Analysis of *NOTCH1* mutations as predictive biomarker for transformation in patients with follicular lymphoma

A 10-year observational retrospective cohort study

Author: Anna Hernández Castillo
Tutor: Dr. Josep Maria Roncero Vidal

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It has been a pleasure to learn from the professionals of the Hematology Department of Hospital Universitari Dr. Josep Trueta and be a testimony to the affection and commitment they profess to their patients.

I would like to express my sincere gratitude to Dr. Josep Mª Roncero Vidal, for his invaluable lessons on Medicine and life.

Last but not least, I would like to thank my loved ones, for their tireless support and inspiration, that always lead me where I need to be.

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ABSTRACT

BACKGROUND

Follicular lymphoma is the most common indolent NHL in the western world. As it is an indolent lymphoma, the typical clinical practice is to only offer treatment when patients develop symptoms or when a high tumour burden is present.

The prognosis of FL is partially determined by the risk of histological transformation to an agressive lymphoma (30% transformation rate at 15-y of follow-up). Recent studies have suggested that early treatment with rituximab could reduce the risk of transformation.

The question that arises is which patients present a higher risk of transformation and consequently, which patients will benefit from early treatment of their lymphoma. At this time there are no predictive biomarkers to identify this subset of patients. Some studies have identified mutations in the *NOTCH1* gene as a risk factor for transformation of other indolent lymphomas and therefore we presume it could be a predictive biomarker of transformation of FL at diagnosis.

OBJECTIVE

To analize the relationship between the mutational status of the *NOTCH1* gene and the risk of transformation in patients with non-transformed follicular lymphoma at the time of diagnosis.

METHODS

The study is designed as a 10-year observational retrospective cohort of patients from *ICO Girona-Hospital Dr. Josep Trueta* in which 224 cryopreserved samples of non-transformed FL will be analyzed for the presence of *NOTCH1* mutations. Posteriorly, we will assess what percentage of mutated and wild-type *NOTCH1* cases suffered transformation.

KEY WORDS

Non-Hodgkin's lymphoma, follicular lymphoma, histological transformation, NOTCH1 mutation, rituximab

ABBREVIATIONS

ABC-DLBCL	Activated B Cell like Diffuse Large B Cell Lymphoma
AlloSCT	Allogenous Stem Cell Transplant
AutoSCT	Autologous Stem Cell Transplant
BCR	B-cell Receptor
CMG	Chromatin Modifying Genes
CPC	Common Precursor Cell
FDC	Follicular Dendritic Cells
FDG	Fluorodeoxyglucose
FISH	Fluorescence in situ hybridization
FL	Follicular lymphoma
FLIPI	Follicular Lymphoma International Prognostic Index
GC	Germinal Centre
GCB-DLBCL	Germinal Centre B Cell like Diffuse Large B Cell Lymphoma
HDT	High Dose Therapy
IgVH	Immunoglobulin heavy chain variable region
ICO	Institut Català d'Oncologia
IPI	International Prognostic Index
LDH	Lactate Dehidrogenase
NHL	Non-Hodgkin's lymphoma
OS	Overall survival
PCR	Polimerase Chain Reaction
PET scan	Positron Emission Tomography Scan
PFS	Progression-free Survival
R-CHOP	Rituximab, Cyclophosphamide, Doxorrubicin, Vincristine, Prednisone
RIT	Radioimmunotherapy
SHM	Somatic Hypermutation
SUV_{max}	Maximum Standarized Uptake Value
TFH	T-Follicular Helper
tFL	Transformed follicular lymphoma

1. INTRODUCTION

Follicular lymphoma (FL) is the second most common type of non-Hodgkin lymphoma (NHL). With the exception of early stage localised disease, FL is generally considered to be an indolent though incurable malignancy, characterised by a pattern of multiple relapses, decreased duration of response and progressive acquisition of resistance to various drugs throughout its natural history (1–3).

With the emergence of new therapies, especially with the introduction of immunotherapy as part of standard treatment, patients can experience long-term survival of almost 19 years (4), with slow progression of the disease. One of the main causes of morbidity and mortality is histological transformation (HT) into a clinically more aggressive lymphoma, typically diffuse large B-cell lymphoma (DLBCL).

