School of Medicine

Department of Health Sciences

Master's Degree in Medical Biotechnologies

CLINICAL IMPACT OF CLONAL HEMATOPOIESIS IN FOLLICULAR LYMPHOMA PATIENTS ENROLLED IN FIL FOLL12 TRIAL

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SUMMARY

Follicular lymphoma (FL) is the most common indolent B-cell non-Hodgkin lymphoma and is characterized by a heterogeneous clinical course. Clonal hematopoiesis (CH), an age-related condition characterized by the expansion of hematopoietic stem cell clones carrying somatic mutations. This study investigated the prevalence, evolution, and clinical impact of CH in patients with newly diagnosed FL enrolled in the phase III FIL FOLL12 trial.

Genomic DNA extracted from peripheral blood samples was analyzed by next-generation sequencing using a targeted panel of CH-associated genes. Baseline and sequential post-treatment samples were evaluated to characterize clonal dynamics following chemoimmunotherapy (CIT). CH was detected in approximately 30% of patients at diagnosis, with *DNMT3A*, *TET2*, and *ASXL1* representing the most frequently mutated genes. Longitudinal analyses demonstrated that CIT increased CH prevalence and promoted the selective expansion of clones harboring mutations in DNA damage response (DDR) genes, particularly *TP53* and *PPM1D*. Bendamustine-containing therapy preferentially favored the expansion of *TP53*-mutated clones. Importantly, baseline DTA mutations and treatment-selected fit DDR-mutated clones were independently associated with an increased risk of second primary malignancies during long-term follow-up. In contrast, CH did not significantly affect progression-free or overall survival.

In conclusion, CH is a frequent and dynamic phenomenon in FL and is profoundly shaped by chemoimmunotherapy. These findings suggest that CH contributes to long-term treatment-related complications and may represent a useful biomarker for risk stratification and patient monitoring.

1. INTRODUCTION

1.1 Follicular lymphoma

Follicular lymphoma (FL) is an indolent form of B-cell non-Hodgkin lymphoma that is generally considered incurable. It is characterized by a follicular growth pattern resembling normal lymphoid follicles and is composed predominantly of centrocytes, along with a variable proportion of centroblasts. The relative number of centroblasts determines the histological grade (grades 1, 2, or 3).

It is the second most common lymphoma in Western countries after diffuse large B-cell lymphoma (DLBCL) and represents the most frequent indolent lymphoma. For this reason, it is often used as a model for developing treatment strategies for other indolent lymphomas. Historically, the median survival was estimated at 9–10 years; however, with advances in therapy and supportive care, it has increased to approximately 14 years (Ghielmini, 2010).

1.2 Pathogenesis

The last years have seen significant progress in understanding of FL lymphomagenesis, which is a multi-step process beginning in the bone marrow during B cell lymphopoiesis with the hallmark t(14;18)(q32;q21) translocation arising from an error during immunoglobulin heavy chain gene (IGH) rearrangement. The t(14;18) translocation places the B-cell lymphoma 2 (*BCL2*) gene under the control of immunoglobulin heavy chain enhancer elements, leading to its constitutive overexpression. As a result, the anti-apoptotic protein BCL2 is upregulated,

promoting cell survival and enabling the accumulation of B cells within germinal centres, ultimately contributing to uncontrolled proliferation (Lackraj et al., 2018a).

Approximately 90% of follicular lymphomas are characterized by the t(14;18)(q32;q21) translocation, which leads to *BCL2* overexpression, even though a minority of FL cases lack this translocation and consequently exhibit reduced or absent *BCL2* expression. These *BCL2*-negative lymphomas account for less than 10% of FL cases and are more commonly associated with grade 3B FL or with predominantly diffuse growth patterns.

In addition to this alteration, FL frequently harbours secondary cytogenetic abnormalities, including deletions of chromosome 6 (approximately 20% of cases) (Schwaenen et al., 2009), deletions involving chromosome 1, which have been associated with an adverse prognosis, and alterations of chromosome 3 affecting the *BCL6* locus, observed in 5–15% of patients (Bosga-Bouwer et al., 2003; Díaz-Alderete et al., 2008; Launay et al., 2012).

Following the initial *BCL2*-driven survival advantage, FL develops through the accumulation of secondary genetic and epigenetic alterations. A defining feature of FL is the high frequency of mutations affecting chromatin-modifying genes, highlighting the central role of epigenetic dysregulation in lymphomagenesis; *KMT2D*, *EZH2* and *CREBBP* are among the most commonly mutated genes.

KMT2D (accounting 70% of cases) encodes a histone methyltransferase responsible for methylation of histone H3 lysine 4 (H3K4), a key mark associated with transcriptional activation, while *CREBBP* (occurring in 60% of cases) encodes a histone acetyltransferase that regulates chromatin accessibility and modulates transcription factors involved in B-cell signalling and immune responses. In FL, both genes are predominantly affected by loss-of-function mutations, leading to impaired regulation of gene expression, defective antigen presentation, and altered interactions with the immune microenvironment. *EZH2* is the third

most frequently mutated chromatin-modifying gene in follicular lymphoma, being altered in approximately 25% of cases. Most *EZH2* mutations are gain-of-function missense variants that enhance its epigenetic repressor activity, promoting germinal centre maintenance and impaired B-cell differentiation. In cooperation with BCL2 overexpression, these alterations contribute to FL pathogenesis (Green, 2018; Lackraj et al., 2018) (**Figure 1.1**).

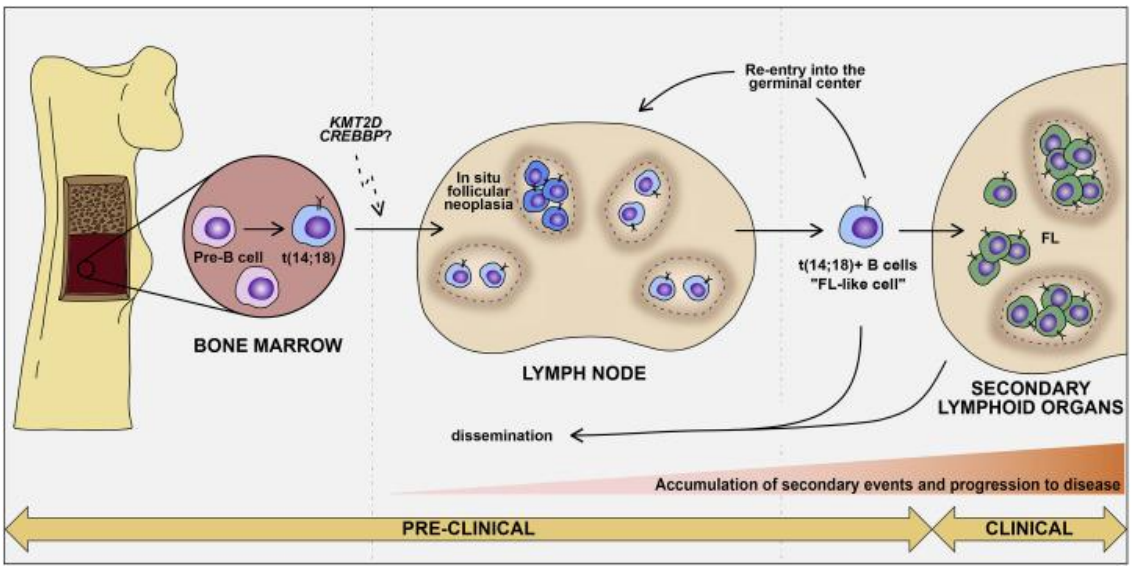

Figure 1.1. Molecular and cellular evolution of follicular lymphoma (Lackraj et al., 2018).

Importantly, these mutations are considered early events in lymphomagenesis and often precede overt clinical disease. Experimental evidence supporting their functional role is largely derived from murine models. In this context, deletion of *KMT2D* has been shown to result in an expansion of germinal centre B cells, particularly when gene inactivation occurs during early B-cell development rather than during the germinal centre reaction. Similarly, loss of *CREBBP* in hematopoietic stem and progenitor cells promotes lymphoid proliferation and lymphoma development.

Beyond epigenetic modifiers, FL is characterized by a heterogeneous mutational landscape involving multiple pathways. Frequently mutated genes include others involved in B-cell receptor (BCR) signalling, immune regulation, and cell survival, such as *STAT6* (found in about 10% of cases), *TNFRSF14* (30-40% of cases) and component of mTOR pathways. Collectively, these alterations contribute to sustained proliferative signalling, immune evasion, microenvironmental remodelling and clonal evolution.

Although FL is characterized by a distinct spectrum of recurrent genetic alterations, disease evolution is thought to be driven by the accumulation of additional genomic events. Histological transformation to a more aggressive lymphoma has been associated with the activation of oncogenes, including *MYC*, *BCL6* and *CCND3*, and the inactivation of tumour suppressor genes such as *TP53*, *CDKN2A*/B and GNA13. Clinically, transformation represents a major determinant of FL-related treatment resistance and mortality and most often manifests as DLBCL (Fischer et al., 2018; Lackraj et al., 2018c; Ott et al., 2002).

1.3 Histological grading

Determining the histological grade is a crucial component of the diagnostic evaluation of follicular lymphoma. The grading system currently accepted in clinical practice and recommended by the 2008 World Health Organization (WHO) Classification of Tumours was proposed by Mann and Berard system; this method is based on counting centroblasts in 10 neoplastic follicles under high-power field (HPF, ×40 objective) magnification. Accordingly, FL is divided into three grades: grade 1 FL, up to 5 centroblasts per HPF; grade 2 FL, 6-15 centroblasts per HPF; grade 3 FL, more than 16 centroblasts per HPF. Grades 1 and 2 FL have been classified together as low grade. Grade 3 FL (high grade FL) is subdivided into grade 3A,

where centrocytes are still present and grade 3B where follicles are composed entirely of centroblasts (Klopčič et al., 2016) (**Figure 1.2**). FL grades 1, 2, and 3A are generally considered part of a continuous morphological spectrum, whereas grade 3B is more closely associated with de novo aggressive lymphomas. Koch and colleagues analyzed these subgroups independently and found that many cases classified as grade 3A included regions histologically similar to lower-grade follicular lymphoma (grades 1 and 2). In contrast, grade 3B cases are more frequently characterized by a diffuse architectural pattern resembling DLBCL, supporting the idea of a biologically distinct pathogenesis. This distinction is clinically meaningful, as grade 3A disease has been linked to a greater risk of transformation in DLBCL (Fischer et al., 2018; Koch et al., 2016). Histologic transformation to a more aggressive lymphoma occurs at an estimated rate of 2–3% per year and is typically associated with rapid clinical deterioration, resistance to standard therapies, and poor clinical outcomes. The most frequent phenotype observed at the time of transformation is DLBCL; however, transformation may result in histologic patterns resembling other lymphomas, such as Burkitt lymphoma, lymphoblastic lymphoma and acute lymphoblastic leukemia (Fischer et al., 2018; Horn et al., 2011; Lossos & Gascoyne, 2011).

