLL and finally to manifest CLL [33, 36].

In both normal and malignant B-cells, signaling through the BCR is essential for proliferation and survival [39]. In CLL there are today several agents available targeting different steps in the pathways downstream the BCR in addition to other available treatments such as antibodies, other small molecules, new cell-based therapies and classical DNA-damaging drugs (Figure 1). These therapies will be described in more detail in following sections.
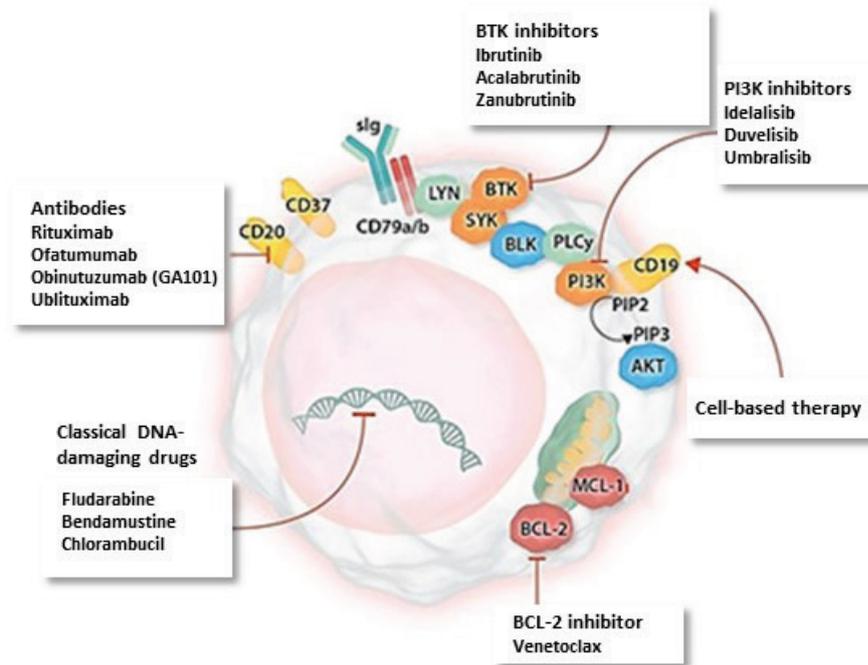


**Figure 1. CLL cell with drug targets and classification of drugs.** AKT= anti-apoptotic protein kinase, BCL-2=B-cell lymphoma 2, BLK=B lymphocyte kinase, BTK=Bruton tyrosine kinase, LYN=LCK/YES novel tyrosine kinase, MCL-1= induced myeloid leukemia cell differentiation protein Mcl-1, PI3K=phosphatidylinositol-4,5-bisphosphate 3-kinase, PIP2=phosphatidylinositol (4,5)-bisphosphate, PIP3=phosphatidylinositol (3,4,5)-trisphosphate, PLC=phospholipase C, sIg=surface immunoglobulin, SYK= spleen tyrosine kinase. *Reprinted in a modified version under the terms of the Creative Commons Attribution 4.0 License, http://creativecommons.org/licenses/by-nc-sa/4.0 from Yosifov et al. Biology to Therapy: The CLL Success Story. HemaSphere, 2019;3:2.*

Antigen binding to the extracellular domain of the BCR activates cascades of intracellular signaling which in turn activates kinases via the Bruton tyrosine kinase (BTK; target for BTK inhibitors e.g. ibrutinib) and the phosphatidylinositol 3-kinase-delta (PI3Kδ; target for PI3Kδ inhibitors, e.g. idelalisib) eventually leading to upregulation of transcription factors, among others nuclear factor-κB (NF-κB) via phospholipase C gamma 2 (PLCγ2). This results in various cellular steps changing the gene expression promoting cellular growth, proliferation and survival. Dysregulation in the pathways of the BCR eventually leads to increased survival and proliferation of the malignant B-cells in CLL [39, 40]. Besides activating mutations in different components in the complex signaling pathways, the tumor environment has been recognized for its involvement in the biology and pathogenesis of CLL. Continuous activation of the BCR stimulated by self-antigens or microbial antigens has been demonstrated and moreover an entwined cross-talk with T-helper cells and nurse-like cells have been described [39, 40].

A pathway not directly activated by the BCR, yet vital in the biology of CLL, acts via proteins encoded for by the B-cell lymphoma 2 (*BCL2*) gene. BCL2 proteins (target for BCL2-inhibitors e.g. venetoclax) are important in the apoptosis regulation and are overexpressed in CLL and further associated with resistance to programmed cell death [41].

### 2.2.3  Genetic alterations

Genetic alterations leading to sustained proliferation and evasion of growth suppression are two of the fundamental features in the genesis of cancer [42]. In CLL an important aspect in survival of CLL cells is recurrent genetic defects [43]. Döhner et al demonstrated 20 years ago, with the use of fluorescent in situ hybridization (FISH), that cytogenetic aberrations could be found in about 80% of CLL patients. The four most common chromosomal aberrations found was deletion in the long arm of chromosome 13 (13q), trisomy of chromosome 12, deletion in the long arm of chromosome 11 (11q) and deletion in the short arm of chromosome 17 (17p). These aberrations were in order significantly associated with worse prognosis. Patients presenting with deletion in 13q alone had a better outcome than patients without any chromosomal aberrations [43].

The tumor suppressor protein 53 (p53) is encoded for on 17p. Deletion in 17p, often abbreviated as del(17p), leads to loss of function in p53 in its central role controlling cell proliferation and apoptosis by affecting various cellular processes for example regulation of DNA repair in case of DNA damage. The function of p53 can be eliminated even if only one allele in the tumor protein 53 (*TP53*) gene is defect. Loss of function of p53 can also occur with different mutations in the *TP53* gene. Together these defects, 17p deletions and mutations in *TP53*, can be referred to as *TP53* aberrations or *TP53* disruptions. Commonly a combination of del(17p) and *TP53* mutations coexists [44, 45].

There has been a huge progress in the knowledge regarding the pathogenesis of CLL with different driving mutations, defects in signaling pathways, as well as several other findings leading to investigation of targeted therapies which has brought great prosperity for many CLL patients and broadly changed the prognosis of CLL [46, 47]. Further insights with whole exome sequencing (WES) and gene expression profiling (GEP) have been made and novel mutations identified [48]. Mutations in over 40 genes have been described,

among them for instance the transcription factor *NOTCH1*, present in 10% of CLL patients. *NOTCH1* mutations have been associated with adverse outcome and a higher risk of transformation into high malignant lymphoma (Richter transformation). Although, so far, the presence of *NOTCH1* mutation has no influence on the treatment decision in clinical routine [15]. However, several targeted therapies are available on the market and therapy recommendations are based on the presence or absence of *TP53* disruptions and the IGHV mutational status. Some of these therapies will be described further in the following sections.

## 2.3 CLINICAL PRESENTATION AND DIAGNOSIS

The clinical course of CLL is highly variable. Many patients lack symptoms at diagnosis and the disease may be indolent for several years and even decades while others have active disease with symptoms already at diagnosis. It is not uncommon for the disease to be detected based on elevated leukocytes or lymphocytes in routine blood samples. Approximately 10% of patients have B-symptoms (fever, unintentional weight loss or night sweats) at diagnosis. Other patients present with enlarged lymph nodes and/or hepatosplenomegaly. Bleedings and increased susceptibility to infections also occur do to bone marrow failure or on immunological basis such as hypogammaglobulinemia [49].

The diagnosis of CLL demands the presence of $\geq 5 \times 10^9$/L monoclonal B-lymphocytes in peripheral blood for a period of at least 3 months according to the World Health Organization (WHO) classification of lymphoid malignancies [50]. The diagnosis is based on morphological examination of peripheral blood, where the CLL-cells typically are small and mature with high nuclear cytoplasmatic ratio (Figure 2) and by using flow cytometry to confirm clonality. The clone either expresses kappa or lambda immunoglobulin light chains on the surface. Co-expression of B cell markers CD5, CD19, CD20 (weak) and CD23 are also characteristic of CLL cells [50]. An aspirate and/or a biopsy of bone marrow is in general not needed to establish the diagnosis but is indicated in case of cytopenia of unknown cause and generally recommended prior to start of treatment [51].
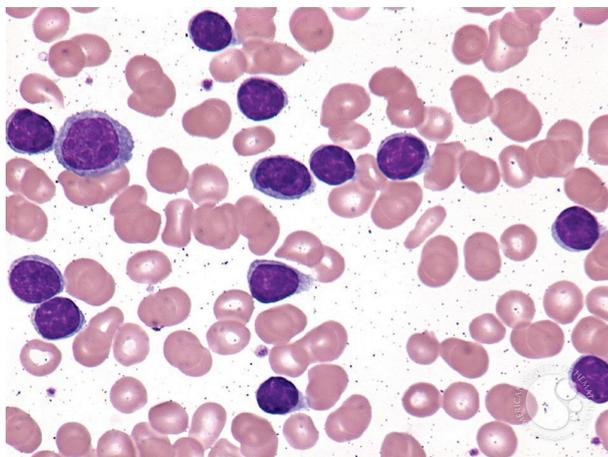


**Figure 2. Morphologic picture of CLL cells.** CLL cells (stained purple) recognized as mature lymphocytes with high nuclear cytoplasmatic ratio. Hematoxylin and eosin staining.
*Reprinted with permission from the ASH Image bank. This image was originally published in ASH Image Bank. Author: Peter Maslak. ASH Image Bank. 2010; image number 00001234. © the American Society of Hematology.*

Small lymphocytic lymphoma (SLL) is a state with lymphadenopathy and/or splenomegaly which differs from CLL by the absence of peripheral lymphocytosis [15].

The tissue morphology and immunophenotype is otherwise the same as for CLL cells but the peripheral blood count of B-lymphocytes must be < $5 \times 10^9$/L for the diagnosis of SLL [50]. SLL further differs from CLL in staging [52] and to some extent in management [53].

In approximately 5-10 % of CLL patients the disease transforms into an aggressive lymphoma, also called Richter transformation (RT). The most frequent transformation is into DLBCL. Transformation into Hodgkin´s lymphoma and other highly malignant disorders are more uncommon. The median time to transformation is about 2 years from CLL diagnosis but CLL can also debut in the form of RT and the range in time to transformation in previous reports is wide (0-12 years) [54, 55]. Most cases (80%) of RT are clonally related to the CLL clone they arose from, while a minority seems clonally unrelated and have features more similar to *de novo* DLBCL. This latter group has been associated with a better outcome [56].

## 2.4    CLINICAL STAGING AND PROGNOSTIC FACTORS

Based on physical examination investigating the presence of lymphadenopathy and hepatosplenomegaly as well as laboratory findings of anemia and/or thrombocytopenia, there are two generally accepted staging systems for CLL: Rai [57] (stage 0-IV) and Binet (stage A-C) [58]. Both systems divide patients into three prognostic subgroups: low, intermediate and high-risk disease. Although these two systems are easy to use in clinical routine, they provide limited information on the risk of disease progression and the risk of requiring treatment. There are several other prognostic markers adding important information including cytogenetic aberrations and IGHV mutational status [15].

Aberration in *TP53*, is both a strong prognostic factor associated with inferior outcome and a predictive factor, influencing the choice of treatment as patients with *TP53* aberrations responds poorly to chemoimmunotherapy (CIT) [45].

Long-term follow-up after CIT with fludarabine, cyclophosphamide and rituximab (FCR), shows that patients with IGHV-M subtype more often have longer remissions and improved OS [59, 60]. Analysis of IGHV status before initiation of treatment has recently been implemented in the Swedish CLL treatment guidelines. The IGHV status is stable during the course of the disease and thus only needs to be analyzed once [27].

Higher levels of serum $\beta_2$-microglobulin, a membrane protein that constitutes a part of the human leukocyte antigen (HLA) class I molecule, has proven to be an independent prognostic marker for worse outcome in CLL [61]. Falling levels down to normal have also been associated with improved PFS in patients treated with ibrutinib while no such association was found with CIT [62]. Currently $\beta_2$-microglobulin has no influence on treatment decisions in Swedish CLL treatment guidelines [27].

The CLL International Prognostic Index (CLL-IPI) is a relatively new prognostic model that integrates five prognostic variables: clinical stage (Binet A or Rai 0 vs Binet B–C or Rai I–IV), age (≤ 65 years vs > 65 years), IGHV mutational status (M vs UM), serum $\beta_2$-microglobulin (≤ 3.5 mg/L vs > 3.5 mg/L) and *TP53* status (no abnormalities vs del(17p) and/or *TP53* mutation). Four prognostic subgroups can be differentiated from this model; low, intermediate, high and very high risk categories with a 5-year OS of 93.2%, 79.3%, 63.3% and 23.3% respectively [63].

Another prognostic score recently presented is the International Prognostic Score for Early-Stage CLL (IPS-E) aiming to predict time to first treatment in patients not requiring treatment directly at diagnosis. Based on IGHV status, absolute lymphocyte count (above or below $15 \times 10^9/L$) and the presence or absence of palpable lymph nodes, IPS-E divides patients into low-, intermediate- and high-risk groups. These groups are separated by different probabilities of initiating treatment within 5 years of 8.4%, 28.4% and 61.2% respectively [64].

There are various factors associated with development of Richter transformation. Some clinical risk factors described are high stage, poor performance status, elevated lactate dehydrogenase (LDH) level, bulky lymphadenopathy as well as an increased risk of RT with several lines of therapy. Biological factors as IGHV-UM, *NOTCH1* mutation and absence of del(13q) have also been linked to a higher risk of RT. The prognosis with RT is considered poor, often less than one year in median OS [54, 56].

## 2.5   TREATMENT AND OUTCOME

### 2.5.1  Treatment indications

So far there is no evidence that CLL patients without active or symptomatic disease gain in overall survival if treated [65-67] why asymptomatic patients with low risk CLL (IPI 0-1) are recommended a strategy of "watch and wait" with regular check-ups [15]. According to the current International Workshop on CLL (iwCLL) Guidelines [15] indication for treatment exists when at least one of following criteria is met: bone marrow failure with aggravation of anemia (Hb < 100 g/L) and/or thrombocytopenia (platelet count < 100 x $10^9/L$), massive ($\geq$ 6 cm below the left costal margin) or progressive or symptomatic splenomegaly, massive ($\geq$ 10 cm) or progressive or symptomatic lymphadenopathy, doubling of lymphocyte counts (applies if the count is > 30 x $10^9/L$) within 6 months or progressive lymphocytosis with an increase of $\geq$ 50% within 2 months, autoimmune induced anemia or thrombocytopenia with poor response to steroids, symptomatic extra nodal involvement or finally at least one of the following symptoms: unintentional weight loss ($\geq$ 10% within 6 months), fatigue ($\geq$ ECOG 2), fever ($\geq$ 38.0°C for 2 or more weeks) or night sweats ($\geq$ 1 month) without signs of infection [15].

If and how long these recommendations will persist after the introduction of new agents remains to be found out. However, a trial randomizing early stage (Binet A) patients without previous treatment to the BTK inhibitor ibrutinib versus placebo could not demonstrate an OS benefit after a median of 31 months but continued follow-up is ongoing [68].

### 2.5.2  Evaluation of treatment

According to the iwCLL guidelines on response assessment [51] the lymphocyte count must be below $4 \times 10^9/L$ for achieving complete remission (CR) and further absence of lymphadenopathy, splenomegaly, hepatomegaly and disease-related constitutional symptoms is required as well as absence of significant cytopenia. A normal bone marrow aspirate or biopsy is needed to confirm CR. Partial remission (PR) requires at least 50%

decrease in lymphocytosis and lymphadenopathy. Progression of lymphadenopathy, splenomegaly, hepatomegaly or rise of any new lesion, increase in lymphocytosis by 50% or more as well as occurrence of cytopenia due to the disease is considered as progressive disease (PD). After a treatment with fixed duration the response assessment should be performed after at least 2 months. If none of the above criteria is met, the response is assessed as stable disease (SD) [51].

In daily clinical practice a bone marrow examination is not always performed if it does not affect further management and should then be registered as PR, although often documented as "clinical CR" in the medical record. If a bone marrow examination is performed and all other criteria for CR is fulfilled but cytopenia is present due to drug toxicity, the category CR with incomplete marrow response (CRi) is sometimes used [51].

### 2.5.2.1 *Minimal residual disease*

In many clinical studies the depth of response to treatment is measured as the count of CLL cells detected in blood or bone marrow after treatment and referred to as minimal residual disease (MRD). MRD has demonstrated a strong prognostic value [69, 70] and the depth of remission after chemoimmunotherapy seems to impact the outcome [71, 72] but will have to be evaluated further before implementation in routine clinical care.

Flow cytometry is the standard method for determining the level of MRD and less than one leukemic cell out of 10.000 leukocytes ($10^{-4}$) is referred to as MRD negativity [73]. Weather more sensitive methods as real-time quantitative polymerase chain reaction (RQ-PCR) and gene sequencing (with detection levels of $10^{-5}$ and $10^{-6}$ respectively) will take place in clinical routine in the future, helping to predict relapses and becoming a tool for individualized and possibly time-limited therapy remains to be proven [74]. MRD and its role as prognostic marker and potential guidance in discontinuation of new treatment strategies are currently being evaluated in prospective settings [75] with new agents and combinations (NCT02401503, NCT02910583).

## 2.5.3  Cytostatic agents

### 2.5.3.1 *Brief history and alkylating agents*

Among the first known papers on CLL treatment was published 1924 describing the reduction in size of enlarged lymph nodes after radiotherapy but without effect on the natural course of the disease [76]. Today, radiotherapy plays a minor role in the treatment of CLL mainly as local control and in debulking large tumor mass in palliative intent. CLL is less sensitive to radiotherapy than other indolent lymphomas for example follicular lymphoma [27, 77].

In the 1950's and 1960's, an antileukemic but transient effect on CLL of the nitrogen mustard compound chlorambucil (CLB) and also corticosteroids was found [78, 79] and since then incredible advances have been made regarding treatment of CLL. During many decades' monotherapy with alkylating agents such as CLB and cyclophosphamide were standard treatments in 1st line for most CLL patients. As new agents emerged, CLB

is today considered only as an option in palliative situations for elderly or fragile patients with the convenience of low toxicity and oral availability [80].

Alkylating drugs exert their antitumor effect by cross-linking DNA thus inducing apoptosis in leukemic cells. Upregulation of *TP53* is a result of DNA alkylation and aberrations in *TP53* is linked to drug resistance and strongly correlates with inferior response upon treatment with alkylating agents [81].

### 2.5.3.2   Fludarabine

Purine nucleoside analogues, inhibiting DNA synthesis and eventually inducing apoptosis, is another group of cytotoxic agents which has played a major role in the treatment of CLL patients over the years [82, 83]. In the beginning of the millennium, fludarabine (F), an intravenously administered fluorinated adenosine analogue, demonstrated improved outcome in treatment naïve patients compared to CLB [84]. However, no significant improvement in OS was seen (median OS 66 months for F vs 56 months for CLB, $p$=0.21). The combination of F and CLB turned out to be too toxic and inclusion in this arm closed prematurely [84]. In the CLL5 trial also investigating F versus CLB in 1st line but in elderly patients (> 65 years), there was a benefit in response rates for F over CLB but no benefit in either PFS or OS. Thus, CLB remained an important option in 1st line for patients older than 65 years [85].

During the 1990´s the combination of fludarabine (F) and cyclophosphamide (C), FC, began to be explored and this combination showed superiority in PFS but no OS benefit over F alone was observed [86, 87]. In the large three-armed CLL4 trial, superior 5-year PFS rates of 36% with FC versus 10% with F and further 10% with CLB ($p$<0.00005) was demonstrated and FC suggested as new standard of care in 1st line. FC was superior even in elderly patients (> 70 years) and in patients with IGHV-U status but patients with del(17p) still had a poor outcome with FC. No OS benefit was demonstrated between the three arms, 5-year OS was 54% with FC, 52% with F and 59% with CLB ($p$=0.2) [88].

### 2.5.3.3   Bendamustine

Bendamustine (B), is an intravenously administered purine antimetabolite which also has cytocidal effects based on cross-linking of DNA by alkylation, leading to defect DNA synthesis and repair, yet in a different way compared to conventional alkylating agents such as CLB [89]. Bendamustine has shown efficacy in both upfront and in relapsed/refractory (R/R) setting of CLL with an acceptable safety profile [90, 91]. Compared to CLB, bendamustine showed a significant increase in response rates and prolonged PFS (median PFS 21.6 months for B vs 8.3 months for CLB, $p$<0.0001) in treatment naïve patients with advanced stage disease (Binet stage B or C). No significant difference in OS was seen after a median follow-up of 35 months [90]. Compared to F in R/R setting bendamustine seemed to have an at least equivalent effect (median PFS of 20.1 months with B vs 14.8 months with F, $p$=0.53) [91].

In today´s treatment arsenal chemotherapy only has a place combined with a monoclonal antibody, referred to as chemoimmunotherapy (CIT) or in combination with targeted agents [27].

## 2.5.4  Monoclonal antibodies

### *2.5.4.1  Anti-CD20 antibodies*

Anti-CD20 antibodies can be divided into two different types (type I and type II) depending on the way they interact with the CD20 antigen (Figure 3) and the main mechanisms they exert on the B-cell which will be described in following sections [92].
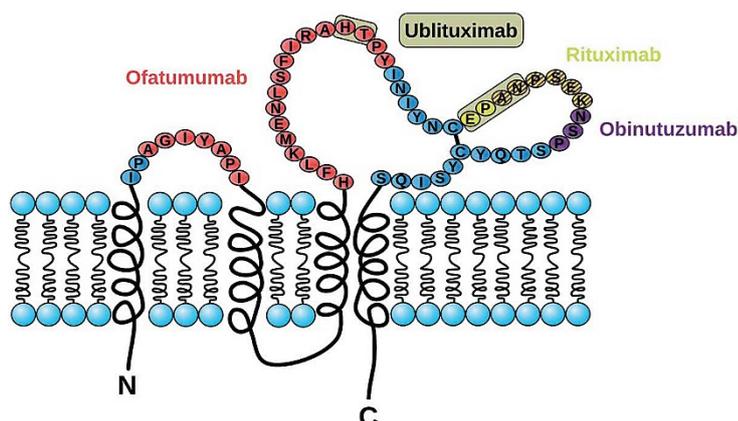


**Figure 3. Different epitopes on the CD20 transmembrane protein recognized by the monoclonal type I antibody rituximab (yellow), the type I antibody ofatumumumab (red), the type II antibody obinutuzumab (purple) and the novel type I antibody ublituximab (boxes).**
*Reprinted with permission from Taylor and Francis from Babiker et al. Ublituximab for the treatment of CD20 positive B-cell malignancies. Expert Opinion on Investigational Drugs. 2018;27:407-412.*

*Rituximab*

Rituximab (R), a type I antibody, was in 1997 the first FDA-approved monoclonal antibody (mAb) for treating non-Hodgkin lymphoma (NHL) after presenting an overall response rate (ORR) of nearly 50% and manageable toxicity used as monotherapy in patients relapsing from indolent lymphomas (CLL excluded) [93]. In CLL, rituximab alone later showed a limited effect but improved ORR in combination with fludarabine [94]. However, rituximab was not approved in CLL until 2010, having showed superiority in both PFS and OS in combination with FC (FCR) over FC alone (3-year OS 87% with FCR vs 83% with FC, $p$=0.01) in frontline setting within the CLL8 trial [95] and moreover superior PFS in R/R setting within the REACH trial [96]. The introduction of rituximab and its combination with other therapy regimens, especially with chemotherapy, has definitely altered the management and outcome in CLL patients [59, 95].