In the setting of the rituximab-era, HT has decreased in comparison to historical cohorts, and it is estimated to appear at a frequeny of 2-3% of patients annually during the first 15 years (5,6), after which the data becomes less reliable (5).

Outcomes following transformation are worse in comparison to those patients that do not experience transformation, mainly due to refractoriness to treatment and rapid clinical progression. Median overall survival post-transformation is improving with the use of rituximab, achieving a median overall survival (OS) at 5 years of 48% (6).

Previous studies have shown that patients with worse prognostic factors (high risk FLIPI and M7-FLIPI) at diagnosis present an increased risk of HT (6–9) but these factors can't predict transformation for the vast majority of patients. For instance, in a recent retrospective study, a high FLIPI score failed to predict transformation in 53% of cases (10).

At this time, it is unclear what biological and molecular factors are implicated in the pathogenesis of HT and thus there are no predictive biomarkers that can accurately identify which patients will transform. Understanding the risk of transformation will be useful to monitor patients and evaluate novel treatment strategies that could potentially prevent transformation (11).

1.1 Epidemiology

Follicular Lymphoma is the most common indolent non-Hodgkin lymphoma (NHL) in Western Europe, accounting for 20% of all NHLs according to the World Health Organisation (WHO) classification of the lymphoid neoplasms (1). The highest incidence rates are reported in USA and western Europe.

The prevalence is of 40/100.000 with similar incidence in men and women. Most cases develop in adults over 50 years and elderly patients. It is rare disease amongst young adults and exceptional in adolescents and children. In Spain, 3,000-5,000 new cases of FL are diagnosed each year.

The incidence of tFL is not well stablished, mainly due to the different criteria for defining and classifying transformation (histological and/or clinical), the follow-up lenght of the studies and the fact that not all studies distinguish between actual transformation and *de novo* DLBCL (12).

Despite the discrepancies mentioned above, the risk of transformation has been estimated to be 2-3% per year from the time of diagnosis for at least 15 years of follow-up. However, overall rates of transformation might be decreasing since the inclusion of rituximab in most chemotherapy regimens (6,13,14).

1.2 Etiology

The exact cause of FL is unknown. Agricultural exposure to pesticides and herbicides has been associated with an increased risk of FL (1).

1.3 Clinical findings of follicular lymphoma

Most cases of disease present with asymptomatic superficial adenopathies (laterocervical, axillary and/or inguinal) in a patient otherwise asymptomatic. The adenopathies are painless and might fluctuate in size. The general status of the patient is typically preserved and only 20% of patients present B symptoms[1] or altered performance status (PS).

It is not uncommon for patients to neglect the growing adenopathies for years so at the time of diagnosis, generalized adenopathies, hepatoesplenomegaly and involvement of the bone marrow are typically detected. The involvement of other extranodal sites is less frequent than in high-grade lymphomas.

The course of the disease is extremely heterogenous. Some patients experience long periods during which the adenopathies grow and shrink and don't require any specific treatment besides

[1] B symptoms include night sweating, >10% weight-loss over 6 months and fever >38º

watchful observation. Other patients will present a disseminated, rapidly growing disease that will require intensive treatment due to large adenopathies or extranodal involvement (15).

Patients will typically present a high response rate to treatment but eventually will relapse. The pattern of multiple remissions and relapses is characteristic of this disease, with each relapse occurring at a shorter interval and progressively losing sensitivity to chemotherapy.

Transformation of FL is considered a common event in the natural history of the disease. This event should be clinically suspected when acute deterioration of the patient is observed. Common symptoms include rapid lymphadenopathy growth, involvement of novel extranodal sites, new B symptoms, a rapid increase in LDH 2 to 3 times normal values and hypercalcemia (6,12,16).

It should be taken into account that these symptoms can also be related to progression without the existence of transformation and that not all cases of transformation will necessarily present with them. For this reason, performing a biopsy is recommended in all cases where transformation is suspected.

1.4 Diagnosis, staging and prognosis factors

General clinical history and physical examination, particularly to check for superficial adenopathies, liver and spleen enlargement should be performed. A blood analysis with complete blood count, routine blood chemistry, including liver and renal function, LDH, uric acid, immunoglobulin and β2-microglobulin levels are recommended, as well as a thoracoabdominal CT scan and a blood marrow biopsy (17).