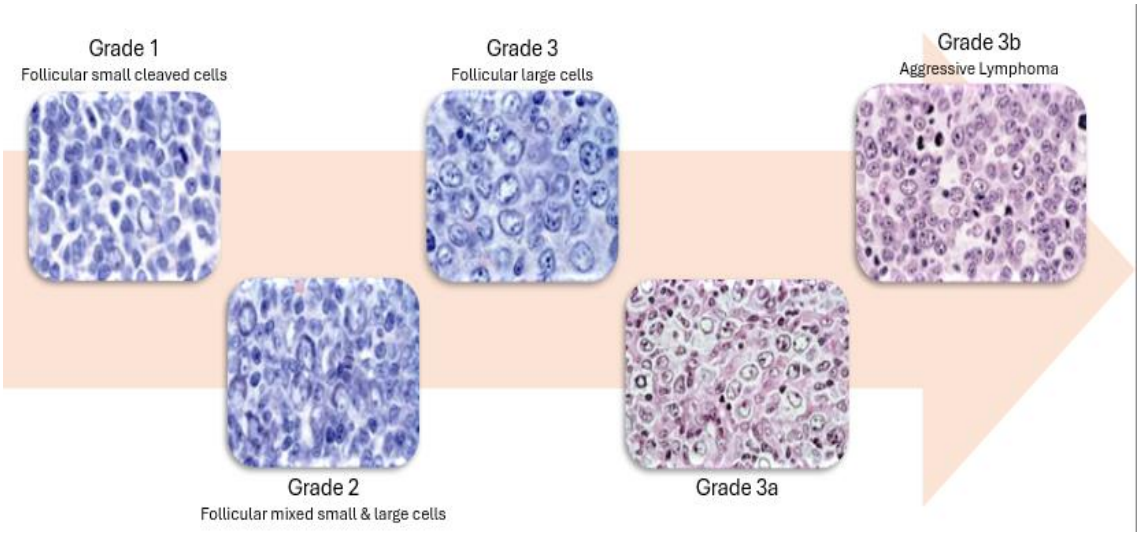

Figure 1.2. Histological representation of all the FL stages, in order from the less to the most aggressive form (Ott et al., 2002).

1.4 Diagnosis

The initial diagnostic workup of follicular lymphoma includes a comprehensive clinical and laboratory evaluation, comprising a detailed physical examination, hematological and biochemical analyses, cross-sectional imaging with computed tomography (CT) of the chest, abdomen, and pelvis; bone marrow aspiration and biopsy is recommended.

FL is characterized by a well-defined nodular growth pattern composed of a mixture of centrocytes and centroblasts; for this reason, excisional biopsy to evaluate lymph node morphology represents the optimal approach for the initial diagnosis.

A comprehensive clinical evaluation including assessment of hepatitis and HIV risk factors, medication use, and social and family history, together with performance status and a thorough physical examination, is routinely performed. All patients should undergo a complete blood count with differential, peripheral blood smear, and evaluation of liver and renal function, along with measurements of lactate dehydrogenase (LDH), electrolytes, and calcium levels.

Immunophenotypic analysis typically demonstrates expression of B-cell–associated antigens, including CD19, CD20, CD22, and CD79a, along with light-chain restriction. CD10 positivity is observed in approximately 60% of cases, while BCL-2 protein is strongly expressed in most grade 1–2 subtypes; The absence of CD5 and CD43 is useful in distinguishing follicular lymphoma from chronic lymphocytic leukemia and mantle cell lymphoma.

Functional imaging with 2-[^{18}F]-fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET), typically combined with CT (PET/CT), may be useful in selected clinical scenarios. In particular, it can aid in confirming suspected early-stage disease that may be amenable to curative-intent radiotherapy, as well as in guiding the diagnostic evaluation when histologic transformation to a more aggressive lymphoma, such as DLBCL, is suspected.

Additional investigations may be performed based on clinical indications: these include radiologic and endoscopic evaluation of the gastrointestinal tract, cervical ultrasonography, and magnetic resonance imaging (MRI) of the orbits and soft tissues, which can facilitate the detection of involvement at these specific anatomical sites (Hayashi et al., 2010; Randall & Fedoriw, 2020; Bargetzi et al., 2018; Dada, 2019).

1.5 Prognosis

Given the marked heterogeneity in clinical presentation and therapeutic response among patients with FL, several prognostic models have been developed to support pre-treatment risk stratification. Among these, the Follicular Lymphoma International Prognostic Index (FLIPI) and its subsequent refinement, FLIPI2, are the most widely utilized tools. FLIPI prognostic index incorporates five parameters (age, stage, number of involved nodal areas, lactate

dehydrogenase (LDH) and hemoglobin levels) to categorize patients into low-, intermediate-, and high-risk groups, correlating with overall survival (OS) and progression-free survival (PFS) respectively. To overcome some limitations of FLIPI and to improve prognostic assessment in the rituximab era, FLIPI2 includes age, hemoglobin, beta-2 microglobulin (B2M) levels, bone marrow involvement and longest diameter of the largest lymph node. However, due to the uncertain utility of FLIPI2, the original FLIPI remains the more widely used and validated prognostic model. Patients identified as high-risk by these models consistently demonstrate inferior clinical outcomes. Despite their clinical utility, FLIPI and FLIPI2 present notable limitations; particularly these models do not account for the underlying molecular heterogeneity of the disease, which has become increasingly relevant with the advent of targeted therapies (Becnel & Nastoupil, 2018; Jacobsen, 2022; Solal-Céligny et al., 2004; Welaya & Casulo, 2019).

The development of prognostic indices such as FLIPI and FLIPI2 was based on the identification of several clinical and laboratory features associated with outcome in patients with FL. Instead, among the biological variables evaluated at diagnosis, only a limited number have been consistently validated as prognostic factors in FL. The initial assessment of a patient includes evaluation of age and performance status, both of which have been recognized as independent predictors of outcome. In addition, disease extent represents one of the most important prognostic determinants and is commonly assessed using the Ann Arbor staging system. This system categorizes disease into four stages according to the distribution of nodal and extranodal involvement, based on findings from physical examination, laboratory investigations, imaging studies, and bone marrow evaluation (Luminari et al., 2012; Makita et al., 2020). Beyond anatomical staging, disease burden may also be estimated indirectly through several clinical and laboratory parameters. These include the presence of B symptoms, low

hemoglobin levels (<10 g/dL), elevated erythrocyte sedimentation rate, and increased serum LDH levels and B2M, all of which have been associated with a less favorable prognosis (Luminari et al., 2012).

1.6 Treatments

Overall survival for patients with FL has significantly improved since the introduction of rituximab (R). According to data from the Swedish Lymphoma Registry, 10-year OS rates between 2003 and 2010 were reported as 92% for patients aged 18–49, 83% for those aged 50–59, 78% for ages 60–69, and 64% for patients aged 70 and older (Junlén et al., 2015).

In the small proportion of patients with limited low tumour burden stages I-II, radiotherapy (RT)-based treatment is the preferred approach with a curative intent. Despite that, a phase III clinical trial have been demonstrated that combination of RT with rituximab based immunochemotherapy R-CVP (rituximab + cyclophosphamide, vincristine and prednisone) improved progression-free survival compared with RT alone (MacManus et al., 2018). Furthermore, a combination of localised irradiation with single-agent rituximab may potentially provide the best balance between efficacy and side-effects as demonstrated by a German group in a phase II study (Herfarth et al., 2018). In selected cases (limited life expectancy/ large abdominal fields and elderly/ medically unfit patients), watch-and-wait or rituximab monotherapy and low-dose RT may be considered respectively.

Systemic treatment is generally recommended for patients presenting with symptomatic FL or a high tumour burden. The introduction of anti-CD20 monoclonal antibodies has significantly improved the management of advanced-stage disease, establishing rituximab and,

more recently, obinutuzumab (glycoengineered anti-CD20 monoclonal antibody useful against refractoriness of indolent NHLs to rituximab) as key components of first-line therapy.

Although rituximab may be administered as a single agent in selected cases, anti-CD20 antibodies are most combined with chemotherapy regimens, including CVP, CHOP (cyclophosphamide, vincristine, doxorubicin and prednisone), bendamustine, and chlorambucil. Multiple randomized studies have demonstrated that the incorporation of anti-CD20 therapy into chemotherapy results in superior clinical outcomes compared with chemotherapy alone (Tobin et al., 2024a) (Dreyling et al., 2021). In the phase III GALLIUM trial demonstrated superiori long-term disease control with obinutuzumab-based immunochemotherapy, with 7-year progression-free survival rates of 63% compared with 55% among patients receiving rituximab-containing. This benefit was further reflected in event-free survival (EFS), with 7-year EFS rates of 62.2% in the obinutuzumab arm and 53.9% in the rituximab arm. However, obinutuzumab treatment was associated with a higher incidence of grade ≥3 adverse events, particularly infections and thrombocytopenia, and did not confer an overall survival benefit (**Figure 1.3**) (Townsend et al., 2023).

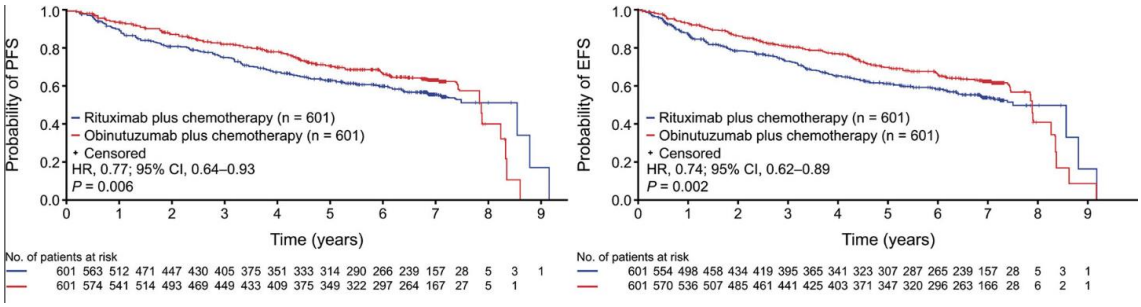

Figure 1.3. PFS and EFS improve in obinutuzumab-based therapy compared to rituximab-based regimen (Townsend et al., 2023).