Rituximab is a genetically engineered chimeric human/mouse anti-CD20 mAb, consisting of human constant regions of the immunoglobulin (Ig) IgG1 and murine variable regions. CD20 is a transmembrane antigen believed to function as a calcium channel engaged in signaling through the BCR. CD20 is presented on most maturation stages of normal B-lymphocytes except in hematopoietic stem cells, the earliest pro-B-cells and later plasma cells. CD20 is also expressed on the surface of lymphocytes from over 90% of CLL patients and patients with other B-cell malignancies [93]. The antigen-binding fragment of rituximab binds to CD20 on B-cells and the constant region of the antibody mediates different effector steps to B-cell lysis primarily via complement-

dependent cytotoxicity (CDC) and through a smaller extent via antibody-dependent cellular cytotoxicity (ADCC) through granulocytes, macrophages and natural killer cells [97].

Rituximab is relatively well tolerated. Infusion-related adverse events (AEs) during the first treatment is the most frequently occurring AE (majority being grade 1-2) but more serious reactions do occur. The risk of infusion-related events can be reduced by giving a lower dose at a slower administration rate the first treatment and by proceeding to subcutaneous administration the following cycles if no complications have occurred [97, 98]. Late onset neutropenia (LON), defined as ≥ grade 3 neutropenia that occurs > 4 weeks after treatment cessation with rituximab, is usually transient and a wide range of incidence (5-30%) is described in NHL patients [99]. Except for inherent drug resistance in the rare CD20 negative cases (1-2%), development of drug resistance can be acquired and other CD20 antibodies have been investigated trying to bypass the mechanisms of resistance to rituximab including structural changes in the CD20 molecule and loss of its expression on the cell-surface [100].

*Ofatumumab*

Ofatumumab, a type I antibody, but in contrast to rituximab, fully humanized and binds a different epitope of the CD20 molecule (Figure 3). Ofatumumab binds with a stronger affinity and induces a more effective CDC. As a single agent it has demonstrated clinical effect in patients refractory to fludarabine with an ORR of nearly 50% but with a short duration of response of approximately 6 months [101]. Currently ofatumumab is not authorized in CLL treatment.

*Obinutuzumab*

Obinutuzumab (initially named GA-101 and sometimes abbreviated G) is a humanized mAb which has demonstrated activity as monotherapy in patients with R/R CLL within the phase 1/2 GAUGUIN-trial [102]. Obinutuzumab is categorized as a type II antibody and in that sense differs from type I antibodies as rituximab and ofatumumab regarding the binding to CD20 (Figure 3) and the cytotoxic mechanisms exerted. The primary killing mechanism of ofatumumab is ADCC and compared to type I antibodies it induces a more intensive activation of programmed cell death [92].

### 2.5.4.2 Anti-CD52 antibody

Alemtuzumab, a humanized antibody targeting the glycoprotein CD52 expressed on most normal lymphocytes and on both B- and T-lymphoma cells, exerts its effect primarily through ADCC and CDC [103]. Alemtuzumab initially received attention in the search for treatment of graft-versus-host disease (GvHD) but later showed promising results in previously treated CLL patients [104] and also in the presence of *TP53* anomalies [105] and further superior PFS, ORR, CR and MRD-negativity rates in relation to CLB in frontline therapy [106]. Alemtuzumab is no longer authorized in CLL but available through a compassionate use program.

## 2.5.5   Chemoimmunotherapy

### 2.5.5.1   *Chemotherapy in combination with rituximab*

*FCR*

FCR, a combination of the purine analogue fludarabine (F), the alkylating agent cyclophosphamide (C) and the mAb rituximab (R) was until recently gold standard in 1st line treatment for most physically fit CLL patients [107] and remains an option for fit patients without *TP53* aberrations, especially in case of IGHV-M status [15].

The addition of rituximab to FC was shown to significantly improve PFS and OS in the CLL8 trial comparing FCR and FC in fit, previously untreated patients with a median age of 61 years. The survival benefit of adding rituximab to FC was demonstrated in most subgroups but not in patients possessing del(17p) where only 38% vs 37% (*p*=0.25) were alive 3 years after randomization [95].

Long-term follow-up after a median of nearly 6 years, showed a median PFS of 57 months for FCR vs 33 months for FC (*p*<0.001). Median OS was still not reached with FCR vs 86 months with FC (p=0.001). Especially patients with IGHV-M status had a clear benefit in outcome with FCR. At 5-year follow-up 86% of patients with IGHV-M status treated with FCR were still alive [59]. In a long-term follow-up of a phase 2 study comparing 1st line FCR to FC, no relapses were observed at 10-year follow-up in 42 patients with IGHV-M status treated with FCR. These findings have given rise to the idea of a possible cure in this patient group [60].

Even if efficient, toxicity issues can complicate the treatment with FCR. Neutropenia and infections ≥ grade 3 has been reported in about one third of patients and more frequently occurring in elderly patients [108].

*BR*

Results from the randomized CLL10 trial comparing 1st line CIT with FCR versus bendamustine in combination with rituximab (BR), in physically fit patients with a median age of 62 years and without del(17p), showed significantly longer PFS with the FCR regimen [108]. Median PFS was 55 months for FCR vs 42 months with BR (*p*=0.0003) at a median follow-up of 37 months. A lower proportion of patients treated with BR achieved CR and MRD negativity but the toxicity with BR was less pronounced. However, in patients older than 65 years no significant difference in PFS was seen but increased toxicity with the FCR regimen [108]. In the extended follow-up (median follow-up 58 months) median OS was still not reached for any arm (5-year OS rates of 80.9% for FCR vs 80.1% for BR, *p*=0.599) but a continued longer PFS for FCR of about 15 months was noted. Proportions of patients developing secondary malignancies were similar for both treatment arms regarding patients ≤ 65 years (13% vs 14%) but more frequently occurring in patients > 65 years treated with FCR (33% vs 17%) [109].

As BR is considered a 1st line option for fit patients older than 65 years, less is known about BR in subgroups such as unfit and very elderly patients. Real-world data has suggested frontline BR to be effective in patients without *TP53* aberrations and Cumulative Illness Rating Scale (CIRS) score > 6 (CIRS scale ranging from 0-56 with higher score associated with worse health status) but for patients with advanced disease,

the targeted agent ibrutinib had a longer PFS in this indirect comparison [110]. However, an indirect comparison in 2nd line on a study population where most patients were older than 65 years, no difference in OS between BR and ibrutinib in patients without *TP53* disruptions treated within clinical routine was observed, indicating that these treatment regimens may have similar effectiveness as first salvage treatment in this population [111]. A real-world study from our own research group, further showed that risk-adapted BR can be a tolerable treatment option even in the very elderly subgroup ($\geq$ 80 years) [112].

*R-CLB*

The combination of rituximab and CLB (R-CLB) was evaluated in a phase 2 trial as many patients with CLL are elderly and comorbid and since more intense chemotherapy had failed to show any major clinical benefit in this population. Treatment with R-CLB indicated improved outcome with an acceptable safety profile [113] inspiring further investigation of CLB in combination with anti-CD20 mAbs.

In the MABLE trial rituximab combined with either bendamustine (BR) or chlorambucil (R-CLB) as 1st line treatment in patients with a median age of 72 years and considered fludarabine-ineligible was studied. Significant advantage regarding CR rates, MRD negativity and PFS for BR over R-CLB was shown. Although no significant improvement in OS was observed [114]. The role for CLB in combination with a mAb is today limited to fragile patients with significant comorbidity burden [27].

### 2.5.5.2 *Chemotherapy in combination with obinutuzumab or ofatumumab*

In the large three-armed CLL11 trial patients with a relatively high comorbidity burden (CIRS > 6) and a median age of 73 years were randomized to CLB alone or in combination with either of the anti-CD20 antibodies rituximab (R-CLB) or obinutuzumab (G-CLB). An OS benefit with G-CLB compared to monotherapy with CLB was shown, introducing G-CLB into a new standard of care in older and/or fragile patients without *TP53* aberrations. MRD negative status was ten times more frequent with G-CLB than with R-CLB. Compared to CLB monotherapy either combination with a mAb was associated with better response rates and improved PFS [115]. In the long-term follow-up of the CLL11 trial at a median follow-up of almost 5 years, G-CLB also demonstrated a significant survival advantage over R-CLB (median OS not reached for G-CLB vs 73 months for R-CLB, *p*=0.0245) [116].

CLB plus ofatumumab also showed a benefit compared to monotherapy with CLB as 1st line treatment in elderly comorbid patients [117]. This was recently confirmed in the 5-year follow-up of the COMPLEMENT 1 trial where a significant PFS improvement was seen (median PFS 23 months with CLB plus ofatumumab vs 15 months with CLB alone, *p*<0.001). There was a trend towards a survival benefit for the chemoimmunotherapy arm although not statistically significant (median OS not reached for CLB plus ofatumumab vs 85 months with CLB alone, *p*=0.363). This might be confounded by following salvage therapies considering the long observation period [118].

## 2.5.6 Targeted agents

Today there are several agents targeting specific pathways in the CLL cells. Targeted therapy regimens have become a favored therapeutic option for many patients requiring treatment [27, 53]. Agents inhibiting the B-cell receptor associated kinases Bruton tyrosine kinase (BTK), phosphatidylinositol 3-kinase-delta (PI3Kδ) and moreover inhibition of the antiapoptotic protein BCL2 has dramatically changed the conditions for many CLL patients [15].

### 2.5.6.1 Ibrutinib

The finding of the orally and thus far continuously administered selective, irreversible inhibitor of BTK, has come to play a major role in the management of CLL [119]. Ibrutinib is the first-in-class BTK inhibitor and by binding to the cysteine-481 residue of the BTK and through this blocking crucial survival pathways in the BCR axis, ibrutinib reduces proliferation, mitigates survival signals from the microenvironment and to some extent also increases apoptosis of CLL-cells [120]. After promising results in phase 1b/2 trials [121, 122] ibrutinib was found to induce both significantly longer PFS and OS compared to ofatumumab in a multicenter phase 3 study (RESONATE) in R/R CLL patients [23]. The RESONATE trial led to FDA-approval of ibrutinib in R/R patients in 2014. Long-term follow-up of up to six years demonstrated continued benefit for ibrutinib over ofatumumab (median PFS of 44.1 months vs 8.1 months, p<0.001). Robust improved outcomes were shown even in patients with high risk genetic features such as *TP53* aberrations and IGHV-U status. A minor, yet significant benefit also remained in OS for ibrutinib vs ofatumumab (67.7 months vs 65.1 months, HR: 0.639; 95% CI: 0.418-0.975) despite that almost 70% of patients on ofatumumab later switched to ibrutinib, confirmed in a sensitivity analysis. Median OS in the subgroup with *TP53* aberrations treated with ibrutinib was 61.8 months [123].

Ibrutinib later showed to be superior compared to monotherapy with CLB in previously untreated patients with a median age of 73 years in the RESONATE-2 trial leading to approval of ibrutinib also in 1st line in 2016 [124]. Recently published 5-year follow-up data from the RESONATE-2 trial confirmed superiority in PFS and OS for ibrutinib over CLB [125].

Commonly reported AEs with ibrutinib are diarrhea, arthralgia and minor bleedings but these are usually transient and of low grade. Most common AEs ≥ grade 3 of hematological origin reported with ibrutinib in the RESONATE-2 trial were neutropenia (25%) followed by thrombocytopenia (10%) and anemia (9%). The most common non-hematological AE ≥ grade 3 was pneumonia (21%). Moreover, hypertension was observed in 9%, diarrhea in 7% and atrial fibrillation in 6%. Importantly, most clinically relevant side effects seemed to diminish with time, hypertension and minor bleedings excluded [125].

Real-world data on ibrutinib from our research group, which was the first published RWD on patients treated with ibrutinib within a compassionate use program and of whom only 50% would have been eligible for the RESONATE trial, showed effectiveness and toxicity similar to results of the pivotal trial. Inferior outcome was observed in patients with *TP53* aberration at 10-months follow-up but at 30-month

follow-up the negative impact of *TP53* aberrations did not seem to last [22, 126]. We also have an ongoing RWS to map the incidence of cardiovascular and bleeding events in CLL patients treated with ibrutinib between 2013 and 2020, but data are not yet published.

A retrospective cohort study on previously untreated patients receiving ibrutinib within routine clinical care further demonstrated that 57% of this cohort would have been ineligible to the pivotal RESONATE-2 trial due to age < 65 years and/or presence of del(17p), two important study populations where RWD are useful to inform and reflect clinical practice on possible differences in outcome of such subgroups. For example, patients with del(17p) had inferior PFS and OS and moreover a higher rate of RT [127]. A greater proportion of patients treated within clinical routine terminated treatment due to AEs compared to the trial population and toxicity was the most common cause for treatment discontinuation both in this cohort and within another large real-world analysis [128]. Moreover, patients with lower start dose than the recommended dose of 420 mg daily used within the pivotal trial, had inferior PFS while dose interruption of eight or more days did not seem to influence PFS [128].

Although PR still dominates as response with ibrutinib as a single agent, the rate of CR increased with time. A third of patients finally reached CR/CRi at 5-year follow-up within the RESONATE-2 trial [125]. Despite its clinical success patients still relapse or progress on ibrutinib. While patients not responding to treatment initially or progress early during the treatment period, more commonly reveals with Richter transformation, patients with later progress or relapse have been found with acquired mutations in the *BTK*- or *PLCγ2*-gene rendering clonal evolution which probably is one of the explanations of drug resistance [129, 130]. A point mutation in the binding pocket of *BTK* (cysteine-481) is the most frequent found mutation and can be detected over a year before clinical progression or relapse. This might become a useful marker to identify patients at risk of developing drug resistance and in need of change in treatment strategy [129, 131].

### *2.5.6.2 Ibrutinib as monotherapy versus combination regimens*

Given the relatively low frequency of CR with ibrutinib alone, indicating that treatment may need to be unlimited, the risk of adverse long-term effects, clonal evolution and further the cost increases with this continuous treatment strategy [19-21]. There is an ongoing pilot study in Sweden coordinated from our research group, on intermittent treatment with ibrutinib with the aim to investigate the effects, adverse events and development of resistance with this ON-OFF strategy (EudraCT 2017-001990-17) but data are not yet published.

One aspect pointing interest towards combination strategies is to diminish the pressure of clonal evolution. Ibrutinib have been and is evaluated in combinations with other agents in order to ameliorate outcomes where examples of some recent trials follow.

Ibrutinib in combination with rituximab (IR) demonstrated earlier and deeper remissions compared to ibrutinib alone but no significant benefit in ORR, CR rate, PFS or OS was presented with the addition of rituximab to ibrutinib in a randomized trial on a study population with mainly relapsed patients. Ibrutinib as monotherapy was therefore proposed to remain standard of care [132].

In the ALLIANCE trial ibrutinib alone (I) or in combination with rituximab (IR), clearly improved PFS compared to CIT with BR in previously untreated patients 65 years or older (estimated 2-year PFS of 87% for I vs 88% for IR vs 74% for BR, $p<0.001$). On the other hand, the addition of rituximab to ibrutinib did not render a significant improvement in PFS over ibrutinib alone and so far, no OS benefit has been demonstrated between the three treatment groups at a median follow-up of 38 months (estimated 2-year OS of 90% for I vs 94% for IR vs 95% for BR, $p \geq 0.65$) [133].

Frontline IR was further compared to FCR in physically fit patients 70 years or younger without *TP53* aberrations in the ECOG-ACRIN trial. Results showed a clear benefit in both PFS and OS for IR over FCR (3-year PFS 89% vs 73%, $p<0.001$, 3-year OS 99% vs 92%, $p<0.001$). Subgroup analysis revealed an even clearer advantage for patients with IGHV-U status while no difference was seen for the IGHV-M group. There was no ibrutinib monotherapy arm in this study [134].

In the iLLUMINATE trial, ibrutinib plus obinutuzumab yielded longer PFS than CLB plus obinutuzumab in treatment-naïve patients ($\geq$ 65 years or younger patients with comorbidities) irrespective of high-risk features such as IGHV-U status. The authors propose ibrutinib plus obinutuzumab as a safe and efficacious chemotherapy-free treatment regimen in 1st line for this patient population [135]. The iLLUMINATE trial completed recruitment before results of for example the CLL10 [108] and ALLIANCE [133] trial data were available and a comparison of ibrutinib plus obinutuzumab vs BR or vs ibrutinib as a single agent in frontline on elderly, comorbid patients would have been valuable.

### 2.5.6.3 Acalabrutinib and zanubrutinib

In attempt to overcome some of the toxicity issues with ibrutinib, 2nd generation BTK inhibitors have been developed [136]. In November 2019, based on PFS data from two phase 3 trials (ASCEND [137] and ELEVATE-TN [138]), the more selective BTK inhibitor acalabrutinib was FDA approved. Acalabrutinib binds to the same residue, cysteine-481, as ibrutinib and does not overcome the issue with drug resistance of acquired mutations in this site. Although, due to enhanced pharmacological characteristics not inhibiting alternative kinases thought to be involved in some of the adverse events, hopefully acalabrutinib will have a better safety profile with reduction in some of these "off-target" effects [137, 138].

In the ASCEND trial, in R/R setting, patients were randomized to acalabrutinib or idelalisib plus rituximab or bendamustine plus rituximab. In the ELEVATE-TN trial monotherapy with acalabrutinib versus acalabrutinib plus obinutuzumab versus CLB plus obinutuzumab was evaluated in frontline setting. None of these studies had an ibrutinib monotherapy arm, which was considered standard of care at the initiation of these studies and whether acalabrutinib has a better safety profile compared to ibrutinib remains to be proven.

A phase 3 trial comparing acalabrutinib and ibrutinib in R/R setting is ongoing with PFS as primary endpoint and toxicity as a secondary endpoint (NCT02477696). Data from real-world studies may also be helpful mapping the safety and optimal use of BTK inhibitors until convincing evidence is available [139].

Zanubrutinib, a next-generation BTK inhibitor with yet optimized selectivity of BTK is currently being compared to ibrutinib in a phase 3 trial (ALPINE) [140]. Investigated in frontline in patients with del(17p) recently published results demonstrating zanubrutinib to be efficacious with an ORR of 94.5%, PFS of 88.6% and OS of 95.1% at 18-month follow-up. Zanubrutinib was further relatively well tolerated [141].

### 2.5.6.4 PI3K inhibitors

Agents inhibiting the phosphatidylinositol 3-kinase-delta (PI3Kδ), mediating signals downstream the BCR, have also showed to be active in CLL [142]. Idelalisib, an oral PI3Kδ inhibitor in combination with rituximab, showed significantly longer PFS and OS in R/R CLL patients (considered ineligible to standard chemotherapy) compared to placebo plus rituximab and lead to FDA approval in this patient population in 2014. Median PFS was not reached in the idelalisib group and was only 5.5 months in the placebo group while median OS rate was 92% and 80% respectively at 12 months follow-up and the study closed in advance due to these preliminary data [143]. Study participants, regardless of initial treatment randomization, could receive idelalisib monotherapy after closing of the study and follow-up data confirmed the efficacy of idelalisib +/- rituximab with improved OS, especially in patients with *TP53* aberrations [144].

Studied in frontline setting, idelalisib has been more frequently associated with assumed to be immune mediated severe AEs such as enterocolitis, transaminitis and pneumonitis while these AEs generally has been milder in R/R setting. In previously untreated patients and also in younger subjects treated with idelalisib, suppression in regulatory T-cells has been observed which is believed to be associated with the increased toxicity [145]. Attention on opportunistic infections must also be payed and prophylaxis against pneumocystis jirovecii is recommended as well as monitoring of cytomegalovirus during treatment with idelalisib [144].

In Sweden idelalisib is a treatment option in 2nd or later lines or as frontline therapy in case of *TP53* aberration and if no other treatment options are appropriate [27].

Duvelisib, another PI3K inhibitor targeting two isoforms of PI3K (PI3Kδ and PI3Kγ) thus blocking both autonomous survival and proliferation of B-cells as well as affecting important supportive mechanisms in the tumor microenvironment. Duvelisib was explored in the DUO trial in R/R setting where superiority in comparison with ofatumumab lead to FDA approval 2018 in patients who have received at least 2 previous lines of therapy [146].

### 2.5.6.5 BCL2 inhibitors

After showing promising effect and manageable safety profile in previously treated and high risk CLL patients [147, 148] venetoclax, an oral inhibitor of BCL2 was approved based on the randomized CLL14 trial [149]. Two years after treatment PFS was significantly higher with venetoclax plus obinutuzumab (88.2%) than with CLB plus obinutuzumab (64,1%) in previously untreated patients with coexisting comorbidities.

Treatment with venetoclax implicates a risk of tumor lysis syndrome although this risk has diminished by strategies such as gradual dose escalation [149].

The combination of venetoclax with obinutuzumab was relatively well tolerated even in a population with most participants having CIRS-score > 6 and more than a third of patients being older than 75 years [149].

In the MURANO trial, venetoclax in combination with rituximab (Ven-R) showed superior outcome and higher rates of MRD negativity over CIT with BR [150]. The superiority with Ven-R remained at the 4-year follow-up where MRD negativity was associated with better outcome [151]. Monotherapy with venetoclax has been associated with accumulated drug resistance in almost half of the patients after 2-3 years of treatment. An acquired mutation in the *BCL2* gene has been identified as a potential cause to this phenomenon [152].

## 2.5.7    First line treatment

With the breakthrough the last decade with novel targeted therapies and combinations offering significant efficacy and improved outcome in many CLL patients, several effective treatment options are available and at present there is not just one generally accepted standard treatment. Various trials are ongoing and will most likely continue to alter the management of CLL moving towards becoming increasingly individualized [15].

Before initiating therapy there are many important aspects to consider. The patients age, performance status, comorbidities, the clinical stage and symptoms of the disease, together with genetic aberrations as well as the patients' preferences in cases where not just one obvious alternative prevails, are all important elements.

Participation in a clinical trial should always be offered for all treatment lines if possible, but if not, the choice of treatment today largely depends on *TP53* mutational status, the physical condition of the patient and more recently also IGHV-mutational status [15].

The physical fitness and comorbidity burden of a patient can be assessed in several ways. The Eastern Cooperative Oncology Group (ECOG) performance status is an easy to use scale of functional status ranging from 0 (fully active) to 4 (completely disabled) and is often employed in clinical routine [153]. The Cumulative Illness Rating Scale (CIRS) ranging from 0-56 is one way of estimating comorbidity burden [154]. With a cut-off of 6, patients with a value ≥ 6 are often considered having significant comorbidity but this does not necessarily correlate to reduced physical fitness or performance status [155].

### 2.5.7.1   Patients without TP53 aberrations

For patients without *TP53* aberrations, regardless of IGHV mutational status and fitness, continuous treatment with ibrutinib (until progression or intolerable side-effects) is currently one of the preferred treatment options in previously untreated CLL patients according to the National Comprehensive Cancer Network (NCCN) [53] and the latest European Society for Medical Oncology (ESMO) [156] guidelines.

In Sweden ibrutinib is not reimbursed in previously untreated patients without *TP53* aberrations and until now IGHV mutational status has not affected the choice of treatment. The recommendation has so far been time-limited CIT with FCR for otherwise healthy individuals up until the age of 65-70 years [27]. FCR is still an option to fit and especially to IGHV-mutated patients according to both the NCCN [53] and ESMO [156]

guidelines, since long remissions and even cure is possible in this subgroup [27, 59, 95]. BR is a reasonable alternative option to patients > 65 years or in younger patients with complicating comorbidities [27, 53, 108, 156].