Diagnosis should be based preferably on an excisional biopsy of a lymph node although a core-needle biopsy can be considered when an excisional biopsy is not possible (e.g retroperitoneal adenopathies). A fine-needle aspirate is insufficient for initial diagnosis.

Pathology (18)

The accumulation of malignant cells enlarges the lymph nodes forming a nodular (follicular) growth pattern recalling the normal follicles. The morphology of FL is described as an effacement of normal lymph node architecture by neoplastic follicles that show attenuated mantle zones and abscence of tingible body macrophages that are usually present in non-neoplastic follicles (18). Although the growth pattern is typically nodular, a diffuse pattern can also be detected.

Outside of lymph nodes, involvement of spleen, peripheral blood and extranodal sites (gastrointestinal tract, liver, testicle) can be seen. The involvement of the bone marrow is very common (70-80% of cases), with the cells forming paratrabecular lymphoid aggregates.

FL cells typically demostrate coexpression of B cell markers such as CD19, CD20 and CD79a, germinal centre markers like CD10 and Bcl-6 and are usually positive for Bcl-2. They are negative for CD43, CD5 and CD23. The characteristic t(14;18) can be detected by fluorescence in situ hybridization (FISH).

The neoplastic cells consist in small cleaved-nucleus cells (centrocytes) and large cells with an oval nucleus and several nuclear membrane-bound nucleoli (centroblasts). The grade of FL is defined by the proportion of centroblasts per high power field (HPF):

- Grade 1: 0-5 centroblasts per HPF
- Grade 2: 6-15 centroblasts per HPF
- Grade 3: >15 centroblasts per HPF
    - Grade 3A: Centrocytes still recognisable
    - Grade 3B: Only sheets of centroblasts present

The ki-67 index in FL generally correlates with histological grade; most grade 1-2 cases have a proliferation index <20% and most grade 3 cases of >20%.

Staging

Standard staging examinations include positron emission tomography (PET)-computed tomography (CT) and bone marrow examination. PET-CT is specially useful to confirm localised disease when considering radiation therapy and to choose the best place to biopsy if HT is suspected.

The Ann Arbor classification *(See Annex I Table 1)* is used for the staging of NHL. It is based on the number of lymph node stations involved, their position with respect to the diaphragm, involvement of extra-lymphatic tissues and presence of B symptoms.

Prognosis

The OS of patients with FL has drastically improved in the last decades (16). A retrospective study by the University of Stanford (4) analyzing the different outcomes of patients with FL during 4 decades showed a median OS of 11 years in the pre-anthracycline era (1960-1975) in comparison with the 18,4 year median OS reported from the more recent data.

To predict FL prognosis, the standard FL International Prognosis Index (FLIPI) and its modified version, FLIPI2[2], are widely used *(See Annex I Tables 2 and 3).*

[2] FLIPI-2 has been designed in a prospective study of patients treated with rituximab

Each risk factor conferes 1 point. A FLIPI score of 0-1 is considered “low risk” and has a 10 year OS of 70,7%. A score of 2 is considered “intermediate risk” with a 10 year OS of 50,9%. Finally, a score of 3 or more is considered “high risk” with a 10 year OS of 35,5% (17).

Recently, a clinicogenetic risk model named m7-FLIPI integrates the mutational status of seven genes (*EZH2, ARID1A, MEF2B, EP300, FOXO1, CREBBP* and *CARD11*), the FL Prognostic Index and the Eastern Cooperative Ongology Group (ECOG) performance status *(See Annex I Table 4).*

This model improves risk stratification for failure-free survival and overall survival in patients with FL recieving first line of immunochemotherapy (R-CHOP or R-CVP). In the validation cohort this index outperformed FLIPI alone and FLIPI+ECOG PS. It has a positive predictive value of 72% and a negative predictive value of 68% at 5 years for failure-free survival. (19)

However, in a recent study, the m7-FLIPI appeared to be dependent on the chosen therapeutic regimen (20). It is currrently not used in clinical practice

1.5 Therapeutic strategies in follicular lymphoma (16,17,21)

First-line treatment of FL will depend on its histological grade, extension of the disease, tumor burden and patients symptoms.

There is no evidence that supports different treatment strategies between grade 1 and 2 FL but different genetic characteristics and clinical behavior suggest that FL grade 3B is a more aggressive disease and should be treated like a DLBCL (22).