The selection of the chemotherapy backbone should be individualized according to treatment objectives, patient age, performance status, and comorbidities, considering the

different toxicity profiles of the available regimens. Compared with R-CVP, R-CHOP has been associated with improved progression-free survival, while demonstrating a more favorable safety profile than rituximab-fludarabine-mitoxantrone–based therapy. Bendamustine combined with rituximab (BR) has shown PFS outcomes that are superior or comparable to those achieved with R-CHOP or R-CVP in large randomized trial (Federico et al., 2013), although no significant differences in overall survival have been observed among the various immunochemotherapy approaches in other studies (Flinn et al., 2014; Rummel et al., 2013). In addition, the chemotherapy-free combination of lenalidomide plus rituximab (R^2) has demonstrated efficacy comparable to that of R-CHOP in the first-line setting (**Figure 1.4**) (Morschhauser et al., 2018).

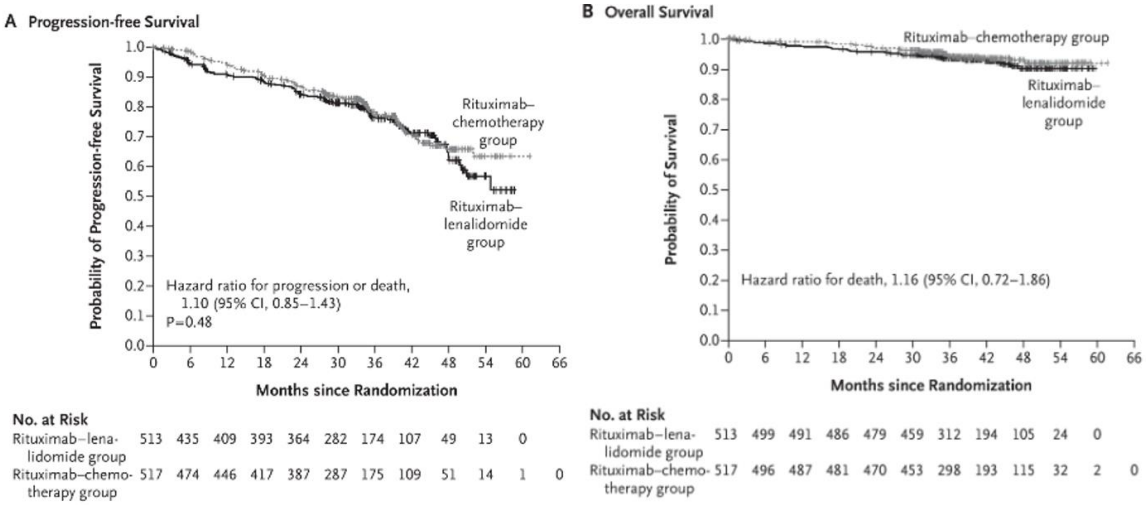

Figure 1.4. PFS and OS in R^2 and R-chemotherapy arm are superimposable (Morschhauser et al., 2018).

Following induction treatment, two years of rituximab maintenance significantly improves PFS compared with observation, although no overall survival advantage has been demonstrated (Bachy et al., 2019; Hirt et al., 2021). Radioimmunotherapy consolidation may also extend PFS

after chemotherapy, but its benefit seems to be less pronounced than that observed with standard rituximab maintenance (Morschhauser et al., 2013).

Although current first-line treatment approaches achieve durable disease control in many patients, FL remains characterized by a relapsing-remitting clinical course, and many patients will ultimately require subsequent lines of therapy. The management of relapsed FL should be individualized according to the extent of relapse, the efficacy and duration of response to previous treatments, and patient-related factors. Localized symptomatic recurrences may be effectively treated with low-dose involved-site radiotherapy, whereas early systemic relapses generally require a non–cross-resistant systemic regimen. Several therapeutic options are available in this setting, including bendamustine-, platinum-, fludarabine-, or alkylating agent–based combinations. Obinutuzumab-based regimens represent an effective alternative in rituximab-refractory disease. In selected patients with low tumour burden, rituximab monotherapy may be appropriate (Dreyling et al., 2021). In addition, combination of lenalidomide and rituximab has demonstrated improved disease control compared with rituximab alone and may be particularly useful in patients with early relapse following immunochemotherapy (Leonard et al., 2019) Moreover, in later relapses, phosphoinositide 3-kinase (PI3K) inhibitors such as idelalisib have demonstrated activity in patients with relapsed/refractory FL after at least two prior lines of therapy, including those with double-refractory disease. Despite high overall response rates, PFS remains relatively modest, and the clinical use of these agents is limited by potentially severe toxicities, including infections, late-onset colitis, hepatitis, and pneumonitis. Finally, in relapsed disease, rituximab maintenance has been associated with prolonged PFS and may also confer an overall survival benefit in relapsed disease.

Recent years have witnessed the development of novel therapeutic strategies for relapsed/refractory FL, including agents targeting B-cell signaling pathways. Although encouraging activity has been reported in phase II studies, their efficacy remains to be confirmed in phase III trials. In contrast, the addition of bortezomib to rituximab has provided limited clinical benefit. CD19-directed CAR-T cell therapy has demonstrated the potential to induce durable remissions; however, owing to its toxicity profile, its use is currently restricted to transformed FL or selected high-risk patients within clinical trial settings(Dreyling et al., 2021; Tobin et al., 2024).

1.7 Clonal Hematopoiesis

The human hematopoietic system is sustained by an estimated 150,000–200,000 hematopoietic stem cells (HSCs). These cells divide relatively infrequently, with each HSC entering the cell cycle approximately once every few months. Because of lifelong replication and exposure to endogenous and environmental mutagenic processes, somatic mutations progressively accumulate over time. Clonal hematopoiesis (CH) refers to the expansion of a hematopoietic cell clone carrying one or more acquired somatic mutations that confer a selective growth advantage. As the mutant clone expands, it may become detectable in peripheral blood despite the absence of overt hematological disease. To distinguish this phenomenon from hematologic malignancies, the term clonal hematopoiesis of indeterminate potential (CHIP) was introduced.

CHIP is defined by the presence of somatic mutations affecting genes commonly associated with hematologic cancers in individuals without evidence of a hematological neoplasm. Traditionally, these mutations are required to be detected at a variant allele frequency (VAF) of at least 2%. However, this VAF threshold is largely operational rather than biologically

defined and remains a matter of debate. Recent improvements in next-generation sequencing (NGS) technologies have enabled the reliable detection of much smaller mutant clones, suggesting that current diagnostic criteria may require revision in the future (Kusne et al., 2022; Weeks & Ebert, 2023).

Several factors have been shown to influence the development and expansion of clonal hematopoiesis, including advancing age, smoking, genetic ancestry, and exposure to chemotherapy or radiotherapy. In addition to its association with these environmental and clinical factors, CH has been linked to an increased risk of developing hematologic malignancies.

The accumulation of somatic mutations within hematopoietic stem cells can give rise to clonally expanded populations of mature blood cells, including monocytes, granulocytes, and lymphocytes. These mutated immune cells may exhibit altered functional properties that contribute to dysregulated immune responses and chronic inflammation, thereby influencing the development and progression of a variety of disease states (Bolton et al., 2020; Jaiswal, 2020).

Consistent with these observations, individuals with CHIP have been shown to carry a substantially increased risk of adverse clinical outcomes, including an approximately 10-fold higher risk of developing hematologic malignancies. Furthermore, CHIP has been associated with a 2- to 4-fold increased risk of atherosclerotic cardiovascular disease and coronary artery calcification, independently of conventional cardiovascular risk factors. These findings suggest that the clinical impact of CHIP extends beyond hematologic cancer predisposition and may involve broader mechanisms linking clonal hematopoiesis to systemic inflammation and age-related diseases (**Figure 1.5**) (Mayerhofer et al., 2023).

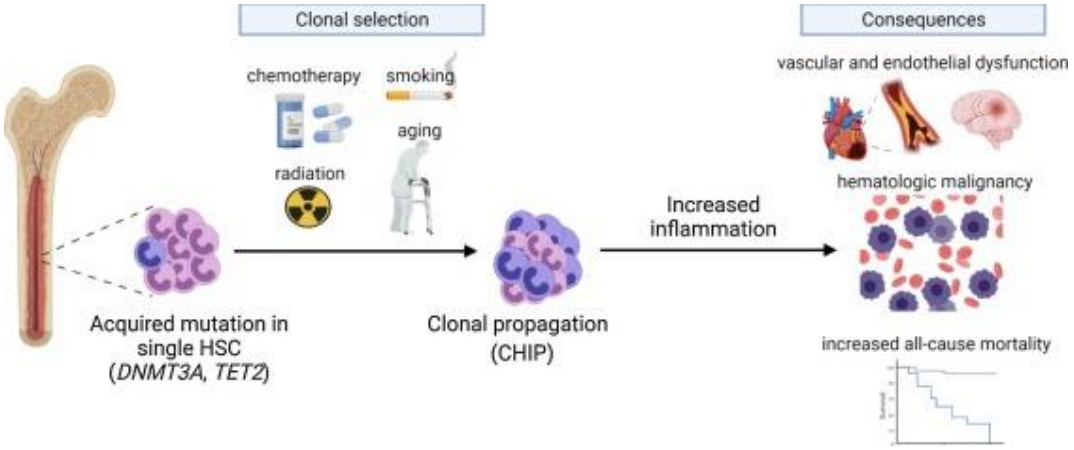

Figure 1.5. Clonal selection in hematopoietic stem cells and its clinical consequences (Kusne et al., 2022).

The majority of mutations identified in clonal hematopoiesis affect genes involved in epigenetic regulation, with alterations in *DNMT3A*, *TET2*, and *ASXL1* accounting for more than 70% of cases. The firsts two genes encode key epigenetic regulators involved in DNA methylation and demethylation, respectively. Most pathogenic DNA methyltransferase 3a (*DNMT3A*) mutations, that encodes an enzyme essential for DNA methylation and epigenetic regulation, are loss-of-function, affecting gene regulation and enhancing HSC self-renewal; moreover, these mutations disrupt normal blood cell differentiation and contribute to inflammation inducing pro-inflammatory T cell polarization. Ten-eleven translocation-2 (*TET2*) deficiency has been associated with a bias toward myeloid differentiation and increased production of pro-inflammatory cytokines, mechanisms that may contribute to the development of cardiovascular disease and accelerated atherosclerosis and, as for *DNMT3A*, its loss of function preferentially leads to differentiation toward myeloid lineages in mouse model. *ASXL1*, another epigenetic regulator frequently altered in CHIP, is involved in chromatin remodeling and transcriptional repression. Mutations in *ASXL1*, most commonly

frameshift or nonsense variants, result in epigenetic dysregulation and further promote clonal fitness (Jaiswal, 2020; Kusne et al., 2022; Marnell et al., 2021).