As acalabrutinib recently was approved by the FDA this is an alternative regimen either as monotherapy or in combination with obinutuzumab according to the NCCN Guidelines [53, 138] but the EMA decision is pending and acalabrutinib is thus not yet approved in Sweden.

Venetoclax plus obinutuzumab, a time-limited chemotherapy-free regimen, was recently approved in frontline by both FDA and EMA based on the CLL14 trial where venetoclax plus obinutuzumab showed prolonged PFS (so far no OS benefit) compared to CLB plus obinutuzumab in patients with CIRS > 6 [149]. There is so far no data on venetoclax plus obinutuzumab compared to CIT with FCR or BR and its place in Sweden will probably be less clear until more data are available also in fit patients and until long-term follow-up data exists. However, the combination of venetoclax and obinutuzumab is approved and reimbursed in Sweden as an option in 1st line. The updated Swedish guidelines are planned to be published soon.

To elderly patients with an impaired physical condition and/or significant comorbidity CLB in combination with a CD20 mAb or BR can be offered [27, 53, 115, 156].

### 2.5.7.2   Patients with TP53 aberrations

A BTK inhibitor as ibrutinib monotherapy or acalabrutinib (the latter not approved in Sweden yet) +/-obinutuzumab or venetoclax plus obinutuzumab or idelalisib plus rituximab are alternative regimens for patients with *TP53* aberrations according to NCCN and ESMO guidelines [53, 149, 156]. CIT shall not be applied in these patients. According to Swedish guidelines ibrutinib has been the preferred regimen so far and if not suitable venetoclax or idelalisib in combination with rituximab has been recommended [27].

## 2.5.8   Relapsed/refractory setting

Refractory disease is defined as treatment failure or progressive disease within less than 6 months from the end of treatment. Relapse is defined as progressive disease more than 6 months after the end of treatment in a patient who previously fulfilled the criteria for CR or PR [51]. In patients where relapse occurs after more than 36 months the 1st line treatment can be repeated. In case of earlier relapse or in refractory cases the treatment regimen should be changed.

As in frontline treatment there is no universal 2nd line treatment recommended and if possible, the patient should be offered participation in a clinical trial. If not, there are several choices depending on which treatment was used in 1st line, the age and fitness of the patient, *TP53* status and whether specific adverse events are desirable to avoid [15].

Some of the potential options are ibrutinib (or acalabrutinib if available and approved), venetoclax plus rituximab or idelalisib plus rituximab [53, 156]. In Sweden ibrutinib is the preferred choice regardless of *TP53* aberrations or not and if intolerance against ibrutinib or in case of progression, a switch to venetoclax has been recommended in parallel to evaluation of allogeneic stem cell transplantation (SCT) [27].

With the access of several new treatment strategies for R/R patients the use of allogeneic SCT has decreased but might still be indicated in patients with del(17p) if in good physical condition and responding to 2nd line treatment [157].

## 2.5.9  Covid-19 aspects

Present year's exceptional circumstances with the coronavirus disease 2019 (Covid-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are affecting virtually all aspects of health care. Cancer care faces numerous difficulties with the management of immunocompromised patients having an increased risk of serious events related to Covid-19 and to keep routine care of patients, treatments and follow-up running in the safest possible way [158]. Collaborations all over the world have been established to exchange experiences and elaborate guidelines on the management of cancer patients during these special and rapidly changing conditions [158]. To be able to maintain pivotal randomized trials open and running as far as possible without compromising the safety of patients, particular guidelines on the conduct of trials have been developed by the FDA [159].

Regarding the impact of Covid-19 on clinical management of CLL in Sweden, temporary recommendations have been developed by the Regional Cancer Center (RCC) and these are continuously updated as the epidemic scenario changes [160]. At present, routine visits should be limited and, if possible, be replaced by phone calls or video meetings, CIT should be avoided whenever possible and orally available agents such as ibrutinib are recommended without the addition of rituximab to further avoid hospital visits and to mitigate the risk of neutropenia [160].

CLL patients generally have an increased risk of infections. Inherent immune abnormalities in the humoral immunity with hypogammaglobulinemia being a common manifestation and moreover defects in the cell-mediated immunity and complement system are associated with the CLL disease itself. Immune defects are associated with all stages of CLL, even in newly diagnosed patients and patients under watchful waiting [161, 162]. Moreover, treatment related suppression of the immune system is common with various effects and a range of different infections more or less commonly associated with specific drugs [162, 163]. An intact immune response has been suggested to be necessary to clear the SARS-CoV-2 virus and it is also known that patients with a defect immune system usually have a poorer response to vaccines [158].

Observational studies can be of great value to increase the understanding of how the Covid-19 disease affect certain subgroups such as immunocompromised cancer patients in a situation where much is unknown, to add to the body of knowledge from ongoing randomized studies [158].

A recent international observational study on outcomes of Covid-19 in 198 CLL patients showed high mortality rates both in previously untreated patients under surveillance and in patients with active disease and ongoing treatment. Forty-five percent had ongoing CLL-treatment at Covid-19 diagnosis, the majority with ibrutinib. Thirty-nine percent were previously untreated and under "watch and wait" at Covid-19 diagnosis. No difference was observed regarding hospitalization rate, need of intensive care or mortality in patients with ongoing treatment or patients under "watch and wait".

Further, ongoing treatment with BTK inhibitor at Covid-19 diagnosis did not seem to impact mortality, although the treatment was temporarily interrupted in most cases [164].

A retrospective analysis of 190 CLL patients diagnosed with Covid-19 in Spain and Italy during this spring demonstrated that almost 80% of the patients needed intensive care or hospital admission for oxygen and of these patients the majority (77%) did not have an active disease or ongoing treatment. Patients on BTK inhibitor had a lower rate of hospitalization compared to patients with other treatment regimens and compared to patients currently off treatment. Although, looking at the characteristics of the patients who died of Covid-19, 19 out of 56 patients had ongoing treatment and of those 19 patients, 12 were on BTK inhibitor [165].

There is also an ongoing real-world analysis of CLL patients diagnosed with Covid-19 in Sweden with a planned subanalysis in the Stockholm region, looking at the immunological response of the disease, but so far, no results are published.

It has been suggested that BTK inhibitors reduce the hyperinflammatory reaction and respiratory failure caused by SARS-CoV-2 [158] and clinical trials are running to gain evidence on the effects of BTK inhibitors in patients with Covid-19 (NCT04346199, NCT04380688, NCT04375397).

## 2.5.10 Novel and emerging treatments and strategies

Even if a part of the CLL patients potentially can be cured and the advent of new targeted agents dramatically has altered the management and outcome of CLL patients with enduring responses, patients still suffer from relapses and toxicity issues. Intensive research is ongoing to find new effective and safe agents, refinement of approved treatments and exploring new combinations and sequences of treatments.

Due to resistance issues even with 2$^{nd}$ generation BTK inhibitors, reversible BTK inhibitors (vecabrutinib, ARQ-531, LOXO-305) are evaluated in phase 1/2 studies (NCT03037645, NCT03162536, NCT03740529) and may get around resistance caused by point mutation in the binding pocket of BTK (cysteine-481) since they inhibit BTK independent of this residue [166].

Novel anti-CD20 mAbs such as ublituximab and novel PI3Kδ-inhibitors such as umbralisib, have also been developed with the aim of less resistance and toxicity. A combination of these two agents (U2) have been investigated in a phase 1 study in R/R setting showing encouraging results [167] and is now evaluated in a phase 3 trial against CLB plus obinututzumab (NCT02612311).

There are several combination and sequential strategies under investigation in trials with the aim of preventing clonal evolution, drug resistance and to reach deep and durable remissions where some data were presented at EHA 2020. In the CLARITY trial (ISRCTN13751862), ibrutinib in combination with venetoclax is evaluated in R/R patients and nearly 50% (24/50) had reached < 0.01% MRD in bone marrow at 26 months follow-up and it seems as if patients reaching MRD negativity faster have more durable responses [168]. In the CAPTIVATE trial (NCT02910583), fixed duration of ibrutinib monotherapy followed by ibrutinib in combination with venetoclax in previously untreated patients is under investigation. Preliminary results show high rates of MRD negativity and tolerability where most patients completed planned treatment [169].

Moreover, obinutuzumab in combination with ibrutinib plus venetoclax in high risk treatment naïve patients is evaluated in the CLL2-GIVE trial (NCT02758665) where 80,5% achieved undetectable MRD at a so far short median follow-up of 18.6 months [170].

There are also several randomized phase 3 trials that either have completed or are under active recruitment or are about to start recruiting patients to different combinations and sequences of therapies. The CLL13 trial (NCT02950051) randomizing treatment naïve fit patients without *TP53* aberrations to four different arms; CIT with FCR/BR versus venetoclax plus rituximab versus venetoclax plus obinutuzumab versus a combination of the three agents obinutuzumab, ibrutinib and venetoclax, closed last year and so far, no results are published. In the upcoming CLL17 trial (EudraCT 2019–003854–99), ibrutinib alone or in combination with venetoclax will be compared to venetoclax in combination with obinutuzumab.

Pembrolizumab, an immune checkpoint inhibiting antibody blocking the programmed death 1 (PD-1) pathway, important to avoid the control of active T-cells has shown effect in many solid tumors, Hodgkin lymphoma and has also indicated efficacy in CLL patients with Richter transformation but will need to be evaluated further [171]. Pembrolizumab is currently being evaluated in combination with the PI3Kδ-inhibitor umbralisib in R/R CLL patients (NCT03283137).

Chimeric antigen receptor (CAR) T-cell therapy, most available being directed against CD19, is another treatment strategy, tested in CLL patients already a decade ago, and with promising results in several other hematological malignancies [172]. However, CAR T-cell therapy has demonstrated lower efficacy in CLL patients [173]. One possible explanation may be T-cell exhaustion in CLL patients. However, there have been attempts trying to potentiate the efficacy by combining CAR T-cell therapy with ibrutinib [173] and there is currently an ongoing phase 1/2 study combining the anti-CD19 CAR T-cell agent lisocabtagene maraleucel with ibrutinib (NCT03331198).

There is also a phase 1/2 study ongoing, investigating the universal CRISPR-Cas9 gene-editing CAR T-cells targeting CD19 (UCART019) in patients with R/R CD19 positive leukemia and lymphoma (NCT03398967), a technique based on the discovery of a tool for genome editing (CRISPR-Cas9) awarded with this year's Nobel Prize in Chemistry [174].

# 3 DIFFUSE LARGE B-CELL LYMPHOMA

## 3.1 INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is a highly malignant disease with origin in different stages under the maturation process of B-cells. It is characterized by large neoplastic B-cells with a nuclear size equal to or exceeding normal macrophage nuclei or more than twice the size of a normal lymphocyte, arranged in a diffuse growth pattern [175] (Figure 4).
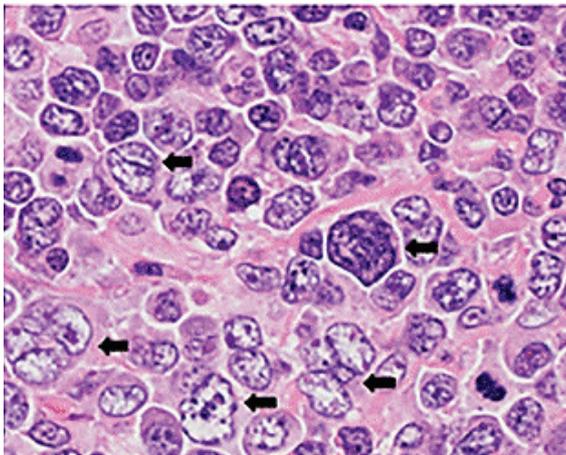


**Figure 4. Morphological picture of diffuse large B-cell lymphoma.** Large malignant lymphocytes (arrows) arranged in a diffuse growth pattern. *Reprinted under the terms of Creative Commons Attribution 4.0 International License from Shimono J. et al. Clinicopathological analysis of polyploid diffuse large B-cell lymphoma. PLoS One. 2018; 13:4. © 2018 Shimono et al.*

DLBCL has been recognized as a heterogeneous disease on several levels, all the way from genetic and molecular aspects through morphological and immunophenotypically features into clinical findings and outcome. Over the years a deeper understanding into the biology of aggressive lymphoma has been reached, subclassifications refined which has yielded several separate diagnostic entities with different management and outcomes (e.g. primary mediastinal large B-cell lymphoma) [176].

In the latest revision of the WHO classification subdivision into cell-of-origin (COO) is required and the former most common group "not otherwise specified" (NOS) should solely be used as a diagnosis of exclusion [177].

DLBCL generally has a quick clinical course with short survival if not treated. The introduction of rituximab (see section *2.5.4.1*) in addition to chemotherapy have improved the prognosis significantly and approximately 60% achieves durable remissions and can be cured with this approach [178]. However, refractoriness and relapses occur in more than a third of the patients and the prognosis with current salvage regimens are generally dismal [179]. Moreover, very elderly patients (> 80 years) remains a challenge, a subgroup presenting with poorer outcome, often complicating comorbidities and generally less studied [14].

## 3.2 EPIDEMIOLOGY AND ETIOLOGY

DLBCL mainly affects elderly individuals and the median age at diagnosis is 70 years. In western countries the disease constitutes about one third of all lymphoma in adults,

making DLBCL the most common subgroup of lymphoma. In Sweden nearly 600 individuals are diagnosed each year [180].

The etiology of DLBCL is not clarified but there are several well-established risk factors. Most cases of DLBCL arises *de novo* but with a rate of a few percent yearly some cases arise in the form of transformation from more indolent lymphoid malignancies such as CLL [54] or follicular lymphoma [181]. The incidence of DLBCL increases with age and slightly more men than women are affected (55% vs 45%) [182]. Whites of European origin seems to have a higher incidence of lymphoma than other populations [183-185]. Moreover, a hereditary predisposition seems to exist with a higher risk for first-degree relatives of DLBCL patients [186, 187].

Both deficient and excessive activation of the immune system are linked to an increased risk of DLBCL. Immune suppression, as in HIV/AIDS or following organ transplantation, is a known risk factor and more often associated with positivity of Epstein-Barr virus (EBV) and with a more aggressive clinical course. Autoimmune and inflammatory diseases, such as rheumatoid arthritis, Sjogren´s syndrome and systemic lupus erythematosus have also been associated with a higher risk. It appears that the greater severity of the underlying inflammatory disease, the greater the risk of developing DLBCL [188]. Obesity, which can be described as a condition of chronic inflammation has moreover been identified as risk factor [188, 189]. Certain anatomical sites have been associated with specific risk factors such as inflammatory bowel disease for gastrointestinal engagement and cigarette smoking for central nervous system (CNS), testicular and cutaneous DLBCL [190].

## 3.3    PATHOGENESIS AND GENETIC FEATURES

### 3.3.1  Cell of origin

As previously stated, DLBCL is frequently described as a very heterogeneous disease in many aspects. On molecular and genetic level this has been confirmed with studies of gene expression profiling (GEP). Two subgroups of DLBCL have been detected corresponding to different stages in the B-cell development from which the subgroups seem to originate; germinal center B-cell (GCB) and activated B-cell (ABC) [191] (Figure 5).

Until recently, the largest subgroup of DLBCL has been "not otherwise specified" (NOS). Subdivision into subgroup based on cell-of-origin (COO) is now required according to the current and revised WHO classification of lymphoid neoplasms [177] and is becoming increasingly relevant since they appear to have both prognostic and predictive impact. The ABC subtype is associated with a worse outcome and different response to treatment [192].

In GCB-DLBCL the COO is believed to be a B-cell in the germinal center of secondary lymphoid tissue, expressing genes characteristic of normal GCBs for example *CD10* and *BCL6*. ABC-DLBCL probably derives from a cell on its way out of the germinal center towards becoming an activated B-cell, expressing genes that are normally expressed in peripheral blood B-cells during their activation, such as *BCL2* and *IRF4* [191].
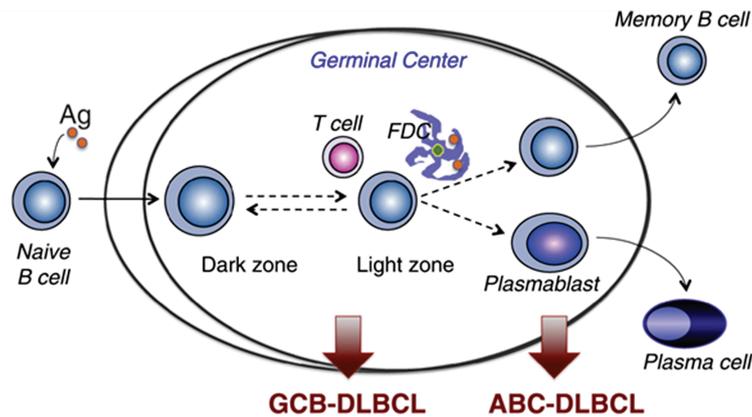
**Figure 5. Schematic picture of the cellular origin of GCB and ABC subtype of DLBCL.**
*Reprinted in a modified version with permission from Blood. Pasqualucci et al. Genetics of diffuse large B-cell lymphoma. Blood; 21:2307-2319.*

### 3.3.2 Genetic alterations and molecular features

Besides COO, ABC and GCB subtypes also differ in their chromosomal alterations and genetic lesions affecting different signaling pathways. GCB-DLBCL is for example more commonly associated with aberrations in *BCL2, EZH2* and *PTEN* while ABC-DLBCL more often is linked to mutations in *CD79A/B* and *MYD88* [193]. Dysregulations in *BCL2* can prevent the cell from programmed cell death, suppressive mutations in *EZH2* interferes in gene methylation and transcription in GCBs resulting in dysregulation in differentiation and proliferation. Deletional mutations in *PTEN* results in activation of the oncogenic PI3K/AKT signaling pathway. *CD79A/B* and *MYD88* are both involved in the activation of the B-cell receptor and Toll-like receptor. Mutations in these genes eventually results in activation of the NF-κB pathway which is important in survival of DLBCL cells [193, 194].

The proto-oncogene *MYC* encodes for a transcription factor that normally induces apoptosis in response to DNA damage. Rearrangements in *MYC,* observed in about 10% of DLBCL-cases leads to loss of its function and allow cells to evade apoptosis. Simultaneous translocations of *MYC* can occur with translocations in the normally antiapoptotic proto-oncogenes *BCL2* and/or *BCL6*, designated "double hit" (DH) or "triple hit" (TH) lymphoma. These subgroups have been associated with a more aggressive clinical course and inferior response to standard treatment with R-CHOP [195] (see section 3.6.1). A relatively recent study suggests the prognosis of these subgroups not being quite as bad as in prior studies, possibly influenced by FISH being performed more frequently in high-risk patients in the past compared to this study where over 5000 patients from prospective trials all were tested [196]. In the latest WHO classification these two groups (DH and TH) are included in the new category "High grade B-cell lymphoma" (HGBL) [197]. There are also cases of both ABC- and GCB-DLBCL in which both *MYC* and *BCL2* are overexpressed, so called "double expressor lymphoma", with an incidence between 19-34%. Double expressor lymphoma are not described to be as aggressive as DH or TH [195].

## 3.4 CLINICAL PRESENTATION AND DIAGNOSIS

Just like at the molecular and genetic level the clinical picture of DLBCL can be very diverse and moreover depends on site of involvement. Symptoms frequently described at diagnosis are fever, night sweats and unintentional weight loss, referred to as B symptoms. Single or multiple rapidly enlarging lymph nodes and fatigue are other symptoms that might occur [16].

DLBCL usually originates in lymph nodes but in around 40% of cases it arises in extra nodal sites where the gastrointestinal tract is the most common location. However, almost any extra nodal site can be affected for example the mediastinum, testis, central nervous system (CNS), breast, bone and skin [194, 198].

The diagnosis of DLBCL should be made on a surgical excision biopsy to ensure that enough material is available to assess morphology, immunohistochemistry and flow cytometry. A core-needle biopsy should if possible be avoided but may be an alternative if surgery is not possible or the risks to the patient are considered to high [52].

Gene expression profiling (GEP) is widely used in clinical trials but is not yet established in clinical routine where different immunohistochemistry methods, although with variable sensitivity and specificity, are available [199]. These algorithms evaluate the expression of different cellular proteins such as CD10, BCL6 and MUM1 as a surrogate for categorization into COO. In clinical routine a classification into the less distinct subgroups GCB DLBCL and non-GCB DLBCL has been frequently employed [200] but new methods are approaching [16].

## 3.5 CLINICAL STAGING AND PROGNOSTIC FACTORS

Stage is an important prognostic factor and commonly established by the Ann Arbor classification system. This classification divides patients into four stages depending on the extent of lymphatic regions and extra nodal tissue involved and into subgroup A and B, based on the absence or presence of B symptoms [201].

In the updated version of the Lugano Classification 2014, PET-CT was incorporated as the preferred way of staging. If not possible a contrast-enhanced CT can be considered. A bone marrow sample is recommended in the case of negative PET-CT and is usually included in clinical trials but not demanded in standard assessment in clinical routine [52].

The most widely used prognostic tool, the International Prognostic Index (IPI), was introduced in the 1990s. Based on five clinical variables (stage, serum LDH, performance status, age and extra nodal engagement), four risk categories are differentiated with an estimated 5-year OS ranging from 26% (4-5 risk factors) to 73% (0-1 risk factors) [202]. In clinical practice the age adjusted IPI (aaIPI) is often used with the cut-off 60 years. This model includes only three clinical variables (stage, LDH and performance status) [202, 203].

To improve risk stratification, new prognostic models have been suggested [204]. NCCN-IPI (National Comprehensive Cancer Network International Prognostic Index), a model where age and LDH have been additionally subcategorized, has shown a range of 5-year OS from 33% in the high-risk group compared to 96% in the low risk group [205].

As a DLBCL relapse or progression in the CNS is usually fatal, the CNS-IPI has been developed as a prognostic tool to identify patients with a higher risk of CNS-

involvement and in whom it is appropriate with prophylactic interventions to reduce this risk [203]. The incidence of CNS relapse varies from less than 1% in young patients with few risk factors up to 35% in patients with several risk factors. The CNS-IPI model is based on the same five clinical variables (stage, serum LDH, performance status, age and extra nodal engagement) as the IPI in addition to involvement of kidney and/or adrenal glands [206]. Involvement of testicles, uterus, breast and dual expression of MYC and BCL2 protein have also been associated with increased risk of CNS involvement [207].

## 3.6 TREATMENT AND OUTCOME

The CHOP regimen, consisting of cyclophosphamide, doxorubicin, vincristine and prednisone, has been the foundation in the treatment of DLBCL in over four decades. Until the anti-CD20 antibody rituximab became available no other intensive chemotherapy regimen had proven to be superior to CHOP. Even if some regimens seemed efficient, advanced schedules, administrative issues, costs and troublesome toxicities remained [208].