Stage I-II disease

Only a 15-25% of patients present with localised stage at diagnosis (1). These patients can benefit of localised treatment based on Involved Field Radiotherapy (24-36 Gy) which results in prolonged remission rates and even curation in some cases. The role of systemic treatment, including immunochemotherapy or chemotherapy, isn’t well established in this context.

First-line treatment in stage III-IV disease

The majority of patients are diagnosed with an advanced stage. In this setting, the first question that arises is if asymptomatic patients without a high tumor burden should be administered treatment immediately or otherwise wait until the eventual progression.

Randomised trials demonstrate that the “watch and wait” strategy allows for chemotherapy to be delayed for years without any survival disadvantage (23). Consequently, in the abscence of risk factors, postponing treatment until progression is a valid option.

The most used criteria to define patients of low tumour burden are those of the GELF (Groupe d’Etudie des Lymphomes Folliculaires) *(See Annex II Table 5)* (24).

In asymptomatic patients for whom W+W is not acceptable, monotherapy with rituximab results in a high response rate and duration, and does not impair quality life.

Symptomatic patients and those with high tumour burden should be treated with systemic therapy. The choice of treatment varies from single agent to intensive chemoimmunotherapy.

Many single agents are active against FL: low-dose RT, radioimmunotherapy (RIT), chlorambucil, rituximab, fludarabine, bendamustine and others. All these agents present similar activity but vary in their toxicity profile. More intensive treatment with combination schemes obtain higher response rates and longer progression-free survival (PFS) but cause more side-effects and don’t improve survival (25).

The addition of rituximab, a monoclonal anti-CD20 agent, to different chemotherapy regimens was shown to improve PFS (26–28) and, in a meta-analysis, also overall survival (OS), becoming therefore the standard treatment (29).

The best chemotherapy to partner with rituximab remains unclear. R-CHOP (Rituximab, Cyclophosphamide, Doxorrubicin, Vincristine and Prednisone) is a very active regimen in FL but it doesn’t improve OS and it has a relatively toxic profile. Other combinations such as R-CVP (Cyclophosphamide, Vincristine, Prednisone) or R-fludarabine were not superior to R-CHOP and a randomised comparison between the three showed that R-CHOP had the best risk-benefit profile (30).

Bendamustine is an increasingly used drug due to its favorable therapeutic index (high response rate and little toxicity). In grade 1-2 FL, R-Bendamustine is as active as R-CHOP but induces less side effects (31).

In patients responding to R-chemo, maintenance treatment with rituximab given every 2 months during 2 years showed an increase of PFS, but not in OS, in the PRIMA trial (32).

In conclusion, as first-line in patients that need treatment, a combination of chemotherapy with rituximab followed by 2 years of rituximab maintenance therapy is the best option. For low risk or unfit patients, the use of single agents, R-monotherapy, RIT or “lighter” chemo regimens such as R-chlorambucil or R-cyclophosphamide are good choices to consider.