Less frequently, mutations occur in genes regulating RNA splicing, such as *SF3B1* and *U2AF1*, as well as in signaling pathways and DNA damage response (DDR) genes, including *TP53* and *PPM1D*; mutations occurring in these last two genes can confer resistance to cellular stress and apoptosis, providing a selective advantage to mutant clones. Notably, *TP53* and *PPM1D* mutations are enriched following exposure to chemotherapy, supporting their role in the development of therapy-related clonal hematopoiesis (Coombs et al., 2017; Kusne et al., 2022; Weeks & Ebert, 2023).

Overall, clonal hematopoiesis is increasingly recognized as a premalignant condition associated with an increased risk of both hematologic and non-hematologic diseases. While its biological and clinical implications have been extensively studied in myeloid neoplasms, its role in lymphoid malignancies remains less well understood. Nevertheless, the prevalence of CHIP in patients with lymphoid cancers is estimated to be approximately 30%, higher than that observed in the general population, potentially reflecting the effects of immune dysregulation and prior exposure to cytotoxic therapies. Further research is needed to better define the clinical significance of CH in lymphoid neoplasms (Bou Zerdan et al., 2022; Husby et al., 2020; Younes et al., 2023).

1.8 FOLL12 TRIAL

The FOLL12 trial aimed to show the noninferiority of a response-adapted postinduction strategy on the basis of FDG-PET and minimal residual disease (MRD) response assessment

after immunochemotherapy (CIT), with 2-year standard maintenance with rituximab in patients with previously untreated advanced FL.

FOLL12 was a prospective randomized open-label multicenter phase III trial designed for patients with previously untreated FL.

The trial included previously untreated patients aged 18-75 years with a histologically confirmed diagnosis of FL grade 1, 2, or 3a according to the WHO classification, Ann Arbor stage II-IV, Eastern Cooperative Oncology Group performance status of 0-2, and Follicular Lymphoma International Prognostic Index 2 (FLIPI2) of > 0.

After enrollment, 786 patients were randomized to either the reference arm (n = 393) or the experimental arm (n = 393). In both groups, patients received induction therapy with either R-CHOP or BR. Subsequently, patients in the reference arm received standard rituximab maintenance, whereas those in the experimental arm underwent a response-adapted post-induction strategy based on complete metabolic response (CMR) and MRD status. After a median follow-up of 53 months, progression-free survival (PFS) was significantly longer in the reference arm than in the experimental arm. The superiority of standard rituximab maintenance was further supported by subgroup analyses, with the greatest benefit observed among patients who achieved both CMR and MRD negativity following induction therapy. (Luminari et al., 2022)

2. AIM OF THE STUDY

Clonal hematopoiesis has emerged as a potential prognostic factor in hematologic malignancies. However, its clinical significance in follicular lymphoma, as well as in B-cell lymphomas more broadly, remains poorly defined. Therefore, the aim of the present study was to investigate the prevalence and clinical implications of CH in patients with FL enrolled in the multicenter phase III FIL FOLL12 trial. Specifically, the objectives were:

i) To evaluate the prevalence of CH and its dynamics during and after treatment.

ii) To assess the association between CH and clinical outcomes, including PFS and OS.

iii) To investigate the relationship between CH and the development of second malignancies in patients with FL.

3. MATERIALS & METHODS

3.1 Patients

The study was conducted on a total of 242 patients enrolled in the FOLL 12 trial. For each patient, a genomic DNA sample (gDNA) was extracted from peripheral blood leukocytes. Clinical and biological data were available for every patient profile like age, sex, B symptoms, FLIPI scores, Ann Arbor staging, nodal sites and peripheral blood count. The study was approved by the Ethics Committee of the Azienda Ospedaliera Universitaria (AOU) Maggiore della Carità di Novara.

3.2 DNA Extraction, Quantification & fragmentation

gDNA was extracted from peripheral blood samples using Maxwell® RSC Blood DNA Kit and was consequently quantified using Quant-iTTM PicoGreen ds DNA Assay Kit (ThermoFisher Scientific, Eugene, OR, USA). PicoGreen is a specific molecule (fluorescent nucleic acid stain) that binds the double strands DNA with high selectivity, permitting a precise evaluation of the DNA amount and showing a maximum emission at 530 nm. The fluorometric reading was performed with Infinite F200 fluorometer (TECAN Männedorf, Switzerland) and the Magellan software.

For gDNA quantification, a standard curve was prepared using DNA of known concentration and performing serial 1:2 scalar dilutions. PicoGreen dye was diluted at a 1:200 ratio. The gDNA was fragmented by sonication using the M220 focused ultrasonicator (Covaris® Woburn, MA, USA) before library preparation to obtain 250/300 base pair fragments, that represent the optimal length for analysis using the MiSeq and NextSeq 550

NGS platforms (Illumina, San Diego, CA,USA). The size of the fragments was checked by usung the 2100 Bioanalyzer Instrument with the High Sensitivity DNA kit (Agilent Technologies, St. Clara, CA, USA). A targeted resequencing gene panel, including coding exons and splice sites of 28 genes (target region: 29710 bp) that are recurrently mutated in CHIP, has been specifically designed for this project.

3.3 Next Generation Sequencing

The mutational analysis in NGS was performed using MiSeq and NextSeq 550, which allows for massive high-throughput sequencing of the genomic regions of interest. The sequencing process steps were: i) generation of libraries with targeted regions; ii) sequencing; and iii) data analysis.

i.*Generation of libraries*

This process consists of generating a collection of DNA fragments suitable for NGS sequencing analysis. For the current study, libraries were prepared by gDNAs. Libraries were obtained through the KAPA HyperPrep kit (Roche Diagnostics, Pleasanton, CA, USA) and enrichment of regions of interest was achieved using a KAPA HyperChoice probe system (Roche Diagnostics, Pleasanton, CA, USA).

ii. *Sequencing*

The MiSeq and NextSeq 550 sequencer exploit the sequencing by synthesis technology, in which the DNA libraries are first transferred onto a solid support (flow cell) and then linked by special adapters. Through a process called “bridge amplification” the libraries are amplified on the flow cell, generating clusters of identical DNA molecules as derivatives of singles amplificated molecules. Sequencing

is based mainly on three steps that resumes a reversible cyclic termination method: incorporation of the nucleotide, fluorescence detection and cleavage. In the first step, DNA polymerase elongates a specific primer by adding a complementary nucleotide with a fluorescent terminator (with color selection related to the nitrogenous base) bound to 3'-OH; in this way the incorporation of another nucleotide is not permitted. Thus, the fluorescence detection step occurs, where the emission of the fluorophore is recognized. At the end, in the cleavage step, the terminator is cutted away from 3'-OH and the polymerase will be able to start a new cycle. The library pool was denatured using 0.2N NaOH. An amount of 6 to 9.5 pM of denatured DNA (for the MiSeq platform) and 1.3 pM (for the NextSeq 550 platform) was loaded into the cartridge, which also contained all the reagents necessary for the sequencing reaction.

iii. *Data analysis*

During the sequencing run, an image analysis and identification of the bases are performed by an integrated software (RTA, Real Time Analysis, Illumina), also assigning a qualitative score to each base for each cycle. After completing the primary analysis, MiSeq Reporter Software (MSR) for MiSeq platform and Local Run Manager for NextSeq 550 platform perform a secondary analysis on RTA generated data through different steps:

- De-multiplexing, that separates data from different sequenced samples and pulled together based on the specific sample index sequences.
- FASTQ generation: files containing all the reads obtained from sequencing.

By using FastUniq v1.1, FASTQ sequencing reads were subjected to deduplication, consequently aligned to the GRCh37/hg19 version of the human genome assembly using the BWA v.0.6.1 software with the default setting and then sorted, indexed and assembled into a mpileup file through SAMtools v.1. Single nucleotide variants (SNVs), insertions

and deletions were analyzed at high-quality nucleotide positions (Phred score >20) by the VarScan2 program comparing gDNA sequences to the GRCh37/hg19 reference genome. All the highlighted variants by VarScan2 were annotated with wANNOVAR software (https://wannovar.wglab.org/). Variants were annotated as single nucleotide polymorphisms (SNPs), according to the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/snp/), with the exception of *TP53* variants that were manually solved and scored as SNPs according to the International Agency for Research on Cancer TP53 database (http://p53.iarc.fr).

Intronic variants, mapping >2 bp before the start or after the end of coding exons, and synonymous variants were filtered out. The protein truncating variants (i.e., indels, splice mutations and stop codon) and missense variants, annotated as somatic in the COSMIC v96 database (https://cancer.sanger.ac.uk/cosmic) and not included in dbSNP, were retained. All the variants were visualized using IGV (Integrative Genomics Viewer) software.

3.4 Statistical analysis

Survival analysis for progression-free survival (PFS) and for overall survival (OS) was performed by the Kaplan-Meier method and compared between strata using the Log-rank test. The Simon–Makuch method was applied to model fit CH clones as a time-dependent variable. For continuous variables, two group comparisons were performed by Mann-Whitney test, while Chi-square test for categorical variables was used to compare patient characteristics and CH presence. The McNemar test was used to compare CH prevalence at baseline and in paired sequential samples. Associations with the development of second primary malignancies were estimated using both Cox proportional hazards regression, with effect expressed as hazard ratio

(HR) with 95% confidence interval (CI) and cumulative incidence function (CIF) analysis by Fine-Gray competing risk modeling with effect expressed as sub distribution HR (sHR) with 95%CI, accounting for death as a competing event. All reported tests were two-sided. Statistics were performed with R version 4.4.2.

4. RESULTS

4.1 Patient Characteristics

A total of 242 patients with follicular lymphoma enrolled in the FIL FOLL12 study were included in the analysis. The median age was 61.5 years (interquartile range [IQR],

51.7–69.0), and 126 patients (52.1%) were female. Most patients presented with advanced-stage disease, with 167 (69.3%) classified as Ann Arbor stage IV, while 34 (14.1%) reported B symptoms. Histological grading showed that 40 patients (19.4%) had grade 1 disease, 116 (56.3%) grade 2, and 50 (24.3%) grade 3A. According to physician choice, 141 patients (58.3%) received R-CHOP and 101 (41.7%) received R-bendamustine (R-Benda) (**Table 4.1**).

Baseline clinical characteristics, including the proportion of patients treated with R-Benda, were comparable between patients included in the present molecular analysis and those enrolled in the FOLL12 trial who were not evaluable for clonal hematopoiesis because of unavailable biological material.