### 3.6.1 Rituximab in combination with CHOP (R-CHOP)

Rituximab was added to the CHOP regimen (R-CHOP) for DLBCL patients in trials just prior to the millennial and evidence of prolonged survival and tolerability was first shown in a phase 3 trial for patients 60-80 years of age published in 2002. After two years of follow-up OS was 70% in patients assigned to R-CHOP compared to 57% in patients assigned to CHOP alone ($p$=0.007) [209]. The 10-year follow-up confirmed maintained survival benefit with the addition of rituximab (10-year OS 43.5% for R-CHOP vs 27.6% for CHOP, median OS 8.4 years for R-CHOP vs 3.5 years for CHOP, $p$<0.0001). Given the elderly age group, a large bulk of patients was diseased at 10-year follow-up; 55% in the R-CHOP group and 71% in the CHOP group. A greater proportion of patients died from what was assessed to be associated with lymphoma progression in patients treated with CHOP (68% vs 56% with CHOP vs R-CHOP). Over 40% among patients assessed to R-CHOP were still alive and in their first complete remission after ten years [178]. The improvement in the elderly subgroup with R-CHOP was confirmed by Habermann and co-authors 2006 who further demonstrated that maintenance therapy with rituximab did not seem to offer additional benefit [210].

Later it was also shown that patients younger than 60 years with none or only one risk factor assessed with aaIPI also had a significant benefit with the R-CHOP combination. In the MInT trial patients who received R-CHOP had a 3-year PFS of 85% compared to 68% in patients who received CHOP alone ($p$<0.0001). OS was 93% for R-CHOP vs 84% for CHOP alone ($p$=0.0001) [211]. The 6-year follow-up demonstrated continued benefit with R-CHOP over CHOP (PFS 80% vs 64%, $p$<0.0001, OS 90% vs 80%, $p$=0.0004). Patients with no risk factors on IPI and further lacking bulky disease (lymphadenopathy ≥ 7.5 cm), composed a group with particularly good outcome compared to other subgroups (OS 95% vs 89%, $p$=0.029) [212].

In Sweden rituximab was introduced within trials in the beginning of the 2000s and has gradually been included in standard treatment for most patients with DLBCL since 2006 [213]. In present guidelines R-CHOP remains the recommended 1st line treatment

regardless of COO or genetic aberrations but if possible, inclusion in clinical trials should be given priority. Patients with CNS-IPI ≥ 4, involvement of ≥ 3 extranodal sites, engagement of testis, uterus, kidneys or adrenal glands as well as intravascular DLBCL should receive CNS-prophylaxis (e.g. methotrexate and cytarabine) in addition to R-CHOP [203].

### 3.6.2  Dose intensity

The matter of administering R-CHOP every other week (R-CHOP-14) or every third week (R-CHOP-21) has been debated. In frontline setting in a multicenter phase 3 study, eight cycles of R-CHOP-21 was compared to 6 cycles of R-CHOP-14 followed by 2 cycles of single rituximab [214]. The study population had a median age of 61 years (range 19-88), predominantly good performance status (PS 0-1 in 87%, PS 2 in 13%), approximately one third were in stage IV, 50% had bulky disease and 65% elevated LDH. Superiority of R-CHOP-14 could not be demonstrated for either PFS or OS after a median follow-up of almost 4 years, neither in any certain subgroups (e.g. aaIPI, COO, genetic rearrangements). Regarding hematological toxicity ≥ grade 3, thrombocytopenia were more frequent for patients treated with R-CHOP-14 (87% of this group received at least 80% of planned dose) while neutropenia and febrile neutropenia were more frequent with R-CHOP-21 (78% received at least 80% of planned dose), probably influenced by the fact that all patients with R-CHOP-14 received granulocyte colony-stimulating factor (G-CSF) prophylactically while it was not obliged with R-CHOP-21 [214]. In a similar trial on elderly patients (60-80 years) no significant difference in efficacy was reported between R-CHOP-14 and R-CHOP-21 [215]. According to Swedish guidelines both options are available. Benefits and disadvantages must be considered in each individual case [203].

Whether six or eight cycles of R-CHOP should be used has also been a matter of debate. In patients 60-80 years eight cycles of R-CHOP-14 was not better regarding survival and further more toxic than six cycles of R-CHOP-14 [216]. In many previous RCTs the strategy with eight cycles every third week (R-CHOP-21) have been employed [209, 210, 214] while six cycles have been empirically used and is recommended in clinical routine in Sweden [203]. In a retrospective population-based study from the Nordic Lymphoma Group, no difference in survival was observed between six or eight cycles of R-CHOP-21 [217].

The advantageous subgroup of patients < 60 years with no risk factors on aaIPI and without bulky disease, were further studied in the FLYER trial published last year [218]. Four cycles of R-CHOP succeeded by two additional cycles of single rituximab was not inferior to previous standard with six cycles of R-CHOP. At a median follow-up of 5.5 years there was no significant difference in PFS or OS. Moreover, reduced toxicity was reported, both hematological and non-hematological. Regarding leucopenia ≥ grade 3 the frequency was diminished by a third [218]. This strategy is now recommended in Swedish guidelines to patients < 80 years without risk factors on aaIPI, even if the study did not include patients older than 60 years of age [203].

### 3.6.3  R-CHOP versus other chemoimmunotherapy regimens

Standard R-CHOP has been compared with intensified chemotherapy with R-ACVBP (rituximab, doxorubicin, cyclophosphamide, vincristine, bleomycin and prednisone) as frontline treatment in patients younger than 60 years and aaIPI=1. An OS benefit was demonstrated for R-ACVBP but to a cost of increased hospitalization and hematological toxicity [219]. Moreover 6 cycles of R-CHOP in 1st line has been compared to 6 cycles of dose adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin and rituximab (DA-EPOCH-R) in a population with a median age of 58 years (range 18-86 years) and a majority of high stage disease (74% in stage III-IV), demonstrating considerable toxicity for the DA-EPOCH-R regimen and no benefit in PFS or OS. This study was underpowered and could not draw firm conclusions on certain genetic high-risk groups [220] whereas an advantage with DA-EPOCH-R was seen in patients with MYC rearrangements in a phase 2 study [221]. According to Swedish guidelines DA-EPOCH-R should be considered to patients with rearrangement of MYC, BCL2 and/or BCL6 [203].

In younger patients with high or intermediate-high aaIPI diagnosed with different aggressive NHL (majority DLBCL) consolidating autologous stem-cell transplantation (SCT) in first remission after R-CHOP (CHOP in CD20-negative cases) showed to improve PFS but not OS [222]. These results were confirmed in a more recent study of patients < 65 years with CD20-positive DLBCL or follicular lymphoma grade 3b where addition of high-dose chemotherapy and autologous SCT could not demonstrate a gain in overall survival [223].

### 3.6.4  CHOP in combination with other antibodies

There have been attempts to replace rituximab with other mAbs. In a recently published update from a phase 3 trial investigating a combination of obinutuzumab (G) (see section *2.5.4.1*) with CHOP (G-CHOP) versus conventional R-CHOP, no benefit in PFS was seen [224]. The authors describe a trend in prolonged PFS in the GCB subgroup treated with G-CHOP and present a possible explanation being the similar origin in the GCBs in parallel to follicular lymphoma where improved PFS with CIT based on obinutuzumab has been observed [225]. Further studies are needed to determine the possible place of obinutuzumab in the treatment arsenal of DLBCL.

There is an actively recruiting double-blind, placebo-controlled randomized phase 3 trial ongoing to evaluate the efficacy and safety of polatuzumab vedotin, a CD79b-directed antibody conjugated with a microtubule inhibitor, in addition to R-CHP (R-CHOP minus vincristine) versus previous standard R-CHOP with results awaited (NCT03274492).

### 3.6.5  Very elderly and fragile patients

Advanced age has been shown to be associated with biologically more aggressive features such as higher prevalence of ABC-subtype suggesting that targeted therapies should be investigated in these patients [226, 227]. It is a challenge to treat elderly patients since they might tolerate treatment worse and sometimes treatment may not even be

started in the oldest group of patients due to toxicity concerns. The very oldest patients ($\geq$ 80 years) are many times underrepresented or excluded from clinical trials due to age, comorbidities and performance status. Data for this patient group can be based on a limited sample size or conclusions extrapolated from studies on younger patients [209, 211].

A phase 2 trial including 150 patients older than 80 years, showed a 2-year PFS of 47% and OS of 59% after a median follow-up of 20 months with a dose-reduced R-CHOP regimen (R-mini-CHOP) [228] which has become the standard frontline treatment to patients over 80 years [203, 229]. Hematological toxicity with $\geq$ grade 3 neutropenia (40%) was the most frequent AE and febrile neutropenia $\geq$ grade 3 occurred in 7% of patients.

Moreover, the combination of ofatumumab (see section *2.5.4.1*) to mini-CHOP was later investigated in 1st line in a phase 2 study on patients $\geq$ 80 years reporting a 2-year OS of 65%. Neutropenia and febrile neutropenia $\geq$ grade 3 were observed in 21% and 6% respectively [230].

To fragile patients with poor performance status or impaired cardiac function, exchange of doxorubicin due to its cardiotoxicity, with gemcitabine, etoposide or liposomal doxorubicin have been suggested [229]. Rituximab in combination with gemcitabine, cyclophosphamide, vincristine and prednisolone (R-GCVP) showed a 2-year PFS of nearly 50% and OS of 56% in a phase 2 study on a population with advanced DLBCL, cardiac comorbidities and a median age of 76.5 years [231]. Cardiac events $\geq$ grade 3 were 10% which is not easily related to other trials where patients with cardiac dysfunction has tended to be excluded or baseline cardiac function not clearly reported, even in trials on elderly patients [209, 210, 228].

There is an ongoing frontline phase 3 trial by the Nordic Lymphoma Group including patients $\geq$ 80 years or $\geq$ 75 and assessed as frail (on a shortened version of the Comprehensive Geriatric Assessment (CGA) scale) investigating R-mini-CHOP versus R-mini-CHP (vincristine removed from CHOP) plus the immunoconjugate polatuzumab vedotin (NCT04332822).

### 3.6.6 Relapsed/refractory setting

The 5-year survival rates are approximately 60-70% in DLBCL after frontline therapy, hence refractoriness and relapses do occur in around a third of patients. In fit patients without complicating comorbidities, the intention is still cure with salvage chemotherapy followed by autologous SCT in responders. However far from all patients in R/R setting will be suitable for transplant and many of those who get transplanted will eventually relapse [232, 233]. Overall, the prognosis is usually very dismal after salvage therapy with figures as low as 6 months in median OS in refractory patients not eligible for SCT [179].

Rituximab in addition to dexamethasone and chemotherapy with high-dose cytarabine and cisplatin (R-DHAP) or rituximab in addition to ifosfamide, carboplatin and etoposide (R-ICE) can be considered as two equal alternative 2nd line treatments and in chemo sensitive patients then succeeded by autologous SCT. No significant difference in outcome was seen in a study comparing these two regimens (3-year PFS 42% vs 31%, *p*=0.4, 3-year OS 51% vs 47%, *p*=0.4 for R-DHAP vs R-ICE). Half of the patients could move on to autologous SCT (3-year PFS 39% versus 14% for patients with and without

SCT respectively). Patients with early relapse after frontline treatment had a worse outcome [233].

R-GDP (rituximab plus gemcitabine, dexamethasone and cisplatin) is another alternative that has demonstrated similar response rates, rates of transplanted patients and survival rates as R-DHAP but with significantly less frequency of febrile neutropenia [234]. There have been attempts in replacing rituximab with another anti-CD20 antibody. Ofatumumab was for example added to CHOP and compared with R-CHOP in R/R DLBCL patients but without indications of better outcome [235].

In patients that does not qualify for SCT strategies combining rituximab with gemcitabine and oxaliplatin (R-GEMOX) [236] or with bendamustine (BR) [237] are potential options. BR in combination with polatuzumab vedotin has recently become another option in patients ineligible for transplantation. Conjugated with a microtubule inhibitor this CD79b-directed antibody was granted accelerated approval by FDA based on CR rates in third or later line [238].

Selinexor is a new type of drug acting as a nuclear transport inhibitor of tumor suppressor and oncogenic proteins recently approved by FDA under accelerated conditions in third or later line for DLBCL. Selinexor is not yet approved by EMA.

Furthermore, maintenance therapy with the immunomodulating agent lenalidomide has indicated effects in elderly patients who had responded to salvage therapy but did not fulfill criteria for SCT or that relapsed after SCT, but needs to be investigated further [239].

### 3.6.7 Covid-19 aspects

Observational reports on the management of DLBCL patients during the pandemic are based on relatively few patients so far. One cohort study of 18 patients with DLBCL and high-grade B-cell lymphoma (HGBL) treated in frontline setting during March to May this year, 11 patients were treated with R-CHOP (all ≤ 70 years), 4 patients received R-mini-CHOP due to age or comorbidities (all ≥ 78 years) and 3 younger patients received DA-EPOCH of which one 27-year old patient also received rituximab in addition. The majority received prophylactic G-CSF. One febrile neutropenia episode was observed day 10 under G-CSF prophylaxis during the first cycle of R-CHOP in a 52-year old previously healthy female. This patient was diagnosed with Covid-19 and received treatment with hydroxychloroquine and antibiotics. CT-scan revealed bilateral ground-glass opacities and consolidations. The patient did not require intensive care or oxygen and could start the second cycle with a delay of 8 days [240].

Another report describes two fatal cases of Covid-19, one DLBCL patient and one patient with mantle cell lymphoma, both with remaining viremia after rituximab-containing treatment. Both patients had received the latest treatment within two weeks before Covid-19 diagnosis, had absolute depletion of B-cells and decreased IgG-levels at this time and further developed a massive inflammatory response and respiratory stress leading to death after 22 and 26 days respectively. Viral load culminated prior to death. The DLBCL patient in addition to rituximab received chemotherapy also affecting the T-cell immunity and the patient with mantle cell lymphoma also had ongoing treatment with ibrutinib at Covid-19 diagnosis [241].

Patients with B-cell malignancies are a very heterogeneous group of patients where treatment decisions always must be adapted to the individual patient and now further complexed with the challenges due to the pandemic. Treatment of aggressive malignant diseases like DLBCL can generally not be delayed due to the rapid natural course of the untreated disease and as evidence on the management during these exceptional circumstances are limited, real-world data can add valuable information and generate hypotheses [158].

### 3.6.8 Novel and emerging treatments and strategies

In recent years, intensive research has been conducted to develop more effective therapies targeting a variety of cellular processes affected by genetic lesions in DLBCL. Many novel approaches are under investigation, all the way from targeting different surface markers, diverse steps in signaling pathways as well as strategies affecting the microenvironment with immunomodulating agents and therapies [223]. However, R-CHOP remains gold standard for most patients.

One field of intensive research has been COO guided approaches. A recent large, randomized phase 3 trial (REMoDL-B) compared the addition of the proteasominhibitor bortezomib to R-CHOP (BR-CHOP) versus R-CHOP alone in frontline setting with no upper age limit. While both groups received the first cycle of R-CHOP, COO was assessed with GEP and patients were then randomly assigned to continue with standard R-CHOP or to addition of bortezomib (BR-CHOP). Even if no improvement in PFS could be demonstrated, not even for the ABC subgroup which was envisioned, the study describes an appealing approach in stratifying patients based on certain risk factors such as COO without delay in the initiation of therapy and thereby mitigating the risk of selecting patients with a less aggressive disease and presumed better outcome [242].

Another study biologically stratifying participants based on COO evaluated the BTK inhibitor ibrutinib (see section *2.5.6.1*) where preclinical trials have indicated effectiveness in non-GC DLBCL [243]. Evaluated in frontline, ibrutinib plus R-CHOP in non-GC DLBCL against standard R-CHOP alone did not show any overall advantage in outcome. However, there was a trend in improved survival in the younger subset of patients with ABC subtype. Reversely, in patients $\geq$ 60 years the combination with ibrutinib and R-CHOP was associated with substantial toxicity and worse outcome [243]. The results may have been biased by selection of the fittest patients due to time delay until inclusion as the median time for immunohistochemistry analyzes from diagnosis to treatment was almost one month longer than in routine practice [243, 244], a bias that probably can be circumvent with a study design as the one used in the REMoDL-B trial[242].

There are ongoing and planned studies with 2$^{nd}$ generation BTK inhibitors as acalabrutinib (see section *2.5.6.3*) in addition to R-CHOP with assumed less toxicity due to reduced off-target effects with 2$^{nd}$ generation BTK inhibitors (NCT03571308, NCT04546620, NCT04529772). Moreover, this treatment regimen will be investigated in patients with Richter transformation (NCT03899337).

CAR T-cell therapy has shown promising results in phase I and II studies in R/R setting [245-247] and phase 3 trials are ongoing (NCT03391466, NCT03570892,

NCT03575351) to gain deeper understanding in the efficacy and safety of these therapies.

A dual antibody directed against both CD19 and CD22 has been developed and administered in a phase 1 study (EudraCT: 2016-004682-11) in combination with pembrolizumab, a monoclonal antibody targeting the interaction between the programmed cell death protein 1 (PD-1) and its ligand (PD-L1). Preliminary data presented at ESMO 2020 was encouraging. Whether responses continue to be durable and safe in long-term follow-up and might circumvent some of the resistance issues observed with CAR T-cells needs to be evaluated further [248].

Another appealing treatment strategy is allogeneic CAR T-cell therapy which would have the advantage of quicker availability to patients in urgent need of salvage therapy [249]. Further there are strategies with bispecific T-cell engaging antibody treatment evaluated in B-cell malignancies in R/R setting, so far with positive results and will be investigated further [250].

Moreover, the efficacy of the immunomodulating agent lenalidomide in addition to R-CHOP has been investigated although with somewhat conflicting results regarding the efficacy in the ABC subgroup [251, 252] and further analyzes from future trials are needed to clarify this.

An association between early progression, based on positive interim PET-CT after two cycles of R-CHOP, with inferior survival has been observed [253]. Even if there were no improvement in outcome with intensified treatment (Burkitt protocol) in the PET-positive group and within current routine care these patients cannot be offered a more efficient therapy, PET-CT nonetheless might serve in differentiating patients resistant to chemotherapy and with an urgent unmet medical need, suitable for ongoing trials. The COO, genetic and molecular nature of PET-positive cases was further studied to be able to distinguish possible candidates for targeted agents [254]. There are also studies on changes in circulating tumor DNA prior to and in real-time under treatment to predict outcomes and adjust treatments individually [255].

The optimal use of COO with emerging agents as well as the optimal way of establishing COO in a practical and economically feasible way in routine clinical practice remains to be found out. Recently, a proposal based on whole genomic analysis presented seven discernible genetic subtypes, that besides possible utilization in prospective trials also might be of value as hypothesis generating in retrospective analyzes [256]. Not entirely unlikely, so far unidentified molecular and genetical characteristics will emerge, subclassifications will probably continue to be refined and open up to potentially novel treatment approaches.

At last, in the future artificial intelligence might provide faster and more reproducible approaches regarding subclassification even in routine clinical practice as we move even closer towards tailored and personalized treatment strategies [257]. Advanced risk assessment methods that continuously evaluates probabilities of outcome in individuals are currently being developed both in aggressive lymphoid malignancies like DLBCL and in more indolent diseases such as CLL [258].

# 4    AIMS OF THE THESIS

The overall aim of this thesis was to compile reliable real-world data in certain subgroups of patients with CLL and DLBCL which might be used for comparison with data from randomized clinical trials and data on new orphan drugs. The specific aims for each paper were:

I.      To obtain real-world data on the effectiveness and safety of 2$^{nd}$ line treatments used in CLL patients in routine health care.

II.     To investigate outcome following 1$^{st}$ line treatment in concecutive unselected CLL patients in Sweden during a ten year period.

III.    To estimate the relative efficacy of ibrutinib versus the effectiveness of previous standard of care in relapsed/refractory CLL patients.

IV.     To obtain knowledge on the outcome, safety and tolerability of 1$^{st}$ line treatment in very elderly patients with DLBCL treated within routine health care.

# 5 MATERIAL AND METHODS

## 5.1 SWEDISH HEALTH CARE SYSTEM AND CANCER CARE

The Swedish health care system is mainly funded by the government and primarily financed through general taxation. Swedish residents all have equal access to public health care services. The private health care sector is relatively small but expanding. Within the public health sector, primary care as well as emergency care is directly available, whereas a referral is needed for specialized care for example cancer care. The central government, the health care regions and municipalities share the responsibilities for health and medical care in Sweden [259].

A main goal regarding cancer prevention and care in Sweden is to create an equal and high-quality care throughout the country. Different aspects of cancer care are managed and coordinated by our six Regional Cancer Centers (RCC) [260].

The unique 10-digit personal identity number that every Swedish citizen receives at birth or is administered to everyone who registers in Sweden, is assigned by the Swedish Tax Agency and further registered in the Swedish Population Registry. This means that we can identify all patients with a certain cancer diagnosis from our cancer registries where this individual civic number is used [261].

## 5.2 CANCER REGISTRIES RELEVANT FOR THE THESIS

### 5.2.1 The Swedish Cancer Registry

The Swedish Cancer Registry (SCR) is held by the Swedish National Board of Health and Welfare and was established in 1958. It covers the whole population and it is mandatory by law for all health care providers, (clinicians, pathologists, cytologists) public as private, to report a newly diagnosed cancer (clinical, morphological and autopsy-based) to the SCR. In the SCR 99% of the cancer diagnoses are based on morphology. Our six RCCs verify data from reported cases before they are delivered to the Swedish National Board of Health and Welfare where further controls are performed before incorporation of data into the SCR [262].

The coverage is about 95% and considered extensive in relation to registries in other countries [262]. The Surveillance, Epidemiology, and End Results Program (SEER) in the United States (U.S), for example covers around 35% of the U.S population [263].

The SCR is used to monitor cancer prevalence, incidence and survival in the country and moreover serves as base for research and international comparisons. In 2018 approximately 68.000 malignant tumors were reported for around 63.000 individuals [262]. The registry contains data related to the patient (personal identification number, sex, place of residence), specific tumor data (site, histological type, stage, basis and date of diagnosis) as well as date and cause of death [264, 265].

Each of the six RCCs linked to one of the six health care regions in Sweden (north, Stockholm-Gotland, south, southeast, Uppsala-Örebro and west), are associated with regional registries to which newly diagnosed cases and corrections regarding previously

recorded patients are reported by the care giver via RCC and then forwarded to the Swedish National Board of Health and Welfare on yearly basis [264].

Only new primary tumors are registered in the SCR. Relapses or metastases of previously known cancers are not included. As a complement to the SCR, there are several national quality registries for different tumor groups administered by RCC and built upon the national IT-platform INCA (Information Network for Cancer Care) [265, 266].

## 5.2.2 National and regional quality registries

### 5.2.2.1 The Swedish Lymphoma Registry

The Swedish Lymphoma Registry (SLR), held by RCC, is a national quality registry established year 2000 on the initiative of the Swedish lymphoma group to optimize the care of patients with malignant lymphoma. It includes all incident lymphoma diagnoses in patients aged ≥ 18 years. Malignant lymphoma is a heterogeneous disease group and the SLR contains as many as 78 different subtypes of lymphoma. Most subtypes cannot be distinguished with certainty from the SCR, making registration in the SLR essential for follow-up of different diagnoses, subgroups and treatments. The most common subgroup in the SLR is DLBCL constituting 33% [180].

The coverage for registration in the SLR is around 95%, although some reporting delay exists. Since 2007, details regarding primary treatment and response have been included in the SLR and since 2010 also information on relapses [180].

### 5.2.2.2 The Swedish CLL Registry

All patients diagnosed with CLL from year 2007 an onwards are reported to a separate quality registry, the Swedish CLL registry [267]. CLL differs in many ways from other lymphoproliferative malignancies justifying a separate registry. The coverage for registration is around 95% and as for most registries, a lag in reporting occurs and so far, data from the CLL registry has not been complete enough to present comprehensive reliable data for example on duration of response after first or later lines of therapy. Registration on first treatment after relapse was implemented 2015.