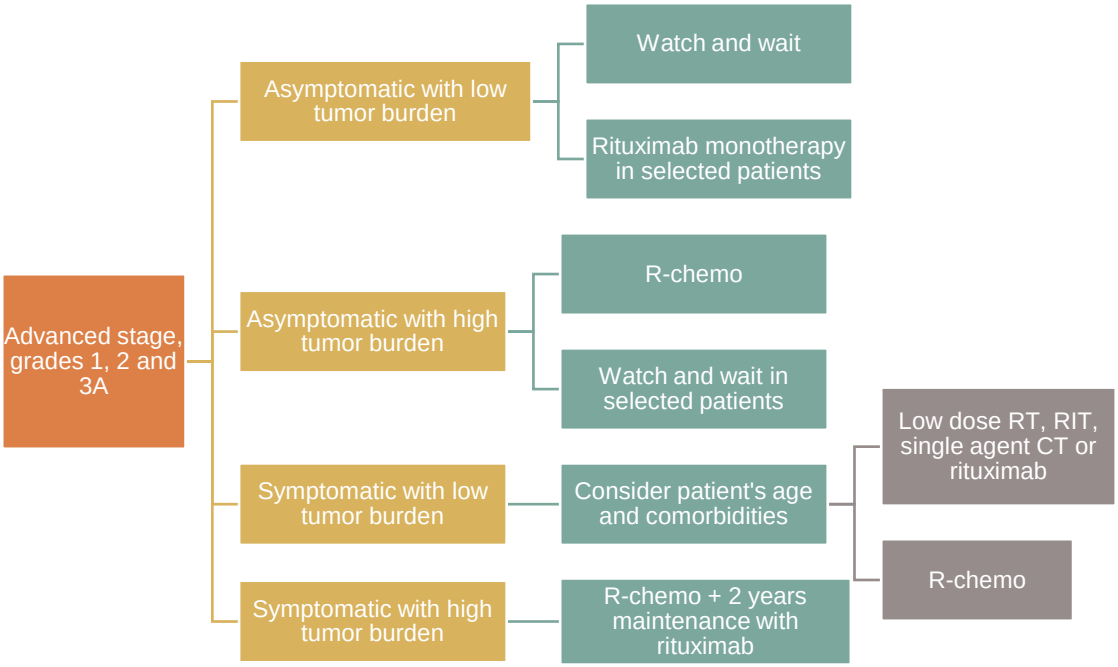


Figure 1. Treatment of advanced stage, low-grade FL

Options at relapse/progression

Before any treatment decision a new biopsy should be performed to rule out HT into aggressive lymphoma, specially if there are indirect signs such as elevated LDH or rapidly growing tumours. Specimens should be tested for MYC translocations with FISH to rule out transformation into high-grade double hit lymphoma.

There isn't a universal standard treatment at relapse, it depends om the efficacy of the previous treatment, duration of time to relapse, fitness of the patient and goals of therapy.

The different options of treatment vary between observation, chemoimmunotherapy, radioimmunotherapy or local RT palliation.

It is possible to re-treat with the same chemotherapy regimen that was effective in the first-line, specially after a long remission. However, a different regimen without cross-resistance (e.g Bendamustine after CHOP) is preferable. The addition of rituximab should be considered if patients were able to achieve a durable remission.

Alternatively a single agent, being radioimmunotherapy, rituximab or chemotherapy, can be sufficient. The choice depends on response duration and prior treatment.

For instance, radioimmunotherapy with ^{90}Y-ibritumomab tiuxetan is an option for patients with nonbulky, indolent B-cell NHL if the bone marrow is minimally involved. Single agent rituximab is

a good option for elderly or fragile patients who will not tolerate cytotoxic agents and are still sensitive to the drug.

New anti-CD20 monoclonal antibodies such as obinutuzumab and ofatumumab could be used if refractoriness to rituximab in combination with bendamustine.

A new option for relapsed FL is the phosphatidylinositol 3-kinase (PI3K) inhibitor idelalisib, that has shown a 57% response rate (RR) in heavily pre-treated FL patients.

If remission lenght is brief (<2 years) high-dose chemotherapy followed by autologous stem cell transplant (AutoSCT) can further prolong remissions and possibly survival. Allogenic stem cell transplant (AlloSCT) can be curative but should only be proposed to relapsed fit and motivated patients, due to the high incidence of severe side effects and mortality.

If patients relapse with few sites of disease and an indolent course, low-dose RT (2 x 2Gy) can obtain a palliative effect.