Characteristic		Value no. (%)
Age (years)	Median	61.5
	Range	51.7–69.0
Gender	Male	116 (47.9)
	Female	126 (52.1)
Ann Arbor stage	II	29 (12.0)
	III	45 (18.7)
	IV	167 (69.3)
B symptoms	No	207 (85.9)
	Yes	34 (14.1)
β2M	≤ULN	117 (48.3)
	>ULN	125 (51.7)
Grading	Grade 1	40 (19.4)
	Grade 2	116 (56.3)
	Grade 3A	50 (24.3)
Hb	≥12 g/dL	203 (83.9)
	<12 g/dL	39 (16.1)
LDH	≤ULN	176 (75.9)
	>ULN	56 (24.1)
Nodal sites	0–4	151 (63.2)
	>4	88 (36.8)
FLIPI	0–1	59 (25.7)
	2	88 (38.3)
	3–5	83 (36.1)
FLIPI2	1–2	146 (60.3)
	3–5	96 (39.7)
Treatment	R-CHOP	141 (58.3)
	R-Benda	101 (41.7)

Table 4.1. Patient characteristics. Abbreviations: β2M, beta-2 microglobulin; Hb, hemoglobin; LDH, lactate dehydrogenase; FLIPI, Follicular Lymphoma International Prognostic Index; ULN, upper limit normal.

4.2 CIT drives selective expansion of CH prevalence

To investigate the impact of chemoimmunotherapy (CIT) on CH dynamics, we analyzed 211 patients with one or more sequential samples available. The median interval between baseline and sequential samples was 30 months (IQR, 12–36 months).

Overall, CIT significantly promoted both the prevalence and clonal burden of CH. The total number of detected CH mutations increased from 116 at baseline to 180 at follow-up ($P = 3.22 \times 10^{-7}$), accompanied by an increase in the proportion of CH-positive patients from 37.4% (N = 79) to 47.4% (N = 100; P = 0.004) (**Figure 4.1A**).

Analysis of treatment-associated changes in CH composition demonstrated a profound remodeling of the CH landscape following CIT, characterized by selective expansion of clones harboring *DNMT3A*, *TP53*, and *PPM1D* mutations (**Figure 4.1B**). The prevalence of *DNMT3A*-mutated patients increased from 18.4% (N = 39) at baseline to 26.5% (N = 56) after treatment (P = 0.005). Even more pronounced expansions were observed for *TP53*-mutated patients, whose prevalence rose from 0.9% (N = 2) to 6.6% (N = 14; P < 0.001), and for *PPM1D*-mutated patients, increasing from 1.9% (N = 4) to 9.9% (N = 21; P < 0.001). Consequently, the prevalence of CH involving DNA damage response (DDR) genes, defined by *TP53* and/or *PPM1D* mutations, increased from 2.8% (N = 6) to 16.6% (N = 35; P < 0.001). Notably, *TP53* and *PPM1D* mutations were almost entirely mutually exclusive, with only a single patient harboring both lesions (**Figure 4.1C**).

Exploiting the distinct genotoxic profiles of R-CHOP and R-Benda allowed the identification of regimen-specific selective pressures on CH. The most striking finding was the exclusive expansion of *TP53*-mutated clones following R-Benda treatment, with the prevalence of *TP53*-mutated patients increasing from 0.9% (N = 1) at baseline to 15.0% (N = 12) after therapy (P < 0.001). In contrast, no change was observed among patients treated with R-CHOP (0.9% at both time points; P = 0.999) (**Figure 4.1.D**). Importantly, all newly detected *TP53* mutations emerged in patients who were minimal residual disease negative, supporting their origin from CH rather than residual FL cells.

By comparison, *PPM1D*-mutated clones expanded under both treatment regimens, with prevalence increasing from 2.5% to 7.6% following R-CHOP (P = 0.031) and from 1.1% to 11.9% following R-Benda (P < 0.001) (**Figure 4.1D**).

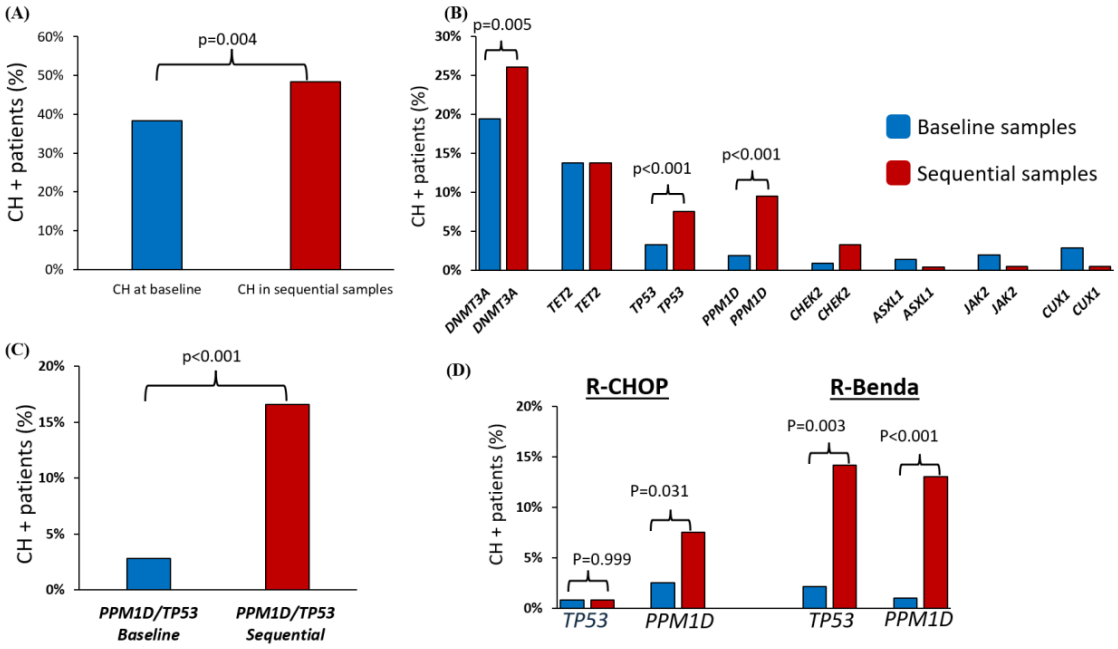

Figure 4.1. Chemoimmunotherapy reshapes the clonal hematopoiesis landscape in follicular lymphoma. (A) Prevalence of CH at baseline and in sequential samples, showing a significant increase following chemoimmunotherapy. **(B)** Distribution of CH mutations before and after treatment, highlighting treatment-associated changes in the mutational spectrum. **(C)** Longitudinal enrichment of DDR gene mutations (*PPM1D* and *TP53*), in sequential samples. **(D)** Treatment-specific effects of R-CHOP and R-Benda on CH evolution, demonstrating preferential expansion of *TP53*-mutated clones after R-Benda and of *PPM1D*-mutated clones under both regimens.

4.3 Clonal fitness of CH differs under R-CHOP versus R-Benda

To further characterize CH dynamics, clonal fitness was estimated for each mutation as the annual change in VAF according to a logistic growth model. Based on their longitudinal behavior, clones were classified as increasing, stable, or decreasing.

Among all CH mutations detected either at baseline or after CIT, 128 (57.1%) displayed progressive expansion over time, whereas 49 (21.9%) showed declining VAFs and 47 (21.0%) remained stable (**Figure 4.2A-B**). These findings indicate that most of CH clones undergo positive selection following treatment.

Analysis of recurrently mutated genes revealed marked differences in clonal fitness across CH subtypes. Variants affecting DDR genes exhibited the strongest selective advantage, with *PPM1D*-, *CHEK2*-, and *TP53*-mutated clones showing the highest median fitness values (1.232, 0.908, and 0.833, respectively). In contrast, mutations in canonical DTA genes displayed substantially lower fitness, particularly *DNMT3A* (0.229) and *TET2* (0.233) (**Figure 4.2C**). Overall, DDR-associated clones demonstrated significantly greater fitness than DTA-associated clones, consistent with preferential expansion under treatment-induced genotoxic stress.

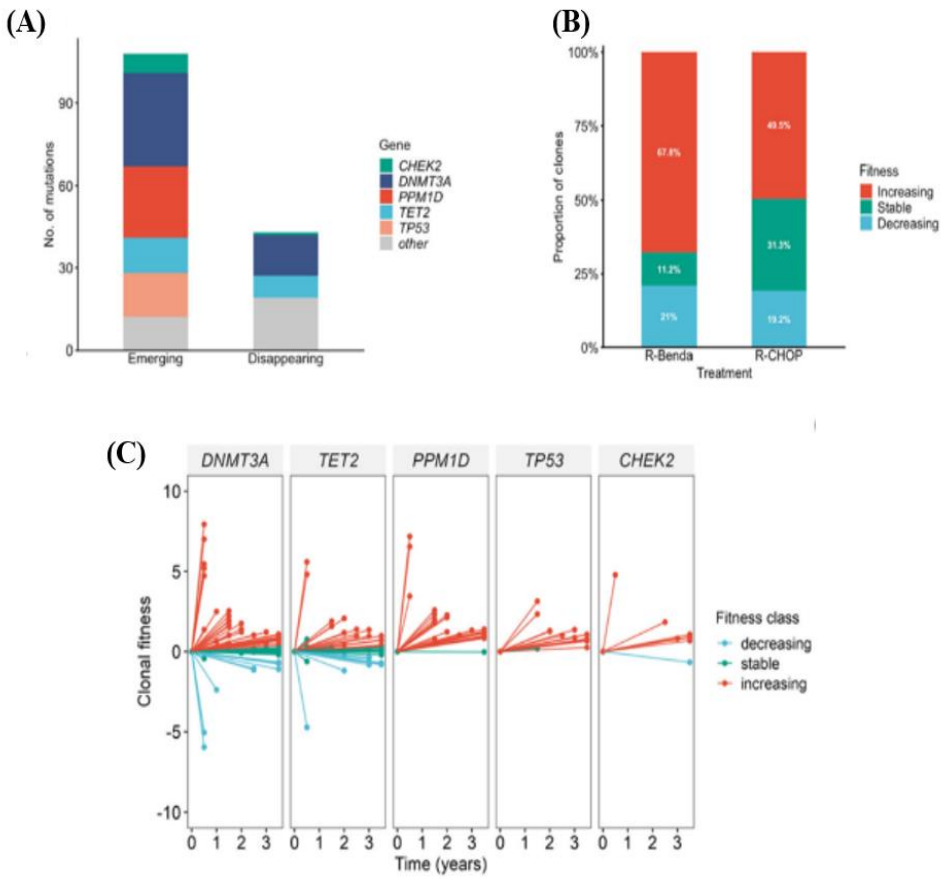

Figure 4.2. Longitudinal dynamics and fitness of clonal hematopoiesis clones following CIT. (A) Emerging and disappearing CH mutations detected in sequential samples. **(B)** Distribution of increasing, stable, and decreasing CH clones following R-CHOP or R-Benda treatment. **(C)** Gene-specific trajectories of CH clonal fitness over time, showing increasing, stable and decreasing clones across major CH-associated genes post-treatment.