The coverage in the SLR and Swedish CLL registry is regularly checked by RCC against the SCR. Potential sources for underreporting are cases where the diagnose is set without cytological or pathological verification, which might occur within primary care. Further, since CLL can be an indolent disease the diagnosis might never be set at all in patients with slightly elevated lymphocytes without other signs or symptoms and moreover in very elderly or fragile patients not in condition for further investigation or treatment. This applies for many other malignancies and hence also a source of underreporting to the SCR. Considering autopsies being performed less frequently the proportion of undiagnosed cases remains unknown [267].

## 5.3  PATIENT COHORTS AND STUDY PROCEDURES

All four studies were real-world studies based on retrospective data on study populations identified through the Swedish CLL registry (paper I-III) and the Swedish Lymphoma Registry (paper IV) and can also be referred to as retrospective observational studies. Unlike traditional registry studies data were collected from individual patient files.

For paper I-III on CLL, the 2008 International Workshop on Chronic Lymphocytic Leukemia (iwCLL) criteria was used to assess treatment response and hematological toxicity [268]. In paper IV treatment response was evaluated according to the 2007 Revised Response Criteria for Malignant Lymphoma [269]. Non-hematological adverse events were graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 in paper I-III. For paper IV AEs were graded according to the NCI CTCAE version 4.0.

### 5.3.1  Paper I

In paper I the Swedish CLL registry via RCC Stockholm-Gotland was used to identify patients diagnosed with CLL between 2003 and 2012. Individual files from 979 patients were available for screening. Patients who met the criteria of R/R CLL according to the current guidelines [268] and who had initiated 2$^{nd}$ line treatment before the end of 2013 were included. In total 137 patients fulfilled the inclusion criteria. Their individual files were reviewed in detail regarding baseline characteristics, treatment regimens used, outcome and toxicity. Data were entered into coded case report forms (CRF) and further into a database for statistical analysis.

### 5.3.2  Paper II

Patients from all the country diagnosed with CLL from the initiation of the Swedish CLL-registry and INCA-database in 2007 until 2013 were identified and individual patient files screened by a representative from each of Sweden´s six health-care regions to include all patients who had started 1$^{st}$ line treatment, before the end of 2013. Patients who only received treatment for autoimmune complications not related to progression of CLL were excluded. Individual files were analyzed in detail and as in paper I, data on patient characteristics, treatment, outcome and toxicity were recorded in coded CRFs. Information regarding participation in clinical trials, type of hospital, geographical region where the patient was treated and adherence to current national guidelines at the time of treatment decision were also gathered.

Two different time periods were compared, 2007-2009 (*early*) vs 2010-2013 (*late*), with the rational of CIT being introduced in the latter time period (FCR in 2010 and BR in 2011) but also with respect to cytogenetic status being a strong prognostic and predictive marker and the implementation of FISH-analysis of cytogenetic aberrations was initiated 2010.

### 5.3.3  Paper III

Paper III included two different patient cohorts. First, the cohort as used in paper I, but now expanded with one more year to include patients diagnosed from 2002 and with investigation of all treatment lines, not just 2nd line. This real-world cohort, (the "Stockholm cohort") receiving standard of care (SOC) between 2002-2013, was compared to a second cohort of 195 patients receiving ibrutinib within the pivotal RESONATE trial [23]. The "Stockholm cohort" consisted of 144 patients who initiated 2nd or later line of therapy between 2002 and 2013. All together these 144 patients constituted 322 started treatment lines. Baseline characteristics, treatments used and outcome for each treatment line from first refractoriness or relapse were entered into coded CRFs. All patients within the RESONATE trial had commenced ibrutinib between 2012 and 2013 and had a median number of previous therapies of 3, ranging from 1-12 [23].

### 5.3.4  Paper IV

In paper IV the Swedish Lymphoma Registry (SLR) via RCC Stockholm-Gotland was used to identify patients diagnosed with DLBCL between 2000 and 2015. Individual patient records were screened to include all patients diagnosed with *de novo* DLBCL and who had received treatment for the disease, regardless of curative or palliative intent. Individual patient records were subjected to detailed analysis and data entered in coded CRFs as described for the previous three papers. Information on patients that did not receive any treatment at all or only received best supportive care as well as patients with transformed DLBCL was managed separately.

## 5.4    STATISTICAL ANALYSIS

Throughout the studies descriptive statistics were used to summarize and present baseline characteristics, treatments used as well as to display adverse events in frequency tables or as bar charts. Analyzing time to events, PFS was calculated from the start date of the treatment line in question until the date of progression on this treatment or until death, whichever came first. OS was calculated from the start date of the line of treatment studied to the date of death. Duration of response (DoR) was calculated from the date of response until the date of progression or start of next therapy, whichever came first, whereas time to next treatment (TNT) was calculated from start date of the treatment studied until start of next therapy. Event free patients were censored at last follow-up. In the inferential part of the statistical analysis all *p*-values were two-sided and *p*-values < 0.05 were considered significant in all four papers.

### 5.4.1  Paper I

Based on when 2nd line treatment was started patients were divided into three time periods, *early* (2003-2007), *middle* (2008-2010) or *late* (2011-2013). Time to events (PFS, OS, DoR and TNT) were analyzed and graphically displayed by using the Kaplan-

Meier method. Survival distributions between the three groups were compared using the log-rank test.

## 5.4.2  Paper II

As in paper I the Kaplan-Meier method was used to estimate and graphically display PFS and OS. To estimate the effect of different variables (e.g. baseline characteristics, treatment, region, type of hospital) on time to event, proportional hazards regression model was performed. Results were presented as hazard ratios (HRs) with 95% confidence intervals (CIs) and *p*-values referred to Wald tests. Multivariate analyzes based on FISH were restricted to the latter time period.

## 5.4.3  Paper III

Proportional hazards regression model was used to calculate unadjusted and adjusted HRs, estimating the relative efficacy of ibrutinib versus the effectiveness of SOC on time to event (progression or death). PFS and OS were graphically displayed by using the Kaplan-Meier method and HRs were graphically presented in forest plots together with 95% CIs.

Initially a univariate analysis using treatment as a sole covariate was performed yielding unadjusted HRs. To account for differences in baseline characteristics (e.g. line of therapy, age, sex, stage, performance status) between the two cohorts' multivariate analysis was thereafter performed yielding adjusted HRs. To control for the clustering of observations in treatment line, since the same patient could be included several times (at start of each new treatment line), the robust sandwich estimator was used generating more conservative CIs. The effect by stepwise adding covariates to the proportional hazards regression model was also analyzed as well as a sensitivity analysis excluding all patients in the real-world cohort commencing treatment before 2012, in order to only compare patients treated during the same years (2012-2013).

## 5.4.4  Paper IV

Statistical analysis was performed in two age subsets with the cut-off of 85 years which was the median age in the study population. End points of interest were response, PFS, OS and AEs. As in the previous papers, the Kaplan-Meier method was used to present PFS and OS, and proportional hazards regression model to estimate the effect of baseline variables and risk factors on time to events, presented as HRs with 95% CIs.

## 5.5    ETHICAL ASPECTS

All studies were performed in accordance with the ethical principles of the Declaration of Helsinki and in compliance with national laws. Approval was obtained by the Regional Ethics Committee (www.etikprovningsmyndigheten.se).

Since all studies were retrospective observational studies no informed patient consent was required. Unlike traditional registry studies, detailed data from each individual patient record were gathered within the studies of this thesis, which can be

considered to violate personal integrity. Since the projects involved thousands of medical records and many patients were deceased at the initiation of the studies it would have been impossible to obtain informed patient consent.

During the performance of the studies each personal identity number was linked to a code, further transferred to the CRF, hence individual patients were unable to be identified. Moreover, all data in the publications are at group level and information on individual patients cannot be recognized why the risk of causing discomfort or harm to individual patients or relatives were considered small in relation to the potential gains with the projects.

# 6 RESULTS, DISCUSSION AND CONCLUSIONS

## 6.1 PAPER I

**"Outcomes of second-line treatment in chronic lymphocytic leukemia - a population-based study from a well-defined geographical region between 2003 and 2013"**

The outcome of 2nd line treatment in R/R CLL patients during a 10-year period was investigated in this paper. At the time of publication this was, to our knowledge, the first real-world report on outcome following 2nd line treatment in consecutive patients from a well-defined geographical region. Since the study was published there has been a tremendous progress and major changes in the management of patients CLL patients, both regarding frontline therapy and treatment of patients with R/R disease.

In our cohort of 137 patients the median age at diagnosis was 72 years with a wide range from 25-94 years, at start of 2nd line therapy. Around 80% of the patients were in good performance status (ECOG 0-1) and slightly more than half of the patients had an advanced stage disease (Binet C). Regarding baseline characteristics such as the wide range of age and inclusion of patients with all scores on the ECOG scale, our cohort differs from typical study populations in randomized trials where patients older than 65-70 years and with ECOG $\geq$ 2 may be excluded [270]. This is important to notice since many patients with CLL are $\geq$ 70 years and multiple comorbidities are not uncommon making them ineligible for many trials further rendering it harder to translate trial data to patients in daily clinical routine.

CLB and FC were the most widely used 2nd line treatments in the *early* time period (2003-2007), accounting for 39% and 30 % respectively. No patients had started FCR during this time period. FC and FCR were most frequently used in the *middle* time period (2008-2010), in 27% and 23% of the treatments respectively, followed by CLB in third place used in 18%. CLB as monotherapy was the most used drug in the *late* time period (2011-2013), used in 24% and FCR in 20%. BR was approved in the *late* time period and accounted for 14% of all 2nd line regimens between 2011–2013. Moreover, several new agents had started to be introduced but were, as expected, only implemented in individual cases in very few percent at this time.

Only a small proportion of patients achieved CR throughout the study period, possibly somewhat underestimated since bone marrow examination to confirm CR can be assumed to be performed less frequently in a generally older population treated within clinical routine compared to a younger selected population within a trial.

There were no major differences regarding infections, hospitalization or hematological toxicity between the time periods, except for neutropenia $\geq$ grade 3, being more frequent in the latter two time periods, probably explained by the introduction of CIT.

Despite the introduction of new agents and possibly better supportive care over this 10-year period, no significant improvement in ORR, PFS or OS following 2nd line therapy was observed. In the case where we compared only two time periods (2003-2007 vs 2008-2013), corresponding to the introduction of CIT, there was still no significant

difference. One possible explanation might be the continued use of CLB that remained high even during the last time period as well as the relatively short follow-up, especially for the latter time period.

Our results differed from the results of a German meta-analysis on prospective trials as well as in Danish population-based registry study that indicated improved survival in the era of CIT [271, 272] and the disparities compared to these as well as other studies [95, 273] might to some degree be explained by the different treatment lines studied (1st vs R/R setting) and different date of baseline (date of diagnosis or date of initiating treatment). Moreover, our study population might be too small to detect differences and impairs the possibility to draw final conclusions.

This study has several other limitations. The therapies administered in 2nd line were very heterogeneous which also had been demonstrated in other studies [272, 274] possibly reflecting the lack of therapeutic standards in R/R setting and that the treatment decision is individual and presumably affected by clinical experience. Moreover, the impact of 1st line and subsequent lines cannot be ignored as CIT has shown to have more impact in frontline [95]. Eight percent of the medical records could not be accessed since the patients were followed at a private hospital where we at this time could not review their medical files. This issue was solved for paper II and IV. However, the probability of these patients fulfilling our inclusion criteria at this time were considered small since at least all intravenous chemotherapy and new agents were administered within the public health care. Further, we gathered information on comorbidities, but they were not evaluated in a systematic way (e.g. CIRS) which was considered in following studies since comorbidities is an important factor that often differs between real-world cohorts and trial cohorts. Probably most important, our study lacked information on genetic risk factors in the majority of patients since analysis of FISH had not yet been implemented in clinical routine, making subgroup analysis based on high-risk features impossible. Several efficacious treatment alternatives now exists in R/R setting and for patients with unfavourable genetic characteristics such as del(17p) [15].

Strengths with our method are the thoroughness regarding the reviews of the patient records to minimize missing data and further that patients are consecutive (not influenced by external referrals) mitigating selection bias. Real-world studies was a relatively new field in our research group and as stated above, this paper is associated with several restraints but gave us valuable experience in our continued work in improving and expanding our method both on regional and national level as well as on other lymphoid malignancies, with the overall aim of obtaining data reflecting outcome in clinical routine in the best possible way to support health authorities, development of national guidelines, assist physicians and also patients in the process of treatment decisions with various new treatments where information on certain subgroups might be limited.

To conclude, the results in this paper must be interpreted with caution but at the time of publication our results highlighted the need for next generation targeted therapies in R/R CLL patients. As patients in clinical trials differ substantially from patients in routine clinical care real-world data like this may be important for interpretation of and comparison with data obtained in early phase and pivotal trials. Our cohort can serve as a real-world historical control before the era of new precision medicines.

## 6.2 PAPER II

**"First-line therapy in chronic lymphocytic leukemia: a Swedish nation-wide real-world study on 1053 consecutive patients treated between 2007 and 2013"**

In this large real-world study of consecutive CLL patients from the whole country receiving 1st line therapy in the time period 2007-2013, we observed an unexpectedly high use of low intensive treatment with CLB and a comparatively short OS as well as regional differences in survival.

Median age were 71 years, around half of the patients had advanced stage disease but most were in good performance status, all of which is expected at initiation of 1st line treatment in clinical routine. Unfortunately, cytogenetic assessment was available in only 57% of patients, reflecting the real-world attributes of the cohort, but impairing the possibility to draw final conclusions based on genetic features. Worth noting yet expected since FISH-analysis was not fully implemented in the CLL work-up, FISH-analysis was more frequently performed in younger patients and at university hospitals. IGHV mutational status was missing for the clear majority since this was not routinely recommended in our national guidelines and at this time did not affect the treatment decision. We now know for example that FCR is very efficacious used as frontline treatment in fit patients without *TP53* aberrations, especially in case of mutated IGHV status, where a possibility to cure exists [134].

In our material CLB (given in combination with an anti-CD20 mAb in only 5%), was overall the most commonly used treatment (39%) followed by CIT with FCR (27%). Patients treated with CLB were significantly older and in poorer performance status than patients receiving other chemotherapy-based treatments. CLB were less frequently used in university hospitals.

There was a tendency but no significant difference in OS between the two time periods studied, 2007-2009 (*early*) vs 2010-2013 (*late*). OS was significantly associated with type of treatment, longest OS for FCR (median OS not reached) and shortest for CLB (median OS 33 months). The results clearly demonstrated that CLB as single treatment is insufficient in 1st line with a median OS < 3 years and more effective alternatives must be offered even to the elderly population and the adequacy of using CLB as a comparative arm in clinical trials must be considered [10]. Although the use of CLB did decrease significantly over time still one third of patients received CLB 2013. In 2016 the use had diminished further to 7 % according to the CLL-registry [267].

The region where the patient was treated was an independent factor for OS whereas type of hospital (city or rural) or compliance to national guidelines did not fall out significantly in the multivariate analysis.

We observed a low rate of response, short PFS and OS in relation to prospective studies [88, 95]. Cytogenetic status is a strong predictive and prognostic factor which unfortunately was unknown in a considerable number of patients which at least might explain the discrepancies to some degree. Moreover, in clinical trials the response is investigated more consistently than in routine care why response rates might be somewhat underestimated in real-world.

A relatively low usage of FCR was seen and BR was introduced during the latter part of the study period. A majority of our elderly cohort receiving CLB did further not

receive the addition of an anti-CD20 mAb. To elderly patients with an impaired physical condition and/or significant comorbidity, CLB is today basically only recommended in combination with a CD20 mAb alternatively BR can be offered [27, 53, 115, 156].

In depth analysis of all individual patient records is a strength in increasing the reliability and accuracy of the data as well as minimizing missing data, but also comes with risks and limitations since data collected in a retrospective manner may be affected by inconsistencies in chart interpretation and differences in clinical experience.

To conclude, this large nation-wide cohort of strictly consecutive 1st line treated CLL patients can serve as a basis for control against data from clinical trials. Increased knowledge about the effectiveness of SOC is important when evaluating new agents and combinations and can support health authorities and regions in deciding which new drugs and indications to be recommended. We also showed that treatment and diagnostics differ between types of hospitals and between regions and that the outcome was dependent on the type of treatment and appears to differ between geographical regions, justifying further investigation in differences between regions and levels of health care to reduce potential regional differences in patient management.

## 6.3   PAPER III

**"Ibrutinib versus previous standard of care: an adjusted comparison in patients with relapsed/refractory chronic lymphocytic leukaemia"**

The BTK inhibitor ibrutinib showed significant improvement in both PFS (HR=0.22, $p$<0.001) and OS (HR=0.43, $p$=0.005) in R/R CLL patients compared to the anti-CD20 mAb ofatumumab in the pivotal RESONATE trial leading to approval of ibrutinib by the FDA 2014 [23]. Due to limited information on comparisons with other widely used treatments in routine health care we explored the relative efficacy of ibrutinib within the trial versus the effectiveness of SOC used in clinical routine in the Stockholm region.

The two cohorts had a similar gender distribution (approximately two thirds were males in both groups) and both cohorts were heavily pretreated but the patients from the "Stockholm cohort" were generally older and had a higher proportion of patients with advanced stage disease as well as poorer performance status at start of each treatment line compared to the "RESONATE cohort".

Both unadjusted (treatment as only covariate) and adjusted (controlled for differences in baseline characteristics at start of respective treatment line) PFS and OS were significantly longer for patients treated with ibrutinib within the "RESONATE cohort" than in patients treated with SOC within the "Stockholm cohort" For PFS the adjusted HR for ibrutinib vs SOC was 0.15 ($p$<0.0001) which can be translated into an 85% lower relative risk for progression with ibrutinib than with SOC. The corresponding HR for OS was 0.36 ($p$<0.0001) and can accordingly be interpreted as a 64% reduced risk of death for the ibrutinib group. HRs from the sensitivity analysis including only patients from the Stockholm-cohort treated within the same time frame as the RESONATE-cohort (2012-2013), were coherent with the base case analysis.

Even if potential confounders were adjusted for and investigated in paper III, being a non-randomized indirect comparison, there is always a risk of differences not being

captured between the groups (residual confounding) introducing bias. For example, the distribution of important risk factors such as *TP53* abnormalities and IGHV mutational status was missing for a vast majority in our cohort and could therefore not be included in the model.

Our results were in line with the pivotal trial and also with other studies performed using data from the RESONATE trial in other indirect comparisons using similar statistical methods [275-277]. There are several recent examples of other studies performing cross-trial comparisons and adjusted comparisons in both frontline and R/R settings when data on direct comparisons are sparse [110, 111, 278]. Of course, all indirect comparisons must be interpreted judiciously considering the associated limitations due to variations in patient characteristics between the study populations, different designs of the studies and disparities in inclusion/exclusion criteria to mention some. These methods can never take the place of RCTs regarding evidence of statistical advantage to one treatment over another. Nevertheless, they may generate hypotheses, add to the body of knowledge and provide complementary information supporting treatment decisions until additional evidence from RCTs and long-term follow-up exists.

Considering the broad spectrum of treatment options already on the market or in transit, it is not feasible neither practically nor economically to compare a new treatment regimen with all or even several frequently used standard alternatives within the frames of RCTs. SOC as well as reference group might also vary across nations where adjusted comparisons at least can provide insight of comparative efficacy and offer support in the navigation among the growing number of treatment options.

To conclude, our study describes a statistical approach to provide a preliminary comparison between treatments used in clinical routine with data from clinical trials of new drugs until direct comparisons from randomized trials are available. As our results implies a significantly improved survival with ibrutinib compared to SOC used during the time period studied, the results and this method must be interpreted and approached with caution since data from two different sources are compared hence residual confounding cannot be excluded.

## 6.4 PAPER IV

**"A real-world study of first-line therapy in 280 consecutive Swedish patients ≥ 80 years with newly diagnosed diffuse large B-cell lymphoma: very elderly (≥ 85 years) do well on curative intended therapy"**

We identified 292 patients diagnosed with *de novo* DLBCL at the age of 80 years or older of which 280 received treatment with curative intent. Since the cohort reached fifteen years back in time both R-CHOP and CHOP was included in this cohort but analyzed separately. The median age was 85 years (range 80-97) which was the cut-off used for comparison between two age subgroups: 80-84 years vs ≥ 85 years. Median age and range were similar between the curative and the palliative intent subgroups. The categorization according to age adjusted IPI (aaIPI) was rather evenly distributed regarding risk groups in the curative intent group while a greater proportion of patients had high aaIPI scores in patients treated with palliative intent.

In patients treated with curative intent the addition of rituximab resulted in a significant improvement in response rates, PFS and OS in both age groups. Moreover, the very oldest patients ≥ 85 years responded to and tolerated R-CHOP similarly to patients aged 80-84 years. Regarding cyclophosphamide/doxorubicin the average delivered relative dose intensity (RDI) in patients treated with R-CHOP was between 50-75% in the majority of patients in both age groups. Neutropenia and infections were the most common ≥ grade 3 AEs in both age groups but numerically actually less frequent in the oldest subgroup. One possible explanation might be that a greater proportion of the younger subgroup received an RDI of > 75% and in median received more cycles than the older subgroup and further biweekly treatment (R-CHOP-14) were more frequent in the younger cohort even if a majority in both groups received treatment every third week (R-CHOP-21).

Another population-based study on DLBCL patients ≥ 80 years showed that 63% of patients receiving a total average RDI over 50% had better outcome and acceptable toxicity profile [279]. Yet another population-based study on DLBCL patients ≥ 75 years further indicated that planned dose-reduction < 80% did not affect OS negatively [280]. Moreover a large retrospective analysis on dose intensity and outcome on elderly DLBCL patients showed a significantly increased risk of progression or death in patients ≥ 80 years with planned full dose intensity of more than 2.5-fold compared to patient aged 70-80 years [281]. R-CHOP can provide cure in very elderly patients, but dose-intensity must be carefully considered and is a challenge in elderly, comorbid patients.

Unfortunately, evaluation of molecular subgroup was only performed in a third of the study population and could therefore not contribute to meaningful analyzes and conclusions. Also, the group treated with palliative intent was small and with various approaches and could only be presented descriptively. Moreover, there are several ways of assessing comorbidities and in our research group we were most familiar with the Cumulative Illness Rating Scale (CIRS) but a more appropriate choice might have been the CIRS-G [14] adapted to the geriatric population and more easily interpretable to the subgroups fit, unfit and frail, both tools commonly used in other studies [15, 115, 282]. There are several other tools more suitable for prospective trials and in therapeutic decisions for example the Comprehensive Geriatric Assessment (CGA) and even a specified version for geriatric cancer patients but these can be time consuming and of course some aspects are not feasible to catch retrospectively such as quality of life [14]. However, these are aspects that might be worth to consider in the planning of future real-world studies [270].

Despite the inherited limitations by being a retrospective analysis and limitations described here and in the published paper, real-world historical cohorts like this can still carry useful insights in comparison with new treatment options that sometimes are approved based on surrogate endpoints via accelerated approval programs in patients with an urgent medical need as patients relapsing from DLBCL [6].

To conclude patients ≥ 85 years responded to and tolerated chemoimmunotherapy equally well as patients aged 80-84 years, highlighting that even very elderly patients benefit from active therapy provided that dose-adaption of chemotherapeutic drugs are performed.

# 7    FUTURE PERSPECTIVES

In the rapid pace of change regarding treatment of lymphoid malignancies, especially in CLL, with the wide array of novel treatment strategies entering the market and under investigation, possible MRD guided length of treatment and further deeper knowledge on the heterogeneous biological features on both CLL and DLBCL, individually tailored treatment are getting closer. However, there are still challenges to overcome and regarding many new treatments reaching the market the information on long-term survival and toxicities are limited at time of approval.

In the quickly and constantly changing treatment landscape of CLL in particular, the standard of care also changes in a speed where RCTs may have difficulties keeping pace with all questions emerging while novel agents and combinations are being implemented in clinical routine care. Before long-term evidence from RCTs and outcome in subgroups not included in pivotal trials are available, real-world data can offer valuable insights on the outcome of treatments used in clinical routine and add to the knowledge on novel treatments in unselected patients in routine care as they might differ in several aspects from patients within trials [7]. Hypotheses generated from RWD may also support in the design of clinical trials investigating combinations or sequencing of novel targeted agents as there to date is no solid evidence on this matter [283].

Novel agents comes with a significant cost [21] and it is important for health care and society at large to find optimal ways to use these treatments and real-world studies can provide feed-back to health care providers based on completely clinically grounded data. Continued elaboration of the generation of RWD might support early and equal access and optimal use of new high-cost treatments. A recent cost-effectiveness analysis on ibrutinib presented calculations that the monthly cost would have to be reduced by 72% to be cost-effective as frontline therapy for unselected CLL patients [21]. In a society with already limited resources, clinically effective treatments and that patients really benefit from new costly drugs is of great importance from a sustainability perspective. Regarding accelerated approvals of drugs in patients with for example R/R lymphoid malignancies with an unmet medical need where trial data are premature and OS benefits and long-term toxicity unknown, RWD might offer valuable insights in some of these aspects.

A continuation of paper II in this thesis is about to commence to see how treatment regimens and management of frontline strategies in CLL patients have changed over the last years (from 2014 and forward) and if outcomes have improved. Hopefully, the implementation of testing for biological risk markers will provide possibilities to make meaningful analyzes and the data contribute to information on long-term outcome in addition to post-authorization studies. The data might further be related to results from ongoing trials combining two or three targeted agents or investigating different sequences of treatments in CLL patients (ISRCTN13751862, NCT02910583, NCT02758665).

There are also plans of a continuation of paper IV on DLBCL patients, but now on all age groups and also in the relapsed setting were for example CAR T-cell therapies have emerged as an alternative salvage treatment. With so far limited data from ongoing phase 3 trials on CAR T-cell therapies (NCT03391466, NCT03570892, NCT03575351), RWD can add valuable information on the outcome of unselected patients to complement data from such trials.

Comprehensive RWD on consecutive unselected patients regarding treatment intensity, dosing, temporary stops, toxicity, response rates, survival data collected in detail from patient records will as far as possible reflect the "true" outcome in routine health care and constitute an important context to relate with data from RCTs. Further RWD can provide guidance on issues outside the scope of clinical trials and for example yield information regarding the adherence of approved/recommended dose and if deviations from these appear to affect the outcome. This may support the discussion within the Swedish Lymphoma- and Swedish CLL-groups regarding national guidelines and might identify possible differences in treatment and outcome within and between regions.

Recently a Nordic CLL study group was formed which opens up to great possibilities of collaborations and expanding studies to include other Nordic countries. There is ongoing work on improving the national quality registries and to harmonize variables between different registries in the Nordic countries. A validation of the SLR is planned next year and it is now possible to access some data online in order to pace up with the rapid evolution in diagnostics, treatments and outcome. However there is still a lag in registration within registries [180].

The majority of cancer patients are today treated in the real world, e.g. outside the context of clinical trials and thus possesses a lot of valuable and important information. High-quality RWD may be time-consuming to achieve and logistic developments of electronic health records with standardized nation-wide entry of clinical data and further innovation of systems transferring data to national cancer registries enabling access to real-time data are warranted. This might facilitate the generation of RWD and moreover help to fill some of the gaps between evidence and clinical practice in a more efficient way and on a larger scale in the era of targeted agents, combinations and sequential treatment strategies [284].

# 8    ACKNOWLEDGEMENTS

I would like to thank all the people who have contributed to the completion of this thesis in different ways. In particular I wish to thank:

*Lotta Hansson***,** my main supervisor, always available, answers texts and e-mails no matter what time of day and who kept pushing me in the right direction. Thank you for all your time and advice regarding everything from research and work to family and kids.

*Sandra Eketorp Sylvan***,** my co-supervisor, for your invaluable advice, support with manuscripts, presentations and for your never-ending encouragement.

*Anders Österborg***,** my co-supervisor, for sharing your broad and deep knowledge and for your inspiration and ideas.

*Håkan Mellstedt,* for giving me the opportunity to be a part of this fantastic research group.

*Leila Relander***,** always kind and helpful. Thank you for sharing your expertise in editing of manuscripts, figures, tables, posters and for teaching me EndNote.

*Gunilla Burén*, for your encouragement and positive energy.

All the members of the *Österborg-Mellstedt* group for teaching me a lot during the years and for valuable input and inspiration during our seminars.

All the *co-authors* of the papers and to *Carl Hansson* for assistance with the database for paper IV.

The whole team behind *"Research school for clinicians in epidemiology"*, generation 16 and 17, for sharing your profound knowledge.

*Carina Nord***,** my mentor, for encouragement and not the least for all informal meetings in the corridors and during lunchbreaks.

*Signe Friesland*, head of the Department of Oncology and *Fernanda Costa Svedman*, head of residents in Oncology, for making it possible to combine research and clinical practice.

*Caroline Staff* and *Josefin Fernebro***,** my clinical supervisors during my residency, for sharing your knowledge in oncology.

*My resident colleagues* for being great co-workers and friends.

*Martina Silivri,* my dear friend, thank you for travelling half the country when I needed it the most, to give me time to work, rest or just hang out.

*Klara Swerup* and *Anders Åslund* for your friendship, for entertaining the kids several weekends and for cooking delicious food.

The extended *family Berggren,* for your cheering and for making me feel like a part of your family from day one.

*Mom* and *Dad*, my loving parents, for being there for me no matter what and for teaching me what´s important in life. Oliver and Ingrid loves you just as much as I do and we hope to see you more soon.

*Daniel Asklid,* my one and only brother, for introducing medicine to me and for your loving support and firm belief in me. Warm thanks to the whole *family Grobecker Asklid* for your encouragement and hospitality.

Foremost and last, my beloved family - *Nicklas Berggren,* my soulmate, *Oliver* and *Ingrid,* our wonderful children. Words are not enough to express my love for you. Thank you for your endless love and for making me happy every day.

# 9 REFERENCES

1. Makady A, de Boer A, Hillege H, et al. What Is Real-World Data? A Review of Definitions Based on Literature and Stakeholder Interviews. Value Health. 2017; 20(7): 858-65.
2. Sherman RE, Anderson SA, Dal Pan GJ, et al. Real-World Evidence - What Is It and What Can It Tell Us? N Engl J Med. 2016; 375(23): 2293-7.
3. RWE Navigator, GetReal glossary of terms and definitions. https://rwe-navigator.eu/rwe-background-getreal-glossary-of-terms-and-definitions. Cited: October 24, 2020.
4. Jarow JP, LaVange L, Woodcock J. Multidimensional Evidence Generation and FDA Regulatory Decision Making: Defining and Using "Real-World" Data. JAMA. 2017; 318(8): 703-4.
5. Lewis JR, Kerridge I, Lipworth W. Use of Real-World Data for the Research, Development, and Evaluation of Oncology Precision Medicines. JCO Precision Oncology. 2017; (1): 1-11.
6. Cave A, Kurz X, Arlett P. Real-World Data for Regulatory Decision Making: Challenges and Possible Solutions for Europe. Clin Pharmacol Ther. 2019; 106(1): 36-9.
7. Islam P, Mato AR. Real-World Evidence for Chronic Lymphocytic Leukemia in the Era of Targeted Therapies. Cancer J. 2019; 25(6): 442-8.
8. Bird ST, Tian F, Flowers N, et al. Idelalisib for Treatment of Relapsed Follicular Lymphoma and Chronic Lymphocytic Leukemia: A Comparison of Treatment Outcomes in Clinical Trial Participants vs Medicare Beneficiaries. JAMA Oncol. 2020; 6(2): 248-54.
9. Khozin S, Blumenthal GM, Pazdur R. Real-world Data for Clinical Evidence Generation in Oncology. J Natl Cancer Inst. 2017; 109(11): 1-5.
10. Kim MS, Prasad V. US Food and Drug Administration approvals for Bruton tyrosine kinase inhibitors in patients with chronic lymphocytic leukemia: Potential inefficiencies in trial design and evidence generation. Cancer. 2020: doi: 10.1002/cncr.33058. Online ahead of print.
11. ElZarrad MK, Corrigan-Curay J. The US Food and Drug Administration's Real-World Evidence Framework: A Commitment for Engagement and Transparency on Real-World Evidence. Clin Pharmacol Ther. 2019; 106(1): 33-5.
12. Fleurence RL, Shuren J. Advances in the Use of Real-World Evidence for Medical Devices: An Update From the National Evaluation System for Health Technology. Clin Pharmacol Ther. 2019; 106(1): 30-3.
13. Nowell WB. Information Patients Can Provide Will Strengthen the Real-World Evidence That Matters to Them. Clin Pharmacol Ther. 2019; 106(1): 49-51.
14. Khan Y, Brem EA. Considerations for the Treatment of Diffuse Large B Cell Lymphoma in the Elderly. Curr Hematol Malig Rep. 2019; 14(4): 228-38.
15. Hallek M. Chronic lymphocytic leukemia: 2020 update on diagnosis, risk stratification and treatment. Am J Hematol. 2019; 94(11): 1266-87.
16. Liu Y, Barta SK. Diffuse large B-cell lymphoma: 2019 update on diagnosis, risk stratification, and treatment. Am J Hematol. 2019; 94(5): 604-16.
17. Kim C, Prasad V. Cancer Drugs Approved on the Basis of a Surrogate End Point and Subsequent Overall Survival: An Analysis of 5 Years of US Food and Drug Administration Approvals. JAMA Intern Med. 2015; 175(12): 1992-4.
18. Davis C, Naci H, Gurpinar E, et al. Availability of evidence of benefits on overall survival and quality of life of cancer drugs approved by European Medicines Agency: retrospective cohort study of drug approvals 2009-13. BMJ. 2017; 359: j4530.
19. Shanafelt TD, Borah BJ, Finnes HD, et al. Impact of ibrutinib and idelalisib on the pharmaceutical cost of treating chronic lymphocytic leukemia at the individual and societal levels. J Oncol Pract. 2015; 11(3): 252-8.
20. Barnes JI, Divi V, Begaye A, et al. Cost-effectiveness of ibrutinib as first-line therapy for chronic lymphocytic leukemia in older adults without deletion 17p. Blood Adv. 2018; 2(15): 1946-56.
21. Patel K, Isufi I, Kothari S, et al. Cost-Effectiveness of First-Line Ibrutinib versus Third-Line in Patients with Untreated Chronic Lymphocytic Leukemia. Blood. 2020: doi: 10.1182/blood.2020004922. Online ahead of print.
22. Winqvist M, Asklid A, Andersson PO, et al. Real-world results of ibrutinib in patients with relapsed or refractory chronic lymphocytic leukemia: data from 95 consecutive patients treated in a compassionate use program. A study from the Swedish Chronic Lymphocytic Leukemia Group. Haematologica. 2016; 101(12): 1573-80.
23. Byrd JC, Brown JR, O'Brien S, et al. Ibrutinib versus ofatumumab in previously treated chronic lymphoid leukemia. N Engl J Med. 2014; 371(3): 213-23.

24.    Sterne JA, Hernan MA, Reeves BC, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. BMJ. 2016; 355: i4919.

25.    Grimes DA, Schulz KF. Bias and causal associations in observational research. Lancet. 2002; 359(9302): 248-52.

26.    Kipps TJ, Stevenson FK, Wu CJ, et al. Chronic lymphocytic leukaemia. Nat Rev Dis Primers. 2017; 3: 16096.

27.    Regionala cancercentrum i samverkan. Nationellt vårdprogram Kronisk lymfatisk leukemi (KLL) version 4.0 2019. https://www.cancercentrum.se/samverkan/cancerdiagnoser/blod-lymfom-myelom/kronisk-lymfatisk-leukemi-kll/vardprogram/. Cited October 24, 2020.

28.    Mattsson M, Sandin F, Kimby E, et al. Increasing prevalence of chronic lymphocytic leukemia with an estimated future rise: A nationwide population-based study. Am J Hematol. 2020; 95(2): E36-E8.

29.    Slager SL, Benavente Y, Blair A, et al. Medical history, lifestyle, family history, and occupational risk factors for chronic lymphocytic leukemia/small lymphocytic lymphoma: the InterLymph Non-Hodgkin Lymphoma Subtypes Project. J Natl Cancer Inst Monogr. 2014; 2014(48): 41-51.

30.    Yang SM, Li JY, Gale RP, et al. The mystery of chronic lymphocytic leukemia (CLL): Why is it absent in Asians and what does this tell us about etiology, pathogenesis and biology? Blood Rev. 2015; 29(3): 205-13.

31.    Karakosta M, Delicha EM, Kouraklis G, et al. Association of various risk factors with chronic lymphocytic leukemia and its cytogenetic characteristics. Arch Environ Occup Health. 2016; 71(6): 317-29.

32.    Goldin LR, Bjorkholm M, Kristinsson SY, et al. Elevated risk of chronic lymphocytic leukemia and other indolent non-Hodgkin's lymphomas among relatives of patients with chronic lymphocytic leukemia. Haematologica. 2009; 94(5): 647-53.

33.    Fabbri G, Dalla-Favera R. The molecular pathogenesis of chronic lymphocytic leukaemia. Nat Rev Cancer. 2016; 16(3): 145-62.

34.    Swerdlow S, Campo E, Harris N. WHO Classification of Tumours of Haematopoetic and Lymphoid Tissues. Revised 4th edition. Lyon: IARC Press. Section: Mature B-cell neoplasms. 2017; 2: 220.

35.    Strati P, Shanafelt TD. Monoclonal B-cell lymphocytosis and early-stage chronic lymphocytic leukemia: diagnosis, natural history, and risk stratification. Blood. 2015; 126(4): 454-62.

36.    Ten Hacken E, Gounari M, Ghia P, et al. The importance of B cell receptor isotypes and stereotypes in chronic lymphocytic leukemia. Leukemia. 2019; 33(2): 287-98.

37.    Hamblin TJ, Davis Z, Gardiner A, et al. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood. 1999; 94(6): 1848-54.

38.    Damle RN, Wasil T, Fais F, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood. 1999; 94(6): 1840-7.

39.    Burger JA, Wiestner A. Targeting B cell receptor signalling in cancer: preclinical and clinical advances. Nat Rev Cancer. 2018; 18(3): 148-67.

40.    Ferrer G, Montserrat E. Critical molecular pathways in CLL therapy. Mol Med. 2018; 24(1): 1-10.

41.    Anderson MA, Deng J, Seymour JF, et al. The BCL2 selective inhibitor venetoclax induces rapid onset apoptosis of CLL cells in patients via a TP53-independent mechanism. Blood. 2016; 127(25): 3215-24.

42.    Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144(5): 646-74.

43.    Dohner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med. 2000; 343(26): 1910-6.

44.    Catherwood MA, Gonzalez D, Donaldson D, et al. Relevance of TP53 for CLL diagnostics. J Clin Pathol. 2019; 72(5): 343-6.

45.    Campo E, Cymbalista F, Ghia P, et al. TP53 aberrations in chronic lymphocytic leukemia: an overview of the clinical implications of improved diagnostics. Haematologica. 2018; 103(12): 1956-68.

46.    Landau DA, Tausch E, Taylor-Weiner AN, et al. Mutations driving CLL and their evolution in progression and relapse. Nature. 2015; 526(7574): 525-30.

47.    Yosifov DY, Wolf C, Stilgenbauer S, et al. From Biology to Therapy: The CLL Success Story. Hemasphere. 2019; 3(2): e175.

48.    Stilgenbauer S, Schnaiter A, Paschka P, et al. Gene mutations and treatment outcome in chronic lymphocytic leukemia: results from the CLL8 trial. Blood. 2014; 123(21): 3247-54.

49.    Nabhan C, Rosen ST. Chronic lymphocytic leukemia: a clinical review. JAMA. 2014; 312(21): 2265-76.

50.  Swerdlow S, Campo E, Harris N. WHO Classification of Tumours of Haematopoetic and Lymphoid Tissues. Revised 4th edition. Lyon: IARC Press. Section: Mature B-cell neoplasms. 2017; 2: 216.

51.  Hallek M, Cheson BD, Catovsky D, et al. iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. Blood. 2018; 131(25): 2745-60.

52.  Cheson BD, Fisher RI, Barrington SF, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. J Clin Oncol. 2014; 32(27): 3059-68.

53.  Wierda WG, Byrd JC, Abramson JS, et al. Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma, Version 4.2020, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2020; 18(2): 185-217.

54.  Jain N, Keating MJ. Richter transformation of CLL. Expert Rev Hematol. 2016; 9(8): 793-801.

55.  Parikh SA, Shanafelt TD. Risk factors for Richter syndrome in chronic lymphocytic leukemia. Curr Hematol Malig Rep. 2014; 9(3): 294-9.

56.  Rossi D, Spina V, Gaidano G. Biology and treatment of Richter syndrome. Blood. 2018; 131(25): 2761-72.

57.  Rai KR, Sawitsky A, Cronkite EP, et al. Clinical staging of chronic lymphocytic leukemia. Blood. 1975; 46(2): 219-34.

58.  Binet JL, Auquier A, Dighiero G, et al. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer. 1981; 48(1): 198-206.

59.  Fischer K, Bahlo J, Fink AM, et al. Long-term remissions after FCR chemoimmunotherapy in previously untreated patients with CLL: updated results of the CLL8 trial. Blood. 2016; 127(2): 208-15.

60.  Thompson PA, Tam CS, O'Brien SM, et al. Fludarabine, cyclophosphamide, and rituximab treatment achieves long-term disease-free survival in IGHV-mutated chronic lymphocytic leukemia. Blood. 2016; 127(3): 303-9.

61.  Hallek M, Wanders L, Ostwald M, et al. Serum beta(2)-microglobulin and serum thymidine kinase are independent predictors of progression-free survival in chronic lymphocytic leukemia and immunocytoma. Leuk Lymphoma. 1996; 22(5-6): 439-47.

62.  Thompson PA, O'Brien SM, Xiao L, et al. beta2 -microglobulin normalization within 6 months of ibrutinib-based treatment is associated with superior progression-free survival in patients with chronic lymphocytic leukemia. Cancer. 2016; 122(4): 565-73.

63.  International CLLIPIwg. An international prognostic index for patients with chronic lymphocytic leukaemia (CLL-IPI): a meta-analysis of individual patient data. Lancet Oncol. 2016; 17(6): 779-90.

64.  Condoluci A, Terzi di Bergamo L, Langerbeins P, et al. International prognostic score for asymptomatic early-stage chronic lymphocytic leukemia. Blood. 2020; 135(21): 1859-69.

65.  Dighiero G, Maloum K, Desablens B, et al. Chlorambucil in indolent chronic lymphocytic leukemia. French Cooperative Group on Chronic Lymphocytic Leukemia. N Engl J Med. 1998; 338(21): 1506-14.

66.  Hoechstetter MA, Busch R, Eichhorst B, et al. Early, risk-adapted treatment with fludarabine in Binet stage A chronic lymphocytic leukemia patients: results of the CLL1 trial of the German CLL study group. Leukemia. 2017; 31(12): 2833-7.

67.  Herling CD, Cymbalista F, Gross-Ophoff-Muller C, et al. Early treatment with FCR versus watch and wait in patients with stage Binet A high-risk chronic lymphocytic leukemia (CLL): a randomized phase 3 trial. Leukemia. 2020; 34(8): 2038-50.

68.  Langerbeins P, Bahlo J, Rhein C, et al. Ibrutinib versus placebo in patients with asymptomatic, treatment-naivë early stage chronic lymphocytic leukemia (CLL): Primary endpoint results of the phase 3 double-blind randomized CLL12 trial. (Abstract LB2602). 24th EHA Congress. Amsterdam, the Netherlands. June 13-16, 2019.

69.  Kovacs G, Robrecht S, Fink AM, et al. Minimal Residual Disease Assessment Improves Prediction of Outcome in Patients With Chronic Lymphocytic Leukemia (CLL) Who Achieve Partial Response: Comprehensive Analysis of Two Phase III Studies of the German CLL Study Group. J Clin Oncol. 2016; 34(31): 3758-65.

70.  Bottcher S, Ritgen M, Fischer K, et al. Minimal residual disease quantification is an independent predictor of progression-free and overall survival in chronic lymphocytic leukemia: a multivariate analysis from the randomized GCLLSG CLL8 trial. J Clin Oncol. 2012; 30(9): 980-8.

71.  Thompson PA, Wierda WG. Eliminating minimal residual disease as a therapeutic end point: working toward cure for patients with CLL. Blood. 2016; 127(3): 279-86.

72. Thompson PA, Srivastava J, Peterson C, et al. Minimal residual disease undetectable by next-generation sequencing predicts improved outcome in CLL after chemoimmunotherapy. Blood. 2019; 134(22): 1951-9.

73. Rawstron AC, Fazi C, Agathangelidis A, et al. A complementary role of multiparameter flow cytometry and high-throughput sequencing for minimal residual disease detection in chronic lymphocytic leukemia: an European Research Initiative on CLL study. Leukemia. 2016; 30(4): 929-36.

74. Furstenau M, De Silva N, Eichhorst B, et al. Minimal Residual Disease Assessment in CLL: Ready for Use in Clinical Routine? Hemasphere. 2019; 3(5): e287.

75. Cramer P, von Tresckow J, Bahlo J, et al. Bendamustine followed by obinutuzumab and venetoclax in chronic lymphocytic leukaemia (CLL2-BAG): primary endpoint analysis of a multicentre, open-label, phase 2 trial. Lancet Oncol. 2018; 19(9): 1215-28.

76. Minot GB, Isaacs RJ. Lymphatic leukemia; age incidence, duration, and benefit derived from irradiation. The Boston Medical Surgical Journal. 1924; 191(1): 1-9.

77. Chan EK, Fung S, Gospodarowicz M, et al. Palliation by low-dose local radiation therapy for indolent non-Hodgkin lymphoma. Int J Radiat Oncol Biol Phys. 2011; 81(5): e781-6.

78. Galton DA, Israels LG, Nabarro JD, et al. Clinical trials of p-(di-2-chloroethylamino)-phenylbutyric acid (CB 1348) in malignant lymphoma. Br Med J. 1955; 2(4949): 1172-6.

79. Shaw R K, Boggs D R, Silberman H R, et al. A Study of Prednisone Therapy in Chronic Lymphocytic Leukemia. Blood. 1961; 17: 182-9.

80. Rai KR, Jain P. Chronic lymphocytic leukemia (CLL)-Then and now. Am J Hematol. 2016; 91(3): 330-40.

81. Begleiter A, Mowat M, Israels LG, et al. Chlorambucil in chronic lymphocytic leukemia: mechanism of action. Leuk Lymphoma. 1996; 23(3-4): 187-201.