4.4 DTA mutations and fit DDR clones predict second primary malignancies in FL

The extended follow-up of the FOLL12 trial provided a unique opportunity to investigate the relationship between CH and late treatment-related toxicities, particularly second primary malignancies (SPMs). After a median follow-up of 98 months (range, 3.3–

141.4), 28 patients developed an SPM, corresponding to a 9-year cumulative incidence of 11.9% (95% CI, 6.3–15.1).

At baseline, patients harboring DTA mutations experienced a significantly higher incidence of SPMs than DTA wild-type patients (9-year cumulative incidence, 21.3% vs. 7.3%; P = 0.0017) (**Figure 4.3A**). Accordingly, multivariable Cox regression analysis identified baseline DTA mutations as an independent predictor of SPM development after adjustment for age and treatment regimen (HR 2.66, 95% CI 1.35–5.67; P = 0.011). Notably, disease progression requiring second-line therapy was not associated with an increased SPM risk (HR 0.25, 95% CI 0.06–1.07; P = 0.062).

In contrast, the presence of DDR mutations at baseline did not influence SPM incidence (P = 0.528). However, following treatment, patients harboring fit DDR-mutated clones exhibited a markedly increased risk of SPMs, with a 9-year cumulative incidence of 28.2% compared with 8.8% among patients without fit DDR mutations (P < 0.001) (**Figure 4.3B**). This association remained significant in multivariable analysis adjusted for baseline DTA status, age, and treatment regimen (HR 2.63, 95% CI 1.07–6.05; P = 0.035) (**Figure 4.3C**).

Given that baseline DTA mutations and post-treatment fit DDR mutations independently predicted SPM development, we next assessed their combined impact. Patients harboring either baseline DTA mutations, fit DDR mutations, or both displayed a substantially higher cumulative incidence of SPMs than patients lacking both features (21.8% vs. 5.9% at 9 years), reaching 32.7% at 10 years (P < 0.001) (**Figure 4.3C**). These findings indicate that baseline CH status and treatment-driven clonal evolution provide complementary information for identifying patients at increased long-term risk of secondary malignancies.

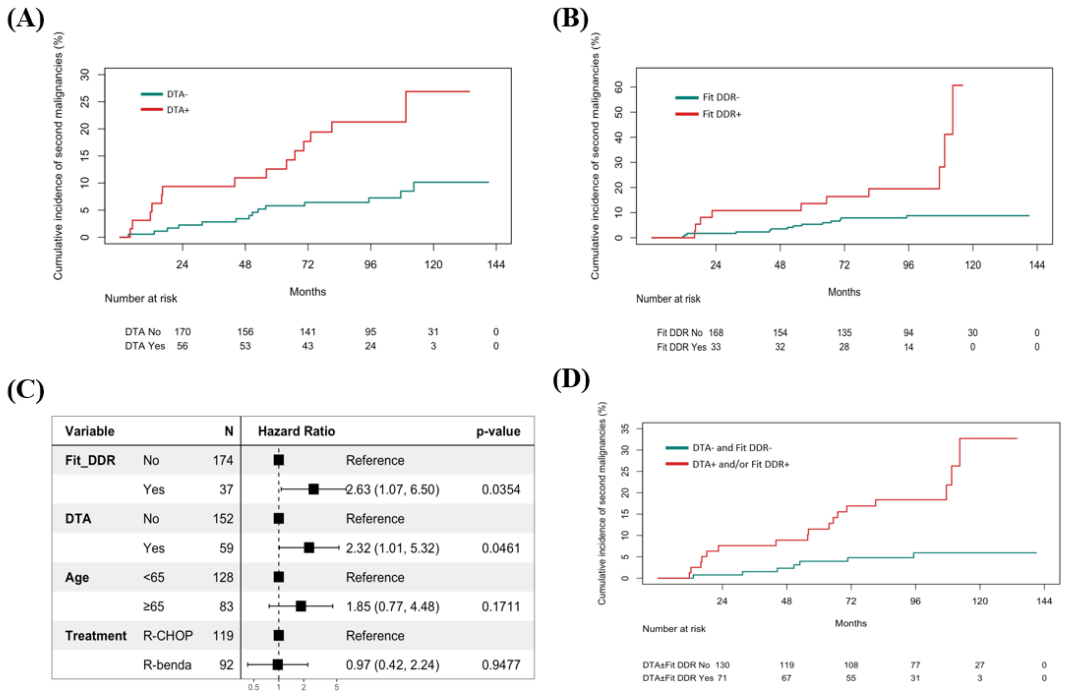

Figure 4.3. Baseline DTA mutations and post-treatment fit DDR clones predict second primary malignancies in follicular lymphoma. (A) Cumulative incidence of second primary malignancies according to baseline DTA mutation status. (B) Cumulative incidence according to the presence of post-CIT fit DDR mutations. (C) Multivariable analysis of risk factors for second primary Malignancies. (D) Integrated cumulative incidence analysis based on baseline DTA mutations and/or fit DDR clones.

5. DISCUSSION

The present study investigated the prevalence, longitudinal evolution and clinical significance of clonal hematopoiesis in 242 patients with newly diagnosed follicular lymphoma enrolled in the FIL FOLL12 trial.

By integrating molecular profiling with long-term clinical follow-up, our findings provide evidence that chemoimmunotherapy not only reshapes the CH landscape through selective clonal expansion but also contributes to the emergence of CH patterns associated with an increased risk of second primary malignancies.

At baseline, CH was detected in a substantial proportion of patients, with predominance of mutations involving *DNMT3A* and other canonical DTA genes as reported in literature (Coombs et al., 2017).

One of the most relevant observations of this study is the marked impact of CIT on CH dynamics. Longitudinal analysis revealed a significant increase in both the prevalence of CH-positive patients and the total number of detectable CH mutations after treatment. These findings indicate that exposure to genotoxic therapy alters the competitive landscape of hematopoietic stem cells, favoring the expansion of selected mutant clones. Such observations are in agreement with previous reports demonstrating that chemotherapy can act as a powerful selective pressure on pre-existing CH populations rather than solely inducing de novo mutations. In this context, treatment appears to function as an evolutionary bottleneck, allowing resistant clones to preferentially survive and expand.

A major finding of this study is that chemoimmunotherapy profoundly reshapes the clonal hematopoiesis landscape in patients with follicular lymphoma. While age-related DTA mutations such as *DNMT3A* expanded after treatment, the most pronounced selective

advantage was observed for mutations affecting DNA damage response genes, particularly *TP53* and *PPM1D*. The marked enrichment of these mutations following therapy supports the concept that genotoxic stress acts as a selective pressure favoring hematopoietic clones with enhanced resistance to DNA damage.

Following treatment, both the prevalence and number of CH mutations increased, suggesting that cytotoxic therapy exerts a selective pressure favoring the expansion of specific hematopoietic clones. In particular, mutations involving DDR genes, such as *TP53* and *PPM1D*, showed marked expansion after treatment. These findings are in agreement with previous studies indicating that clones harboring DDR mutations possess a survival advantage under genotoxic stress and may represent a substrate for therapy-related clonal evolution.

Interestingly, different treatment regimens appeared to influence clonal selection differently. *TP53*-mutated clones expanded predominantly in patients treated with bendamustine-containing regimens, whereas *PPM1D* mutations increased following both R-Benda and R-CHOP. Notably, R-Benda appeared to impose a stronger selective bottleneck on hematopoietic cells, uniquely promoting the expansion of highly fit *TP53*-mutated clones. This differential selective pressure may be related to the dual mechanism of action of bendamustine, which combines alkylating and purine analogue-like properties, thereby inducing a distinct pattern of DNA damage that favors the outgrowth of *TP53*-deficient hematopoietic clones (Lalic et al., 2022). Furthermore, DDR-associated mutations exhibited higher clonal fitness than mutations involving epigenetic regulators such as *DNMT3A* and *TET2*, highlighting the importance of treatment-induced selective pressure in shaping clonal dynamics.

A major finding of this study is the association between CH and the development of second primary malignancies. Baseline DTA mutations (*DNMT3A*, *TET2*, and *ASXL1*) were associated with a significantly increased risk of secondary cancers, while the presence of fit DDR clones

after treatment independently predicted a higher cumulative incidence of second malignancies. These observations suggest that both pre-existing clonal hematopoiesis and treatment-driven clonal evolution contribute to long-term oncologic risk in FL patients. Notably, most second malignancies observed in this cohort were solid tumors, supporting the hypothesis that CH may promote susceptibility to secondary cancers beyond the hematopoietic compartment. This association may be mediated through CH-driven systemic inflammation and alterations of the tumour microenvironment, mechanisms that have been increasingly recognized as contributors to cancer development and progression (Pich et al., 2025; Liu X. et al., 2022; Yang F. et al., 2023; Nguyen et al., 2021; Pan et al., 2017).

The strengths of this study include the large, well-characterized cohort, the long follow-up period, and the availability of sequential samples that enabled evaluation of clonal evolution over time. However, some limitations should be acknowledged, including the use of a targeted gene panel and the relatively limited number of second malignancy events.

In conclusion, CH is a frequent finding in patients with FL and undergoes significant treatment-driven evolution after chemoimmunotherapy. Although CH does not affect survival outcomes, baseline DTA mutations and treatment-selected DDR clones identify patients at increased risk of developing second primary malignancies. These findings support the potential role of CH assessment as a tool for long-term risk stratification and surveillance in patients with follicular lymphoma.