82. Robak T. Therapy of chronic lymphocytic leukaemia with purine nucleoside analogues: facts and controversies. Drugs Aging. 2005; 22(12): 983-1012.

83. Keating MJ, Kantarjian H, Talpaz M, et al. Fludarabine: a new agent with major activity against chronic lymphocytic leukemia. Blood. 1989; 74(1): 19-25.

84. Rai KR, Peterson BL, Appelbaum FR, et al. Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukemia. N Engl J Med. 2000; 343(24): 1750-7.

85. Eichhorst BF, Busch R, Stilgenbauer S, et al. First-line therapy with fludarabine compared with chlorambucil does not result in a major benefit for elderly patients with advanced chronic lymphocytic leukemia. Blood. 2009; 114(16): 3382-91.

86. Eichhorst BF, Busch R, Hopfinger G, et al. Fludarabine plus cyclophosphamide versus fludarabine alone in first-line therapy of younger patients with chronic lymphocytic leukemia. Blood. 2006; 107(3): 885-91.

87. Flinn IW, Neuberg DS, Grever MR, et al. Phase III trial of fludarabine plus cyclophosphamide compared with fludarabine for patients with previously untreated chronic lymphocytic leukemia: US Intergroup Trial E2997. J Clin Oncol. 2007; 25(7): 793-8.

88. Catovsky D, Richards S, Matutes E, et al. Assessment of fludarabine plus cyclophosphamide for patients with chronic lymphocytic leukaemia (the LRF CLL4 Trial): a randomised controlled trial. Lancet. 2007; 370(9583): 230-9.

89. Cheson BD, Leoni L. Bendamustine: mechanism of action and clinical data. Clin Adv Hematol Oncol. 2011; 9(8 Suppl 19): 1-11.

90. Knauf WU, Lissichkov T, Aldaoud A, 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.

91. Niederle N, Megdenberg D, Balleisen L, et al. Bendamustine compared to fludarabine as second-line treatment in chronic lymphocytic leukemia. Ann Hematol. 2013; 92(5): 653-60.

92. Klein C, Lammens A, Schafer W, et al. Epitope interactions of monoclonal antibodies targeting CD20 and their relationship to functional properties. MAbs. 2013; 5(1): 22-33.

93. McLaughlin P, Grillo-Lopez AJ, Link BK, et al. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol. 1998; 16(8): 2825-33.

94. Byrd JC, Peterson BL, Morrison VA, et al. Randomized phase 2 study of fludarabine with concurrent versus sequential treatment with rituximab in symptomatic, untreated patients with B-cell chronic lymphocytic leukemia: results from Cancer and Leukemia Group B 9712 (CALGB 9712). Blood. 2003; 101(1): 6-14.

95.    Hallek M, Fischer K, Fingerle-Rowson G, et al. Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. Lancet. 2010; 376(9747): 1164-74.

96.    Robak T, Dmoszynska A, Solal-Celigny P, et al. Rituximab plus fludarabine and cyclophosphamide prolongs progression-free survival compared with fludarabine and cyclophosphamide alone in previously treated chronic lymphocytic leukemia. J Clin Oncol. 2010; 28(10): 1756-65.

97.    Maloney DG. Anti-CD20 antibody therapy for B-cell lymphomas. N Engl J Med. 2012; 366(21): 2008-16.

98.    Chanan-Khan A, Kipps T, Stilgenbauer S. Clinical roundtable monograph. New alternatives in CLL therapy: managing adverse events. Clin Adv Hematol Oncol. 2010; 8(8): suppl 1-15.

99.    Aguiar-Bujanda D, Blanco-Sanchez MJ, Hernandez-Sosa M, et al. Late-Onset Neutropenia After Rituximab-Containing Therapy for Non-Hodgkin Lymphoma. Clin Lymphoma Myeloma Leuk. 2015; 15(12): 761-5.

100.   Stolz C, Schuler M. Molecular mechanisms of resistance to Rituximab and pharmacologic strategies for its circumvention. Leuk Lymphoma. 2009; 50(6): 873-85.

101.   Osterborg A, Jewell RC, Padmanabhan-Iyer S, et al. Ofatumumab monotherapy in fludarabine-refractory chronic lymphocytic leukemia: final results from a pivotal study. Haematologica. 2015; 100(8): e311-4.

102.   Cartron G, de Guibert S, Dilhuydy MS, et al. Obinutuzumab (GA101) in relapsed/refractory chronic lymphocytic leukemia: final data from the phase 1/2 GAUGUIN study. Blood. 2014; 124(14): 2196-202.

103.   Hale G. The CD52 antigen and development of the CAMPATH antibodies. Cytotherapy. 2001; 3(3): 137-43.

104.   Osterborg A, Dyer MJ, Bunjes D, et al. Phase II multicenter study of human CD52 antibody in previously treated chronic lymphocytic leukemia. European Study Group of CAMPATH-1H Treatment in Chronic Lymphocytic Leukemia. J Clin Oncol. 1997; 15(4): 1567-74.

105.   Stilgenbauer S, Dohner H. Campath-1H-induced complete remission of chronic lymphocytic leukemia despite p53 gene mutation and resistance to chemotherapy. N Engl J Med. 2002; 347(6): 452-3.

106.   Hillmen P, Skotnicki AB, Robak T, et al. Alemtuzumab compared with chlorambucil as first-line therapy for chronic lymphocytic leukemia. J Clin Oncol. 2007; 25(35): 5616-23.

107.   Hallek M. Chronic lymphocytic leukemia: 2017 update on diagnosis, risk stratification, and treatment. Am J Hematol. 2017; 92(9): 946-65.

108.   Eichhorst B, Fink AM, Bahlo J, 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; 17(7): 928-42.

109.   Kutsch N, Bahlo J, Robrecht S, et al. Long Term Follow-up Data and Health-Related Quality of Life in Frontline Therapy of Fit Patients Treated With FCR Versus BR (CLL10 Trial of the GCLLSG). Hemasphere. 2020; 4(1): e336.

110.   Cuneo A, Mato AR, Rigolin GM, et al. Efficacy of bendamustine and rituximab in unfit patients with previously untreated chronic lymphocytic leukemia. Indirect comparison with ibrutinib in a real-world setting. A GIMEMA-ERIC and US study. Cancer Med. 2020; Sep 24: doi: 10.1002/cam4.3470. Online ahead of print.

111.   Cuneo A, Follows G, Rigolin GM, et al. Efficacy of bendamustine and rituximab as first salvage treatment in chronic lymphocytic leukemia and indirect comparison with ibrutinib: a GIMEMA, ERIC and UK CLL FORUM study. Haematologica. 2018; 103(7): 1209-17.

112.   Mattsson A, Sylvan SE, Asklid A, et al. Risk-adapted bendamustine + rituximab is a tolerable treatment alternative for elderly patients with chronic lymphocytic leukaemia: a regional real-world report on 141 consecutive Swedish patients. Br J Haematol. 2020; Aug 10: doi: 10.1111/bjh.17032. Online ahead of print.

113.   Hillmen P, Gribben JG, Follows GA, et al. Rituximab plus chlorambucil as first-line treatment for chronic lymphocytic leukemia: Final analysis of an open-label phase II study. J Clin Oncol. 2014; 32(12): 1236-41.

114.   Michallet AS, Aktan M, Hiddemann W, et al. Rituximab plus bendamustine or chlorambucil for chronic lymphocytic leukemia: primary analysis of the randomized, open-label MABLE study. Haematologica. 2018; 103(4): 698-706.

115.   Goede V, Fischer K, Busch R, et al. Obinutuzumab plus chlorambucil in patients with CLL and coexisting conditions. N Engl J Med. 2014; 370(12): 1101-10.

116. Goede V, Fischer K, Dyer M, et al. Overall survival benefit of obinutuzumab over rituximab when combined with chlorambucil in patients with chronic lymphocytic leukemia and comorbidities: Final survival analysis of the CLL11 study. (Abstract S151). 23rd EHA Congress. Stockholm, Sweden. June 14-17, 2018.

117. Hillmen P, Robak T, Janssens A, et al. Chlorambucil plus ofatumumab versus chlorambucil alone in previously untreated patients with chronic lymphocytic leukaemia (COMPLEMENT 1): a randomised, multicentre, open-label phase 3 trial. Lancet. 2015; 385(9980): 1873-83.

118. Offner F, Robak T, Janssens A, et al. A five-year follow-up of untreated patients with chronic lymphocytic leukaemia treated with ofatumumab and chlorambucil: final analysis of the Complement 1 phase 3 trial. Br J Haematol. 2020; 190(5): 736-40.

119. Pan Z, Scheerens H, Li SJ, et al. Discovery of selective irreversible inhibitors for Bruton's tyrosine kinase. ChemMedChem. 2007; 2(1): 58-61.

120. Patel V, Balakrishnan K, Bibikova E, et al. Comparison of Acalabrutinib, A Selective Bruton Tyrosine Kinase Inhibitor, with Ibrutinib in Chronic Lymphocytic Leukemia Cells. Clin Cancer Res. 2017; 23(14): 3734-43.

121. Byrd JC, Furman RR, Coutre SE, et al. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. N Engl J Med. 2013; 369(1): 32-42.

122. O'Brien S, Furman RR, Coutre SE, et al. Ibrutinib as initial therapy for elderly patients with chronic lymphocytic leukaemia or small lymphocytic lymphoma: an open-label, multicentre, phase 1b/2 trial. Lancet Oncol. 2014; 15(1): 48-58.

123. Munir T, Brown JR, O'Brien S, et al. Final analysis from RESONATE: Up to six years of follow-up on ibrutinib in patients with previously treated chronic lymphocytic leukemia or small lymphocytic lymphoma. Am J Hematol. 2019; 94(12): 1353-63.

124. Burger JA, Tedeschi A, Barr PM, et al. Ibrutinib as Initial Therapy for Patients with Chronic Lymphocytic Leukemia. N Engl J Med. 2015; 373(25): 2425-37.

125. Burger JA, Barr PM, Robak T, et al. Long-term efficacy and safety of first-line ibrutinib treatment for patients with CLL/SLL: 5 years of follow-up from the phase 3 RESONATE-2 study. Leukemia. 2020; 34(3): 787-98.

126. Winqvist M, Andersson PO, Asklid A, et al. Long-term real-world results of ibrutinib therapy in patients with relapsed or refractory chronic lymphocytic leukemia: 30-month follow up of the Swedish compassionate use cohort. Haematologica. 2019; 104(5): e208-e10.

127. Mato AR, Roeker LE, Allan JN, et al. Outcomes of front-line ibrutinib treated CLL patients excluded from landmark clinical trial. Am J Hematol. 2018; 93(11): 1394-401.

128. Mato AR, Nabhan C, Thompson MC, et al. Toxicities and outcomes of 616 ibrutinib-treated patients in the United States: a real-world analysis. Haematologica. 2018; 103(5): 874-9.

129. Woyach JA, Ruppert AS, Guinn D, et al. BTK(C481S)-Mediated Resistance to Ibrutinib in Chronic Lymphocytic Leukemia. J Clin Oncol. 2017; 35(13): 1437-43.

130. Jain P, Thompson PA, Keating M, et al. Long-term outcomes for patients with chronic lymphocytic leukemia who discontinue ibrutinib. Cancer. 2017; 123(12): 2268-73.

131. Ahn IE, Underbayev C, Albitar A, et al. Clonal evolution leading to ibrutinib resistance in chronic lymphocytic leukemia. Blood. 2017; 129(11): 1469-79.

132. Burger JA, Sivina M, Jain N, et al. Randomized trial of ibrutinib vs ibrutinib plus rituximab in patients with chronic lymphocytic leukemia. Blood. 2019; 133(10): 1011-9.

133. Woyach JA, Ruppert AS, Heerema NA, et al. Ibrutinib Regimens versus Chemoimmunotherapy in Older Patients with Untreated CLL. N Engl J Med. 2018; 379(26): 2517-28.

134. Shanafelt TD, Wang XV, Kay NE, et al. Ibrutinib-Rituximab or Chemoimmunotherapy for Chronic Lymphocytic Leukemia. N Engl J Med. 2019; 381(5): 432-43.

135. Moreno C, Greil R, Demirkan F, et al. Ibrutinib plus obinutuzumab versus chlorambucil plus obinutuzumab in first-line treatment of chronic lymphocytic leukaemia (iLLUMINATE): a multicentre, randomised, open-label, phase 3 trial. Lancet Oncol. 2019; 20(1): 43-56.

136. Byrd JC, Harrington B, O'Brien S, et al. Acalabrutinib (ACP-196) in Relapsed Chronic Lymphocytic Leukemia. N Engl J Med. 2016; 374(4): 323-32.

137. Ghia P, Pluta A, Wach M, et al. ASCEND: Phase III, Randomized Trial of Acalabrutinib Versus Idelalisib Plus Rituximab or Bendamustine Plus Rituximab in Relapsed or Refractory Chronic Lymphocytic Leukemia. J Clin Oncol. 2020; 38(25): 2849-61.

138. Sharman JP, Egyed M, Jurczak W, et al. Acalabrutinib with or without obinutuzumab versus chlorambucil and obinutuzmab for treatment-naive chronic lymphocytic leukaemia (ELEVATE TN): a randomised, controlled, phase 3 trial. Lancet. 2020; 395(10232): 1278-91.

139. Isaac K, Mato AR. Acalabrutinib and Its Therapeutic Potential in the Treatment of Chronic Lymphocytic Leukemia: A Short Review on Emerging Data. Cancer Manag Res. 2020; 12: 2079-85.

140. Hillmen P, Brown JR, Eichhorst BF, et al. ALPINE: zanubrutinib versus ibrutinib in relapsed/refractory chronic lymphocytic leukemia/small lymphocytic lymphoma. Future Oncol. 2020; 16(10): 517-23.

141. Tam CS, Robak T, Ghia P, et al. Zanubrutinib monotherapy for patients with treatment naive chronic lymphocytic leukemia and 17p deletion. . Haematologica. 2020: 10.3324/haematol.2020.259432. Online ahead of print.

142. Hoellenriegel J, Meadows SA, Sivina M, et al. The phosphoinositide 3'-kinase delta inhibitor, CAL-101, inhibits B-cell receptor signaling and chemokine networks in chronic lymphocytic leukemia. Blood. 2011; 118(13): 3603-12.

143. Furman RR, Sharman JP, Coutre SE, et al. Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. N Engl J Med. 2014; 370(11): 997-1007.

144. Sharman JP, Coutre SE, Furman RR, et al. Final Results of a Randomized, Phase III Study of Rituximab With or Without Idelalisib Followed by Open-Label Idelalisib in Patients With Relapsed Chronic Lymphocytic Leukemia. J Clin Oncol. 2019; 37(16): 1391-402.

145. Lampson BL, Kasar SN, Matos TR, et al. Idelalisib given front-line for treatment of chronic lymphocytic leukemia causes frequent immune-mediated hepatotoxicity. Blood. 2016; 128(2): 195-203.

146. Flinn IW, Hillmen P, Montillo M, et al. The phase 3 DUO trial: duvelisib vs ofatumumab in relapsed and refractory CLL/SLL. Blood. 2018; 132(23): 2446-55.

147. Stilgenbauer S, Eichhorst B, Schetelig J, et al. Venetoclax in relapsed or refractory chronic lymphocytic leukaemia with 17p deletion: a multicentre, open-label, phase 2 study. Lancet Oncol. 2016; 17(6): 768-78.

148. Roberts AW, Davids MS, Pagel JM, et al. Targeting BCL2 with Venetoclax in Relapsed Chronic Lymphocytic Leukemia. N Engl J Med. 2016; 374(4): 311-22.

149. Fischer K, Al-Sawaf O, Bahlo J, et al. Venetoclax and Obinutuzumab in Patients with CLL and Coexisting Conditions. N Engl J Med. 2019; 380(23): 2225-36.

150. Seymour JF, Kipps TJ, Eichhorst B, et al. Venetoclax-Rituximab in Relapsed or Refractory Chronic Lymphocytic Leukemia. N Engl J Med. 2018; 378(12): 1107-20.

151. Kater AP, Wu JQ, Kipps T, et al. Venetoclax Plus Rituximab in Relapsed Chronic Lymphocytic Leukemia: 4-Year Results and Evaluation of Impact of Genomic Complexity and Gene Mutations From the MURANO Phase III Study. J Clin Oncol. 2020; Sep 28: doi: 10.1200/JCO.20.00948. Online ahead of print.

152. Tausch E, Close W, Dolnik A, et al. Venetoclax resistance and acquired BCL2 mutations in chronic lymphocytic leukemia. Haematologica. 2019; 104(9): e434-e7.

153. Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol. 1982; 5(6): 649-55.

154. Goede V, Bahlo J, Chataline V, et al. Evaluation of geriatric assessment in patients with chronic lymphocytic leukemia: Results of the CLL9 trial of the German CLL study group. Leuk Lymphoma. 2016; 57(4): 789-96.

155. Parmelee PA, Thuras PD, Katz IR, et al. Validation of the Cumulative Illness Rating Scale in a geriatric residential population. J Am Geriatr Soc. 1995; 43(2): 130-7.

156. Eichhorst B, Robak T, Montserrat E, et al. Chronic lymphocytic leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2020 Oct 19:S0923-7534(20)42469-X. doi: 10.1016/j.annonc.2020.09.019. Online ahead of print.

157. Dreger P, Ghia P, Schetelig J, et al. High-risk chronic lymphocytic leukemia in the era of pathway inhibitors: integrating molecular and cellular therapies. Blood. 2018; 132(9): 892-902.

158. Gosain R, Abdou Y, Singh A, et al. COVID-19 and Cancer: a Comprehensive Review. Curr Oncol Rep. 2020; 22(5): 53.

159. Clinical Trial Conduc During the COVID-19 Pandemic. https://www.fda.gov/drugs/coronavirus-covid-19-drugs/clinical-trial-conduct-during-covid-19-pandemic. Cited: October 26, 2020.

160. Tillfälliga avsteg från gällande rekommendationer i nationellt vårdprogram KLL med anledning av Covid-19. https://www.cancercentrum.se/globalassets/covid-19/kll_avvikelse_covid19_8sept20.pdf. Cited: October 26, 2020.

161. Forconi F, Moss P. Perturbation of the normal immune system in patients with CLL. Blood. 2015; 126(5): 573-81.

162. Ravandi F, O'Brien S. Immune defects in patients with chronic lymphocytic leukemia. Cancer Immunol Immunother. 2006; 55(2): 197-209.

163. 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; 32(5): 387-99.

164. Mato AR, Roeker LE, Lamanna N, et al. Outcomes of COVID-19 in patients with CLL: a multicenter international experience. Blood. 2020; 136(10): 1134-43.

165. Scarfo L, Chatzikonstantinou T, Rigolin GM, 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.

166. Bond DA, Woyach JA. Targeting BTK in CLL: Beyond Ibrutinib. Curr Hematol Malig Rep. 2019; 14(3): 197-205.

167. Lunning M, Vose J, Nastoupil L, et al. Ublituximab and umbralisib in relapsed/refractory B-cell non-Hodgkin lymphoma and chronic lymphocytic leukemia. Blood. 2019; 134(21): 1811-20.

168. Rawstron A, Webster N, Dalal S, et al. Kinetics of response in the peripheral blood predicts long term responses to ibrutinib + venetoclax treatment for relapsed/refractory CLL in the Bloodwise TAP CLARITY trial.  (Abstract S164). 25th EHA Congress, virtual. June 11-14, 2020.

169. Siddiqi T, Tam C, Allan J, et al. First-line ibrutinib (IBR) + venetoclax (VEN) for patients (pts) with chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL): efficacy and safety results from CAPTIVATE MRD cohort. (Abstract S158). 25th EHA congress, virtual. June 11-14, 2020.

170. Huber H, Edenhofer S, von Tresckow J, et al. CLL2-GIVE, A prospective, open-label, multicenter phase-II trial of obinutuzumab (GA101, G), Ibrutinib (I), plus venetoclax (VE) in untreated patients with CLL with 17P deletion/TP53 mutation. (Abstract S157). 25th EHA Congress, virtual. June 11-14, 2020.

171. Ding W, LaPlant BR, Call TG, et al. Pembrolizumab in patients with CLL and Richter transformation or with relapsed CLL. Blood. 2017; 129(26): 3419-27.

172. Porter DL, Levine BL, Kalos M, et al. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med. 2011; 365(8): 725-33.

173. Roex G, Feys T, Beguin Y, et al. Chimeric Antigen Receptor-T-Cell Therapy for B-Cell Hematological Malignancies: An Update of the Pivotal Clinical Trial Data. Pharmaceutics. 2020; 12(2): 194.

174. Doudna JA, Charpentier E. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014; 346(6213): 1258096.

175. Swerdlow S, Campo E, Harris N, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. WHO Classification of Tumours. 2008; 2: Page 233. Revised 4th Edition. Lyon. IARC Publications.

176. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016; 127(20): 2375-90.

177. Swerdlow S, Campo E, Harris N. WHO Classification of Tumours of Haematopoetic and Lymphoid Tissues. Revised 4th edition. Lyon: IARC Press. Section: Mature B-cell neoplasms. 2017; 2: 291.

178. Coiffier B, Thieblemont C, Van Den Neste E, et al. Long-term outcome of patients in the LNH-98.5 trial, the first randomized study comparing rituximab-CHOP to standard CHOP chemotherapy in DLBCL patients: a study by the Groupe d'Etudes des Lymphomes de l'Adulte. Blood. 2010; 116(12): 2040-5.

179. Crump M, Neelapu SS, Farooq U, et al. Outcomes in refractory diffuse large B-cell lymphoma: results from the international SCHOLAR-1 study. Blood. 2017; 130(16): 1800-8.

180. Regionala Cancercentrum i samverkan. Nationella kvalitetsregistret för lymfom; Årsrapport från Nationella kvalitetsregistret för lymfom, diagnosperioden 2000-2019. https://www.cancercentrum.se/samverkan/cancerdiagnoser/blod-lymfom-myelom/lymfom-lymfkortelcancer/kvalitetsregister/.

181. Fischer T, Zing NPC, Chiattone CS, et al. Transformed follicular lymphoma. Ann Hematol. 2018; 97(1): 17-29.

182. Szekely E, Hagberg O, Arnljots K, et al. Improvement in survival of diffuse large B-cell lymphoma in relation to age, gender, International Prognostic Index and extranodal presentation: a population based Swedish Lymphoma Registry study. Leuk Lymphoma. 2014; 55(8): 1838-43.

183. Morton LM, Wang SS, Devesa SS, et al. Lymphoma incidence patterns by WHO subtype in the United States, 1992-2001. Blood. 2006; 107(1): 265-76.

184. van Leeuwen MT, Turner JJ, Joske DJ, et al. Lymphoid neoplasm incidence by WHO subtype in Australia 1982-2006. Int J Cancer. 2014; 135(9): 2146-56.

185. Diumenjo MC, Abriata G, Forman D, et al. The burden of non-Hodgkin lymphoma in Central and South America. Cancer Epidemiol. 2016; 44 Suppl 1: S168-S77.

186. Goldin LR, Bjorkholm M, Kristinsson SY, et al. Highly increased familial risks for specific lymphoma subtypes. Br J Haematol. 2009; 146(1): 91-4.

187. Altieri A, Bermejo JL, Hemminki K. Familial risk for non-Hodgkin lymphoma and other lymphoproliferative malignancies by histopathologic subtype: the Swedish Family-Cancer Database. Blood. 2005; 106(2): 668-72.