BIBLIOGRAPHY

Bachy, E., Seymour, J. F., Feugier, P., Offner, F., López-Guillermo, A., Belada, D., Xerri, L., Catalano, J. V., Brice, P., Lemonnier, F., Martin, A., Casasnovas, O., Pedersen, L. M., Dorvaux, V., Simpson, D., Leppa, S.,

Gabarre, J., da Silva, M. G., Glaisner, S., … Salles, G. (2019). Sustained Progression-Free Survival Benefit of Rituximab Maintenance in Patients With Follicular Lymphoma: Long-Term Results of the PRIMA Study. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, *37*(31), 2815–2824. https://doi.org/10.1200/JCO.19.01073

Becnel, M. R., & Nastoupil, L. J. (2018). Follicular Lymphoma: Past, Present, and Future. *Current Treatment Options in Oncology*, *19*(7), 32. https://doi.org/10.1007/s11864-018-0550-0

Bolton, K. L., Ptashkin, R. N., Gao, T., Braunstein, L., Devlin, S. M., Kelly, D., Patel, M., Berthon, A., Syed, A., Yabe, M., Coombs, C. C., Caltabellotta, N. M., Walsh, M., Offit, K., Stadler, Z., Mandelker, D., Schulman, J., Patel, A., Philip, J., … Papaemmanuil, E. (2020). Cancer therapy shapes the fitness landscape of clonal hematopoiesis. *Nature Genetics*, *52*(11), 1219–1226. https://doi.org/10.1038/s41588-020-00710-0

Bosga-Bouwer, A. G., van Imhoff, G. W., Boonstra, R., van der Veen, A., Haralambieva, E., van den Berg, A., de Jong, B., Krause, V., Palmer, M. C., Coupland, R., Kluin, P. M., van den Berg, E., & Poppema, S. (2003). Follicular lymphoma grade 3B includes 3 cytogenetically defined subgroups with primary t(14;18), 3q27, or other translocations: T(14;18) and 3q27 are mutually exclusive. *Blood*, *101*(3), 1149–1154. https://doi.org/10.1182/blood.V101.3.1149

Bou Zerdan, M., Nasr, L., Saba, L., Meouchy, P., Safi, N., Allam, S., Bhandari, J., & Chaulagain, C. P. (2022). A Synopsis Clonal Hematopoiesis of Indeterminate Potential in Hematology. *Cancers*, *14*(15), 3663. https://doi.org/10.3390/cancers14153663

Coombs, C. C., Zehir, A., Devlin, S. M., Kishtagari, A., Syed, A., Jonsson, P., Hyman, D. M., Solit, D. B., Robson, M. E., Baselga, J., Arcila, M. E., Ladanyi, M., Tallman, M. S., Levine, R. L., & Berger, M. F. (2017). Therapy-Related Clonal Hematopoiesis in Patients with Non-hematologic Cancers Is Common and Associated with Adverse Clinical Outcomes. *Cell Stem Cell*, *21*(3), 374-382.e4. https://doi.org/10.1016/j.stem.2017.07.010

Díaz-Alderete, A., Doval, A., Camacho, F., Verde, L., Sabin, P., Arranz-Sáez, R., Bellas, C., Corbacho, C., Gil, J., Perez-Martín, M., Ruiz-Marcellán, M., Gonzalez, L., Montalbán, C., Piris, M., & Menarguez, J.

(2008). Frequency of BCL2 and BCL6 translocations in follicular lymphoma: Relation with histological and clinical features. *Leukemia & Lymphoma*, *49*(1), 95–101. https://doi.org/10.1080/10428190701742472

Dreyling, M., Ghielmini, M., Rule, S., Salles, G., Ladetto, M., Tonino, S. H., Herfarth, K., Seymour, J. F., Jerkeman, M., & ESMO Guidelines Committee. Electronic address: clinicalguidelines@esmo.org. (2021). Newly diagnosed and relapsed follicular lymphoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of Oncology: Official Journal of the European Society for Medical Oncology*, *32*(3), 298–308. https://doi.org/10.1016/j.annonc.2020.11.008

Federico, M., Luminari, S., Dondi, A., Tucci, A., Vitolo, U., Rigacci, L., Di Raimondo, F., Carella, A. M., Pulsoni, A., Merli, F., Arcaini, L., Angrilli, F., Stelitano, C., Gaidano, G., Dell'olio, M., Marcheselli, L., Franco, V., Galimberti, S., Sacchi, S., & Brugiatelli, M. (2013). R-CVP versus R-CHOP versus R-FM for the initial treatment of patients with advanced-stage follicular lymphoma: Results of the FOLL05 trial conducted by the Fondazione Italiana Linfomi. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, *31*(12), 1506–1513. https://doi.org/10.1200/JCO.2012.45.0866

Fischer, T., Zing, N. P. C., Chiattone, C. S., Federico, M., & Luminari, S. (2018). Transformed follicular lymphoma. *Annals of Hematology*, *97*(1), 17–29. https://doi.org/10.1007/s00277-017-3151-2

Flinn, I. W., van der Jagt, R., Kahl, B. S., Wood, P., Hawkins, T. E., Macdonald, D., Hertzberg, M., Kwan, Y.-L., Simpson, D., Craig, M., Kolibaba, K., Issa, S., Clementi, R., Hallman, D. M., Munteanu, M., Chen, L., & Burke, J. M. (2014). Randomized trial of bendamustine-rituximab or R-CHOP/R-CVP in first-line treatment of indolent NHL or MCL: The BRIGHT study. *Blood*, *123*(19), 2944–2952. https://doi.org/10.1182/blood-2013-11-531327

Ghielmini, M. (2010). Follicular lymphoma. *Annals of Oncology: Official Journal of the European Society for Medical Oncology*, *21 Suppl 7*, vii151-153. https://doi.org/10.1093/annonc/mdq287

Green, M. R. (2018). Chromatin modifying gene mutations in follicular lymphoma. *Blood*, *131*(6), 595–604. https://doi.org/10.1182/blood-2017-08-737361

Herfarth, K., Borchmann, P., Schnaidt, S., Hohloch, K., Budach, V., Engelhard, M., Viardot, A., Engenhart-Cabillic, R., Keller, U., Reinartz,

G., Eich, H.-T., Witzens-Harig, M., Hess, C. F., Dörken, B., Dürig, J., Wiegel, T., Hiddemann, W., Hoster, E., Pott, C., & Dreyling, M. (2018). Rituximab With Involved Field Irradiation for Early-stage Nodal Follicular Lymphoma: Results of the MIR Study. *HemaSphere*, *2*(6), e160. https://doi.org/10.1097/HS9.0000000000000160

Hirt, C., Hoster, E., Unterhalt, M., Hänel, M., Prange-Krex, G., Forstpointner, R., Florschütz, A., Graeven, U., Frickhofen, N., Wulf, G., Lengfelder, E., Lerchenmüller, C., Schlag, R., Dierlamm, J., Fischer von Weikersthal, L., Ahmed, A., Harich, H.-D., Rosenwald, A., Klapper, W., … Herold, M. (2021). Rituximab Maintenance Versus Observation After Immunochemotherapy (R-CHOP, R-MCP, and R-FCM) in Untreated Follicular Lymphoma Patients: A Randomized Trial of the Ostdeutsche Studiengruppe Hämatologie und Onkologie and the German Low-Grade Lymphoma Study Group. *HemaSphere*, *5*(7), e600. https://doi.org/10.1097/HS9.0000000000000600

Horn, H., Schmelter, C., Leich, E., Salaverria, I., Katzenberger, T., Ott, M. M., Kalla, J., Romero, M., Siebert, R., Rosenwald, A., & Ott, G. (2011). Follicular lymphoma grade 3B is a distinct neoplasm according to cytogenetic and immunohistochemical profiles. *Haematologica*, *96*(9), 1327–1334. https://doi.org/10.3324/haematol.2011.042531

Husby, S., Favero, F., Nielsen, C., Sørensen, B. S., Bæch, J., Grell, K., Hansen, J. W., Rodriguez-Gonzalez, F. G., Haastrup, E. K., Fischer-Nielsen, A., Andersen, P., Arboe, B., Sækmose, S. G., Hansen, P. B., Christiansen, I., Clasen-Linde, E., Meldgaard, L., Ebbesen, L. H., Segel, E. K., … Grønbæk, K. (2020). Clinical impact of clonal hematopoiesis in patients with lymphoma undergoing ASCT: A national population-based cohort study. *Leukemia*, *34*(12), 3256–3268. https://doi.org/10.1038/s41375-020-0795-z

Jacobsen, E. (2022). Follicular lymphoma: 2023 update on diagnosis and management. *American Journal of Hematology*, *97*(12), 1638–1651. https://doi.org/10.1002/ajh.26737

Jaiswal, S. (2020). Clonal hematopoiesis and nonhematologic disorders. *Blood*, *136*(14), 1606–1614. https://doi.org/10.1182/blood.2019000989

Junlén, H. R., Peterson, S., Kimby, E., Lockmer, S., Lindén, O., Nilsson-Ehle, H., Erlanson, M., Hagberg, H., Rådlund, A., Hagberg, O., & Wahlin, B. E. (2015). Follicular lymphoma in Sweden: Nationwide improved survival in the rituximab era, particularly in elderly women: a

Swedish Lymphoma Registry study. *Leukemia*, *29*(3), 668–676. https://doi.org/10.1038/leu.2014.251

Klopčič, U., Lavrenčak, J., Gašljević, G., Bračko, M., Pohar-Marinšek, Ž., & Kloboves-Prevodnik, V. (2016). Grading of follicular lymphoma in cytological samples. *Cytopathology: Official Journal of the British Society for Clinical Cytology*, *27*(6), 390–397. https://doi.org/10.1111/cyt.12319

Koch, K., Hoster, E., Ziepert, M., Unterhalt, M., Ott, G., Rosenwald, A., Hansmann, M. L., Bernd, W., Stein, H., Pöschel, V., Dreyling, M., Trümper, L., Löffler, M., Schmitz, N., Hiddemann, W., Pfreundschuh, M., & Klapper, W. (2016). Clinical, pathological and genetic features of follicular lymphoma grade 3A: A joint analysis of the German low-grade and high-grade lymphoma study groups GLSG and DSHNHL. *Annals of Oncology: Official Journal of the European Society for Medical Oncology*, *27*(7), 1323–1329. https://doi.org/10.1093/annonc/mdw185

Kusne, Y., Xie, Z., & Patnaik, M. M. (2022). Clonal hematopoiesis: Molecular and clinical implications. *Leukemia Research*, *113*, 106787. https://doi.org/10.1016/j.leukres.2022.106787

Lackraj, T., Goswami, R., & Kridel, R. (2018a). Pathogenesis of follicular lymphoma. *Best Practice & Research. Clinical Haematology*, *31*(1), 2–14. https://doi.org/10.1016/j.beha.2017.10.006

Lalic H, Aurer I, Batinic D, Visnjic D, Smoljo T and Babic A: Bendamustine: A review of pharmacology, clinical use and immunological effects (Review). Oncol Rep 47: 114, 2022.

Launay, E., Pangault, C., Bertrand, P., Jardin, F., Lamy, T., Tilly, H., Tarte, K., Bastard, C., & Fest, T. (2012). High rate of TNFRSF14 gene alterations related to 1p36 region in de novo follicular lymphoma and impact on prognosis. *Leukemia*, *26*(3), 559–562. https://doi.org/10.1038/leu.2011.266

Leonard, J. P., Trneny, M., Izutsu, K., Fowler, N. H., Hong, X., Zhu, J., Zhang, H., Offner, F., Scheliga, A., Nowakowski, G. S., Pinto, A., Re, F., Fogliatto, L. M., Scheinberg, P., Flinn, I. W., Moreira, C., Cabeçadas, J., Liu, D., Kalambakas, S., … AUGMENT Trial Investigators. (2019). AUGMENT: A Phase III Study of Lenalidomide Plus Rituximab Versus Placebo Plus Rituximab in Relapsed or Refractory Indolent Lymphoma. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, *37*(14), 1188–1199. https://doi.org/10.1200/JCO.19.00010

Liu X, Sato N, Shimosato Y, et al. CHIP-associated mutant ASXL1 in blood cells promotes solid tumor progression. *Cancer Sci*. 2022;113:1182–1194. doi:10.1111/cas.15294

Lossos, I. S., & Gascoyne, R. D. (2011). Transformation of follicular lymphoma. *Best Practice & Research. Clinical Haematology*, *24*(2), 147–163. https://doi.org/10.1016/j.beha.2011.02.006

Luminari, S., Bellei, M., Biasoli, I., & Federico, M. (2012). Follicular lymphoma—Treatment and prognostic factors. *Revista Brasileira De Hematologia E Hemoterapia*, *34*(1), 54–59. https://doi.org/10.5581/1516-8484.20120015

Luminari, S., Manni, M., Galimberti, S., Versari, A., Tucci, A., Boccomini, C., Farina, L., Olivieri, J., Marcheselli, L., Guerra, L., Ferrero, S., Arcaini, L., Cavallo, F., Kovalchuk, S., Skrypets, T., Del Giudice, I., Chauvie, S., Patti, C., Stelitano, C., … Fondazione Italiana Linfomi. (2022). Response-Adapted Postinduction Strategy in Patients With Advanced-Stage Follicular Lymphoma: The FOLL12 Study. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, *40*(7), 729–739. https://doi.org/10.1200/JCO.21.01234

MacManus, M., Fisher, R., Roos, D., O'Brien, P., Macann, A., Davis, S., Tsang, R., Christie, D., McClure, B., Joseph, D., Jayamohan, J., & Seymour, J. F. (2018). Randomized Trial of Systemic Therapy After Involved-Field Radiotherapy in Patients With Early-Stage Follicular Lymphoma: TROG 99.03. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, *36*(29), 2918–2925. https://doi.org/10.1200/JCO.2018.77.9892

Makita, S., Maruyama, D., Maeshima, A. M., Taniguchi, H., Yuda, S., Toyoda, K., Yamauchi, N., Fukuhara, S., Munakata, W., Kobayashi, Y., Kurihara, H., Izutsu, K., & Tobinai, K. (2020). A comparison of clinical staging using the Lugano versus Ann Arbor classifications in Japanese patients with Hodgkin lymphoma. *Asia-Pacific Journal of Clinical Oncology*, *16*(3), 108–114. https://doi.org/10.1111/ajco.13248

Marnell, C. S., Bick, A., & Natarajan, P. (2021). Clonal hematopoiesis of indeterminate potential (CHIP): Linking somatic mutations, hematopoiesis, chronic inflammation and cardiovascular disease. *Journal of Molecular and Cellular Cardiology*, *161*, 98–105. https://doi.org/10.1016/j.yjmcc.2021.07.004

Mayerhofer, E., Strecker, C., Becker, H., Georgakis, M. K., Uddin, M. M., Hoffmann, M. M., Nadarajah, N., Meggendorfer, M., Haferlach, T., Rosand, J., Natarajan, P., Anderson, C. D., Harloff, A., & Hoermann, G. (2023). Prevalence and Therapeutic Implications of Clonal Hematopoiesis of Indeterminate Potential in Young Patients With Stroke. *Stroke*, *54*(4), 938–946. https://doi.org/10.1161/STROKEAHA.122.041416

Morschhauser, F., Fowler, N. H., Feugier, P., Bouabdallah, R., Tilly, H., Palomba, M. L., Fruchart, C., Libby, E. N., Casasnovas, R.-O., Flinn, I. W., Haioun, C., Maisonneuve, H., Ysebaert, L., Bartlett, N. L., Bouabdallah, K., Brice, P., Ribrag, V., Daguindau, N., Le Gouill, S., … RELEVANCE Trial Investigators. (2018). Rituximab plus Lenalidomide in Advanced Untreated Follicular Lymphoma. *The New England Journal of Medicine*, *379*(10), 934–947. https://doi.org/10.1056/NEJMoa1805104

Morschhauser, F., Radford, J., Van Hoof, A., Botto, B., Rohatiner, A. Z. S., Salles, G., Soubeyran, P., Tilly, H., Bischof-Delaloye, A., van Putten, W. L. J., Kylstra, J. W., & Hagenbeek, A. (2013). 90Yttrium-ibritumomab tiuxetan consolidation of first remission in advanced-stage follicular non-Hodgkin lymphoma: Updated results after a median follow-up of 7.3 years from the International, Randomized, Phase III First-LineIndolent trial. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, *31*(16), 1977–1983. https://doi.org/10.1200/JCO.2012.45.6400

Mozas, P., Sorigué, M., & López-Guillermo, A. (2021). Follicular lymphoma: An update on diagnosis, prognosis, and management. *Medicina Clinica*, *157*(9), 440–448. https://doi.org/10.1016/j.medcli.2021.03.041

Nguyen YTM, Fujisawa M, Nguyen TB, et al. Tet2 deficiency in immune cells exacerbates tumor progression by increasing angiogenesis in a lung cancer model. *Cancer Sci*. 2021;112:49314943. https://doi.org/10.1111/cas.15165

Ott, G., Katzenberger, T., Lohr, A., Kindelberger, S., Rüdiger, T., Wilhelm, M., Kalla, J., Rosenwald, A., Müller, J. G., Ott, M. M., & Müller-Hermelink, H. K. (2002). Cytomorphologic, immunohistochemical, and cytogenetic profiles of follicular lymphoma: 2 types of follicular lymphoma grade 3. *Blood*, *99*(10), 3806–3812. https://doi.org/10.1182/blood.v99.10.3806

Rummel, M. J., Niederle, N., Maschmeyer, G., Banat, G. A., von Grünhagen, U., Losem, C., Kofahl-Krause, D., Heil, G., Welslau, M., Balser, C., Kaiser, U., Weidmann, E., Dürk, H., Ballo, H., Stauch, M., Roller, F., Barth, J., Hoelzer, D., Hinke, A., … Study group indolent Lymphomas (StiL). (2013). Bendamustine plus rituximab versus CHOP plus rituximab as first-line treatment for patients with indolent and mantle-cell lymphomas: An open-label, multicentre, randomised, phase 3 non-inferiority trial. *Lancet*, *381*(9873), 1203–1210. https://doi.org/10.1016/S0140-6736(12)61763-2

Schwaenen, C., Viardot, A., Berger, H., Barth, T. F. E., Bentink, S., Döhner, H., Enz, M., Feller, A. C., Hansmann, M.-L., Hummel, M., Kestler, H. A., Klapper, W., Kreuz, M., Lenze, D., Loeffler, M., Möller, P., Müller-Hermelink, H.-K., Ott, G., Rosolowski, M., … Molecular Mechanisms in Malignant Lymphomas Network Project of the Deutsche Krebshilfe. (2009). Microarray-based genomic profiling reveals novel genomic aberrations in follicular lymphoma which associate with patient survival and gene expression status. *Genes, Chromosomes & Cancer*, *48*(1), 39–54. https://doi.org/10.1002/gcc.20617

Solal-Céligny, P., Roy, P., Colombat, P., White, J., Armitage, J. O., Arranz-Saez, R., Au, W. Y., Bellei, M., Brice, P., Caballero, D., Coiffier, B., Conde-Garcia, E., Doyen, C., Federico, M., Fisher, R. I., Garcia-Conde, J. F., Guglielmi, C., Hagenbeek, A., Haïoun, C., … Montserrat, E. (2004). Follicular lymphoma international prognostic index. *Blood*, *104*(5), 1258–1265. https://doi.org/10.1182/blood-2003-12-4434

Tobin, J. W. D., Hapgood, G., Johnston, A., Cheah, C. Y., Lee, S. T., Trotman, J., Inam, S., Campbell, B. A., Norris, D., MacManus, M., Hertzberg, M., & Hawkes, E. (2024a). Diagnosis, management and follow-up of follicular lymphoma: A consensus practice statement from the Australasian Lymphoma Alliance. *Internal Medicine Journal*, *54*(8), 1384–1395. https://doi.org/10.1111/imj.16454

Townsend, W., Hiddemann, W., Buske, C., Cartron, G., Cunningham, D., Dyer, M. J. S., Gribben, J. G., Phillips, E. H., Dreyling, M., Seymour, J. F., Grigg, A., Trotman, J., Lin, T.-Y., Hong, X.-N., Kingbiel, D., Nielsen, T. G., Knapp, A., Herold, M., & Marcus, R. (2023). Obinutuzumab Versus Rituximab Immunochemotherapy in Previously Untreated iNHL: Final Results From the GALLIUM Study. *HemaSphere*, *7*(7), e919. https://doi.org/10.1097/HS9.0000000000000919

Weeks, L. D., & Ebert, B. L. (2023a). Causes and consequences of clonal hematopoiesis. *Blood*, *142*(26), 2235–2246. https://doi.org/10.1182/blood.2023022222

Welaya, K., & Casulo, C. (2019). Follicular Lymphoma: Redefining Prognosis, Current Treatment Options, and Unmet Needs. *Hematology/Oncology Clinics of North America*, *33*(4), 627–638. https://doi.org/10.1016/j.hoc.2019.03.003

Yang Feng, Qingchen Yuan, Rachel C. Newsome, Troy Robinson, Robert L. Bowman, Ashley N. Zuniga, Kendra N. Hall, Cassandra M. Bernsten, Daniil E. Shabashvili, Kathryn I. Krajcik, Chamara Gunaratne, Zachary J. Zaroogian, Kartika Venugopal, Heidi L. Casellas Roman, Ross L. Levine, Walid K. Chatila, Rona Yaeger, Alberto Riva, Christian Jobin, Daniel Kopinke, Dorina Avram, Olga A. Guryanova; Hematopoietic-specific heterozygous loss of *Dnmt3a* exacerbates colitis-associated colon cancer. *J Exp Med* 6 November 2023; 220 (11): e20230011. doi: https://doi.org/10.1084/jem.20230011

Younes, I. E., Syler, L., & Hamed, A. (2023). Review of clonal hematopoiesis, subtypes and its role in neoplasia and different morbidities. *Leukemia Research*, *130*, 107307. https://doi.org/10.1016/j.leukres.2023.107307