188. Smedby KE, Ponzoni M. The aetiology of B-cell lymphoid malignancies with a focus on chronic inflammation and infections. J Intern Med. 2017; 282(5): 360-70.

189. Castillo JJ, Ingham RR, Reagan JL, et al. Obesity is associated with increased relative risk of diffuse large B-cell lymphoma: a meta-analysis of observational studies. Clin Lymphoma Myeloma Leuk. 2014; 14(2): 122-30.

190. Chihara D, Nastoupil LJ, Williams JN, et al. New insights into the epidemiology of non-Hodgkin lymphoma and implications for therapy. Expert Rev Anticancer Ther. 2015; 15(5): 531-44.

191. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature. 2000; 403(6769): 503-11.

192. Sehn LH, Gascoyne RD. Diffuse large B-cell lymphoma: optimizing outcome in the context of clinical and biologic heterogeneity. Blood. 2015; 125(1): 22-32.

193. Testoni M, Zucca E, Young KH, et al. Genetic lesions in diffuse large B-cell lymphomas. Ann Oncol. 2015; 26(6): 1069-80.

194. Li S, Young KH, Medeiros LJ. Diffuse large B-cell lymphoma. Pathology. 2018; 50(1): 74-87.

195. Rosenthal A, Younes A. High grade B-cell lymphoma with rearrangements of MYC and BCL2 and/or BCL6: Double hit and triple hit lymphomas and double expressing lymphoma. Blood Rev. 2017; 31(2): 37-42.

196. Rosenwald A, Bens S, Advani R, et al. Prognostic Significance of MYC Rearrangement and Translocation Partner in Diffuse Large B-Cell Lymphoma: A Study by the Lunenburg Lymphoma Biomarker Consortium. J Clin Oncol. 2019; 37(35): 3359-68.

197. Swerdlow S, Campo E, Harris N. WHO Classification of Tumours of Haematopoetic and Lymphoid Tissues. Revised 4th edition. Lyon: IARC Press. Section: Mature B-cell neoplasms. 2017; 2: 335.

198. Martelli M, Ferreri AJ, Agostinelli C, et al. Diffuse large B-cell lymphoma. Crit Rev Oncol Hematol. 2013; 87(2): 146-71.

199. Coutinho R, Clear AJ, Owen A, et al. Poor concordance among nine immunohistochemistry classifiers of cell-of-origin for diffuse large B-cell lymphoma: implications for therapeutic strategies. Clin Cancer Res. 2013; 19(24): 6686-95.

200. Hans CP, Weisenburger DD, Greiner TC, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood. 2004; 103(1): 275-82.

201. Rosenberg SA. Validity of the Ann Arbor staging classification for the non-Hodgkin's lymphomas. Cancer Treat Rep. 1977; 61(6): 1023-7.

202. International Non-Hodgkin's Lymphoma Prognostic Factors P. A predictive model for aggressive non-Hodgkin's lymphoma. N Engl J Med. 1993; 329(14): 987-94.

203. Regionalt Cancercentrum i samverkan. Nationellt vårdprogram Aggressiva B-cellslymfom. 2019-09-10 Version: 4.0. https://wwwcancercentrumse/samverkan/cancerdiagnoser/blod-lymfom-myelom/lymfom-lymfkortelcancer/vardprogram/gallande-vardprogram-aggressiva-b-cellslymfom/.

204. Jelicic J, Larsen TS, Maksimovic M, et al. Available prognostic models for risk stratification of diffuse large B cell lymphoma patients: a systematic review. Crit Rev Oncol Hematol. 2019; 133: 1-16.

205. Zhou Z, Sehn LH, Rademaker AW, et al. An enhanced International Prognostic Index (NCCN-IPI) for patients with diffuse large B-cell lymphoma treated in the rituximab era. Blood. 2014; 123(6): 837-42.

206. Schmitz N, Zeynalova S, Nickelsen M, et al. CNS International Prognostic Index: A Risk Model for CNS Relapse in Patients With Diffuse Large B-Cell Lymphoma Treated With R-CHOP. J Clin Oncol. 2016; 34(26): 3150-6.

207. Savage KJ. Secondary CNS relapse in diffuse large B-cell lymphoma: defining high-risk patients and optimization of prophylaxis strategies. Hematology Am Soc Hematol Educ Program. 2017; 2017(1): 578-86.

208. Fisher RI, Gaynor ER, Dahlberg S, et al. Comparison of a standard regimen (CHOP) with three intensive chemotherapy regimens for advanced non-Hodgkin's lymphoma. N Engl J Med. 1993; 328(14): 1002-6.
209. Coiffier B, Lepage E, Briere J, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med. 2002; 346(4): 235-42.
210. Habermann TM, Weller EA, Morrison VA, et al. Rituximab-CHOP versus CHOP alone or with maintenance rituximab in older patients with diffuse large B-cell lymphoma. J Clin Oncol. 2006; 24(19): 3121-7.
211. Pfreundschuh M, Trumper L, Osterborg A, et al. CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large-B-cell lymphoma: a randomised controlled trial by the MabThera International Trial (MInT) Group. Lancet Oncol. 2006; 7(5): 379-91.
212. Pfreundschuh M, Kuhnt E, Trumper L, et al. CHOP-like chemotherapy with or without rituximab in young patients with good-prognosis diffuse large-B-cell lymphoma: 6-year results of an open-label randomised study of the MabThera International Trial (MInT) Group. Lancet Oncol. 2011; 12(11): 1013-22.
213. Ekberg S, Jerkeman M, Andersson PO, et al. Long-term survival and loss in expectancy of life in a population-based cohort of 7114 patients with diffuse large B-cell lymphoma. Am J Hematol. 2018; May 17: doi: 10.1002/ajh.25147. Online ahead of print.
214. Cunningham D, Hawkes EA, Jack A, et al. Rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisolone in patients with newly diagnosed diffuse large B-cell non-Hodgkin lymphoma: a phase 3 comparison of dose intensification with 14-day versus 21-day cycles. Lancet. 2013; 381(9880): 1817-26.
215. Delarue R, Tilly H, Mounier N, et al. Dose-dense rituximab-CHOP compared with standard rituximab-CHOP in elderly patients with diffuse large B-cell lymphoma (the LNH03-6B study): a randomised phase 3 trial. Lancet Oncol. 2013; 14(6): 525-33.
216. Pfreundschuh M, Schubert J, Ziepert M, et al. Six versus eight cycles of bi-weekly CHOP-14 with or without rituximab in elderly patients with aggressive CD20+ B-cell lymphomas: a randomised controlled trial (RICOVER-60). Lancet Oncol. 2008; 9(2): 105-16.
217. Wasterlid T, Biccler JL, Brown PN, et al. Six cycles of R-CHOP-21 are not inferior to eight cycles for treatment of diffuse large B-cell lymphoma: a Nordic Lymphoma Group Population-based Study. Ann Oncol. 2018; 29(8): 1882-3.
218. Poeschel V, Held G, Ziepert M, et al. Four versus six cycles of CHOP chemotherapy in combination with six applications of rituximab in patients with aggressive B-cell lymphoma with favourable prognosis (FLYER): a randomised, phase 3, non-inferiority trial. Lancet. 2019; 394(10216): 2271-81.
219. Recher C, Coiffier B, Haioun C, et al. Intensified chemotherapy with ACVBP plus rituximab versus standard CHOP plus rituximab for the treatment of diffuse large B-cell lymphoma (LNH03-2B): an open-label randomised phase 3 trial. Lancet. 2011; 378(9806): 1858-67.
220. Bartlett NL, Wilson WH, Jung SH, et al. Dose-Adjusted EPOCH-R Compared With R-CHOP as Frontline Therapy for Diffuse Large B-Cell Lymphoma: Clinical Outcomes of the Phase III Intergroup Trial Alliance/CALGB 50303. J Clin Oncol. 2019; 37(21): 1790-9.
221. Dunleavy K, Fanale MA, Abramson JS, et al. Dose-adjusted EPOCH-R (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab) in untreated aggressive diffuse large B-cell lymphoma with MYC rearrangement: a prospective, multicentre, single-arm phase 2 study. Lancet Haematol. 2018; 5(12): e609-e17.
222. Stiff PJ, Unger JM, Cook JR, et al. Autologous transplantation as consolidation for aggressive non-Hodgkin's lymphoma. N Engl J Med. 2013; 369(18): 1681-90.
223. Chiappella A, Santambrogio E, Castellino A, et al. Integrating novel drugs to chemoimmunotherapy in diffuse large B-cell lymphoma. Expert Rev Hematol. 2017; 10(8): 697-705.
224. Sehn LH, Martelli M, Trneny M, et al. A randomized, open-label, Phase III study of obinutuzumab or rituximab plus CHOP in patients with previously untreated diffuse large B-Cell lymphoma: final analysis of GOYA. J Hematol Oncol. 2020; 13(1): 71.
225. Marcus R, Davies A, Ando K, et al. Obinutuzumab for the First-Line Treatment of Follicular Lymphoma. N Engl J Med. 2017; 377(14): 1331-44.
226. Klapper W, Kreuz M, Kohler CW, et al. Patient age at diagnosis is associated with the molecular characteristics of diffuse large B-cell lymphoma. Blood. 2012; 119(8): 1882-7.

227. Paul U, Richter J, Stuhlmann-Laiesz C, et al. Advanced patient age at diagnosis of diffuse large B-cell lymphoma is associated with molecular characteristics including ABC-subtype and high expression of MYC. Leuk Lymphoma. 2018; 59(5): 1213-21.

228. Peyrade F, Jardin F, Thieblemont C, et al. Attenuated immunochemotherapy regimen (R-miniCHOP) in elderly patients older than 80 years with diffuse large B-cell lymphoma: a multicentre, single-arm, phase 2 trial. Lancet Oncol. 2011; 12(5): 460-8.

229. Tilly H, Gomes da Silva M, Vitolo U, et al. Diffuse large B-cell lymphoma (DLBCL): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2015; 26 Suppl 5: v116-25.

230. Peyrade F, Bologna S, Delwail V, et al. Combination of ofatumumab and reduced-dose CHOP for diffuse large B-cell lymphomas in patients aged 80 years or older: an open-label, multicentre, single-arm, phase 2 trial from the LYSA group. Lancet Haematol. 2017; 4(1): e46-e55.

231. Fields PA, Townsend W, Webb A, et al. De novo treatment of diffuse large B-cell lymphoma with rituximab, cyclophosphamide, vincristine, gemcitabine, and prednisolone in patients with cardiac comorbidity: a United Kingdom National Cancer Research Institute trial. J Clin Oncol. 2014; 32(4): 282-7.

232. Vardhana SA, Sauter CS, Matasar MJ, et al. Outcomes of primary refractory diffuse large B-cell lymphoma (DLBCL) treated with salvage chemotherapy and intention to transplant in the rituximab era. Br J Haematol. 2017; 176(4): 591-9.

233. Gisselbrecht C, Glass B, Mounier N, et al. Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. J Clin Oncol. 2010; 28(27): 4184-90.

234. Crump M, Kuruvilla J, Couban S, et al. Randomized comparison of gemcitabine, dexamethasone, and cisplatin versus dexamethasone, cytarabine, and cisplatin chemotherapy before autologous stem-cell transplantation for relapsed and refractory aggressive lymphomas: NCIC-CTG LY.12. J Clin Oncol. 2014; 32(31): 3490-6.

235. van Imhoff GW, McMillan A, Matasar MJ, et al. Ofatumumab Versus Rituximab Salvage Chemoimmunotherapy in Relapsed or Refractory Diffuse Large B-Cell Lymphoma: The ORCHARRD Study. J Clin Oncol. 2017; 35(5): 544-51.

236. Mounier N, El Gnaoui T, Tilly H, et al. Rituximab plus gemcitabine and oxaliplatin in patients with refractory/relapsed diffuse large B-cell lymphoma who are not candidates for high-dose therapy. A phase II Lymphoma Study Association trial. Haematologica. 2013; 98(11): 1726-31.

237. Arcari A, Chiappella A, Spina M, et al. Safety and efficacy of rituximab plus bendamustine in relapsed or refractory diffuse large B-cell lymphoma patients: an Italian retrospective multicenter study. Leuk Lymphoma. 2016; 57(8): 1823-30.

238. Sehn LH, Herrera AF, Flowers CR, et al. Polatuzumab Vedotin in Relapsed or Refractory Diffuse Large B-Cell Lymphoma. J Clin Oncol. 2020; 38(2): 155-65.

239. Ferreri AJM, Sassone M, Angelillo P, et al. Long-lasting efficacy and safety of lenalidomide maintenance in patients with relapsed diffuse large B-cell lymphoma who are not eligible for or failed autologous transplantation. Hematol Oncol. 2020; 38(3): 257-65.

240. de la Cruz-Benito B, Lazaro-Del Campo P, Ramirez-Lopez A, et al. Managing the front-line treatment for diffuse large B-cell lymphoma and high-grade B-cell lymphoma during the COVID-19 outbreak. Br J Haematol. 2020 Aug 6;10.1111/bjh.17066. doi: 10.1111/bjh.. Online ahead of print.

241. Tepasse PR, Hafezi W, Lutz M, et al. Persisting SARS-CoV-2 viraemia after rituximab therapy: two cases with fatal outcome and a review of the literature. Br J Haematol. 2020; 190(2): 185-8.

242. Davies A, Cummin TE, Barrans S, et al. Gene-expression profiling of bortezomib added to standard chemoimmunotherapy for diffuse large B-cell lymphoma (REMoDL-B): an open-label, randomised, phase 3 trial. Lancet Oncol. 2019; 20(5): 649-62.

243. Younes A, Sehn LH, Johnson P, et al. Randomized Phase III Trial of Ibrutinib and Rituximab Plus Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone in Non-Germinal Center B-Cell Diffuse Large B-Cell Lymphoma. J Clin Oncol. 2019; 37(15): 1285-95.

244. Maurer MJ, Ghesquieres H, Link BK, et al. Diagnosis-to-Treatment Interval Is an Important Clinical Factor in Newly Diagnosed Diffuse Large B-Cell Lymphoma and Has Implication for Bias in Clinical Trials. J Clin Oncol. 2018; 36(16): 1603-10.

245. Locke FL, Ghobadi A, Jacobson CA, et al. Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1-2 trial. Lancet Oncol. 2019; 20(1): 31-42.

246. Schuster SJ, Bishop MR, Tam CS, et al. Tisagenlecleucel in Adult Relapsed or Refractory Diffuse Large B-Cell Lymphoma. N Engl J Med. 2019; 380(1): 45-56.

247. Abramson JS, Palomba ML, Gordon LI, et al. Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. Lancet. 2020; 396(10254): 839-52.

248. Tholouli E, Osborne W, Bachier C, et al. Phase 1 Alexander Study OF AUTO3, the first CD19/CD22 dual targeting CAR-T cell, with pembrolizumab in patients with relapsed/refractory (R/R) DLBCL. (Abstract 890MO). ESMO Virtual Congress. September 19-21, 2020.

249. Neelapu S, Munoz J, Locke F, et al. First-in-human data of ALLO-501 and ALLO-647 in relapsed/refractory large B-cell or follicular lymphoma (R/R LBCL/FL): ALPHA study. (Abstract 8002). ASCO Virtual Scientific Program. May 29–31, 2020.

250. Dufner V, Sayehli CM, Chatterjee M, et al. Long-term outcome of patients with relapsed/refractory B-cell non-Hodgkin lymphoma treated with blinatumomab. Blood Adv. 2019; 3(16): 2491-8.

251. Nowakowski G, Hong F, Scott D, et al. Addition of lenalidomide to RCHOP (R2CHOP) improves outcomes in newly diagnosed diffuse large B-cell lymphoma (DLBCL): first report of ECOG-ACRIN1412 a randomized phase 2 US intergroup study of R2CHOP vs R-CHOP. Hematol Oncol. 2019; S2(37): e38.

252. Vitolo U, Witzig T, Gascoyne R, et al. ROBUST: First report of phase III randomized study of Lenalidomide/R-CHOP (R2-CHOP) vs placebo/R-CHOP in previously untreated ABC-type diffuse large B-cell lymphoma Hematol Oncol. 2019; 37: 36-7.

253. Duhrsen U, Muller S, Hertenstein B, et al. Positron Emission Tomography-Guided Therapy of Aggressive Non-Hodgkin Lymphomas (PETAL): A Multicenter, Randomized Phase III Trial. J Clin Oncol. 2018; 36(20): 2024-34.

254. Richter J, Huttmann A, Rekowski J, et al. Molecular characteristics of diffuse large B-cell lymphoma in the Positron Emission Tomography-Guided Therapy of Aggressive Non-Hodgkin lymphomas (PETAL) trial: correlation with interim PET and outcome. Blood Cancer J. 2019; 9(9): 67.

255. Kurtz DM, Scherer F, Jin MC, et al. Circulating Tumor DNA Measurements As Early Outcome Predictors in Diffuse Large B-Cell Lymphoma. J Clin Oncol. 2018; 36(28): 2845-53.

256. Wright GW, Huang DW, Phelan JD, et al. A Probabilistic Classification Tool for Genetic Subtypes of Diffuse Large B Cell Lymphoma with Therapeutic Implications. Cancer Cell. 2020; 37(4): 551-68 e14.

257. Xu-Monette ZY, Zhang H, Zhu F, et al. A refined cell-of-origin classifier with targeted NGS and artificial intelligence shows robust predictive value in DLBCL. Blood Adv. 2020; 4(14): 3391-404.

258. Kurtz DM, Esfahani MS, Scherer F, et al. Dynamic Risk Profiling Using Serial Tumor Biomarkers for Personalized Outcome Prediction. Cell. 2019; 178(3): 699-713 e19.

259. About the Swedish healthcare system. https://www.socialstyrelsen.se/en/about-us/healthcare-for-visitors-to-sweden/about-the-swedish-healthcare-system/. Cited: October 26, 2020.

260. Regionala Cancercentrum i samverkan. Våra uppdrag. https://www.cancercentrum.se/samverkan/vara-uppdrag. Cited: October 26, 2020.

261. Skatteverket. Personnummer och samordningsnummer. https://skatteverket.se/privat/folkbokforing/personnummerochsamordningsnummer. Cited: October 26, 2020.

262. Socialstyrelsen. Cancerregistret. https://www.socialstyrelsen.se/statistik-och-data/register/alla-register/cancerregistret/. Cited: October 26, 2020.

263. Overview of the SEER Program. https://seer.cancer.gov/about/overview.html. Cited: October 26, 2020.

264. Barlow L, Westergren K, Holmberg L, et al. The completeness of the Swedish Cancer Register: a sample survey for year 1998. Acta Oncol. 2009; 48(1): 27-33.

265. Kodning i cancerregistret 2020. https://www.socialstyrelsen.se/globalassets/sharepoint-dokument/artikelkatalog/ovrigt/2020-1-6567.pdf. Cited: October 26, 2020.

266. Regionala Cancercentrum i samverkan. Om INCA. https://www.cancercentrum.se/samverkan/vara-uppdrag/kunskapsstyrning/kvalitetsregister/om-inca/. Cited: October 26, 2020.

267. Regionala Cancercentrum i samverkan. Nationellt kvalitetsregister kronisk lymfatisk leukemi. Nationell KLL rapport diagnosår 2007-2016. https://wwwcancercentrumse/samverkan/cancerdiagnoser/blod-lymfom-myelom/kronisk-lymfatisk-leukemi-kll/kvalitetsregister/.

268. Hallek M, Cheson BD, Catovsky D, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood. 2008; 111(12): 5446-56.

269. Cheson BD, Pfistner B, Juweid ME, et al. Revised response criteria for malignant lymphoma. J Clin Oncol. 2007; 25(5): 579-86.

270. Islam P, Mato AR. Utilizing Real-World Evidence (RWE) to Improve Care in Chronic Lymphocytic Leukemia: Challenges and Opportunities. Curr Hematol Malig Rep. 2020; 15(4): 254-60.

271. da Cunha-Bang C, Simonsen J, Rostgaard K, et al. Improved survival for patients diagnosed with chronic lymphocytic leukemia in the era of chemo-immunotherapy: a Danish population-based study of 10455 patients. Blood Cancer J. 2016; 6(11): e499.

272. Cramer P, Isfort S, Bahlo J, et al. Outcome of advanced chronic lymphocytic leukemia following different first-line and relapse therapies: a meta-analysis of five prospective trials by the German CLL Study Group (GCLLSG). Haematologica. 2015; 100(11): 1451-9.

273. Pulte D, Castro FA, Jansen L, et al. Trends in survival of chronic lymphocytic leukemia patients in Germany and the USA in the first decade of the twenty-first century. J Hematol Oncol. 2016; 9: 28.

274. Tam CS, O'Brien S, Plunkett W, et al. Long-term results of first salvage treatment in CLL patients treated initially with FCR (fludarabine, cyclophosphamide, rituximab). Blood. 2014; 124(20): 3059-64.

275. Hillmen P, Fraser G, Jones J, et al. Comparing single-agent ibrutinib, bendamustine plus rituximab (BR) and ibrutinib plus BR in patients with previously treated chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL): an indirect comparison of the RESONATE and HELIOS trials. Blood. 2015; ASH, 57th Annual Meeting, (Abstract 126): 23.

276. Doubek M, Obrtlikova P, Spacek M, et al. Single-agent ibrutinib vs standard of care for patients with relapsed/refractory (R/R) chronic lymphocytic leukemia (CLL): an adjusted comparison of resonate (TM) with the CLLEAR database. . Haematologica 2016; 101: 230–31.

277. Tam C, Sorensen S, Sengupta N, et al. Indirect treatment comparison of ibrutinib versus physician's choice for treatment of relapsed or refractory chronic lymphocytic leukemia. XVI International Workshop on Chronic Lymphocytic Leukaemia. (Abstract). Sydney, Australia. September 7-9, 2015.

278. Sorensen S, Wildgust M, Sengupta N, et al. Indirect Treatment Comparisons of Ibrutinib Versus Physician's Choice and Idelalisib Plus Ofatumumab in Patients With Previously Treated Chronic Lymphocytic Leukemia. Clin Ther. 2017; 39(1): 178-89 e5.

279. Lee S, Fujita K, Negoro E, et al. Impact of relative dose intensity of standard regimens on survival in elderly patients aged 80 years and older with diffuse large B-cell lymphoma. Haematologica. 2020; 105(8): e415-e8.

280. Juul MB, Jensen PH, Engberg H, et al. Treatment strategies and outcomes in diffuse large B-cell lymphoma among 1011 patients aged 75 years or older: A Danish population-based cohort study. Eur J Cancer. 2018; 99: 86-96.

281. Eyre TA, Salisbury R, Eyre DW, et al. Results of a large retrospective analysis of the effect of intended dose intensity of R-CHOP on outcome in a cohort of consecutive, unselected elderly patients with de novo diffuse large B cell lymphoma. Br J Haematol. 2016; 173(3): 487-91.

282. Tucci A, Ferrari S, Bottelli C, et al. A comprehensive geriatric assessment is more effective than clinical judgment to identify elderly diffuse large cell lymphoma patients who benefit from aggressive therapy. Cancer. 2009; 115(19): 4547-53.

283. Mato AR, Hill BT, Lamanna N, et al. Optimal sequencing of ibrutinib, idelalisib, and venetoclax in chronic lymphocytic leukemia: results from a multicenter study of 683 patients. Ann Oncol. 2017; 28(5): 1050-6.

284. Booth CM, Karim S, Mackillop WJ. Real-world data: towards achieving the achievable in cancer care. Nat Rev Clin Oncol. 2019; 16(5): 312-25.