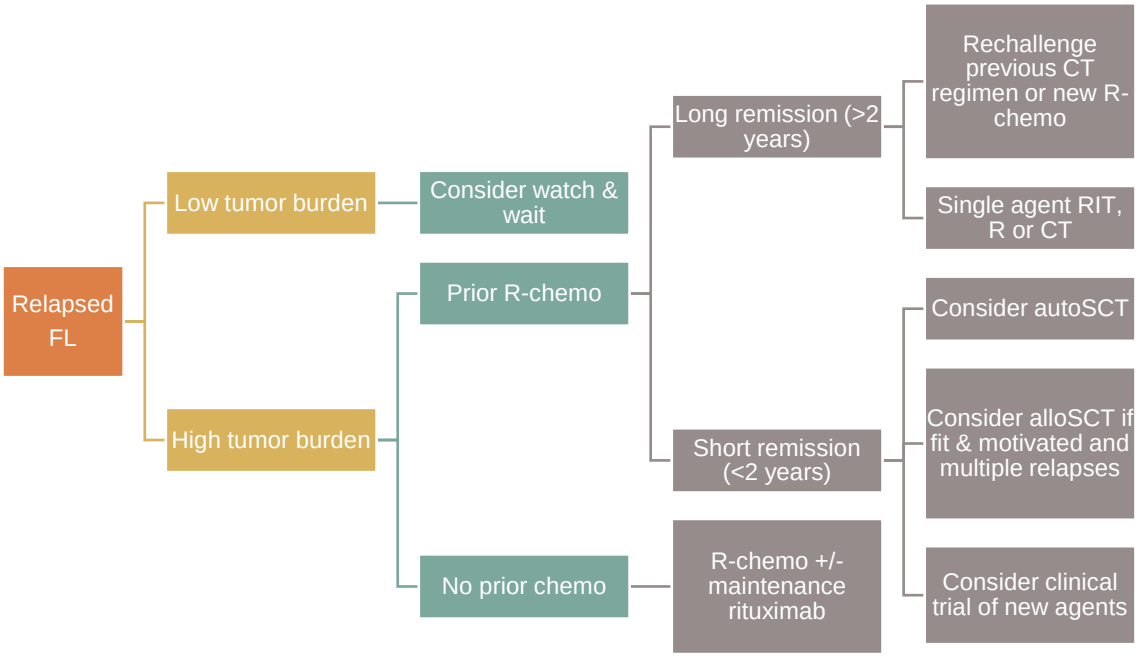


Figure 2. Treatment options for relapsed FL

Definition of transformation

The gold standard definition of transformation is the histologically confirmed progression of a low-grade FL (grades 1, 2 and 3A) to a high-grade lymphoma, by the documentation of an increased number of large cells and disruption of the follicular structure (33)(7,12).

Identifying the best location for biopsy isn't always simple, as HT may occur only in a subset of affected nodal or extranodal sites and biopsying the most accessible adenopathy could fail to identify transformation (21).

Ideally, a PET-CT scan should be performed prior to biopsy for assessment of higher standarized uptake value (SUV_{max}) areas, as research shows the majority of transformed lymphomas present high SUV_{max}, similar to DLBCL. Biopsies should be directed to the sites with most FDG avidity (34).

HT to a high-grade lymphoma is most frequently to DLBCL (80%) and less commonly Burkitt lymphoma or with features intermediate of DLBCL and Burkitt lymphoma. Transformation typically involves additional genetic abnormalities, specially *MYC* translocations. The combination of BCL2 and *MYC* rearrangement is associated with a particularly agressive course and should be diagnosed as high-grade B-cell lymphoma with double hit (1).

Treatment at transformation (21)

There are still great differences between the outcome of patients with non-transformed follicular lymphoma and tFL with the new therapy regimens but the median post-transformation survival has increased up to 5 years (6,13).

The treatment of HT has to be individualized, as there are no randomized studies to guide practice. The decision depends essentially on the clinical scenario and the treatment of the prior indolent lymphoma.

There are several clinical scenarios:

a) Patients presenting with indolent and transformed lymphoma simultaneously
b) Patients with known FL that are treatment naïve before transformation. It would be the case of “watch and wait” strategy
c) Patients with FL that have been treated previously and have undergone histological transformation

Patients who are treatment naïve have a better chance of survival, as well as those who have recieved anthracycline-free CIT for the indolent disease. With the increasing use of bendamustine-based regimens for initial treatment of FL, more patients will be anthracycline naïve at the time of transformation but will have recieved another form of CIT. A question that arises is if R-CHOP will still be a good option at this point and if stem cell transplant would improve progression and/or OS.

Another aspect that has changed since the introduction of rituximab is the necessity of consolidative high-dose chemotherapy followed by autoSCT. In the groups where patients have a simultaneous diagnosis of indolent and agressive disease, as well as those who are treatment naïve, studies showed no difference in OS between being only treated with R-CHOP or R-CHOP followed by autoSCT (6). Thus the recommendation is to not offer autoSCT for patients who haven’t been treated for the indolent lymphoma.

There is a lack of evidence regarding the need of consolidation with autoSCT following R-CHOP in patients who have been previously treated with anthracycline-free regimens. For this reason a discussion regarding its pros and cons at each individual case is encouraged.

In patients who have transformed after treatment for indolent disease, salvage chemotherapy plus autoSCT, similar strategy to relapsed DLBCL, is a good option.

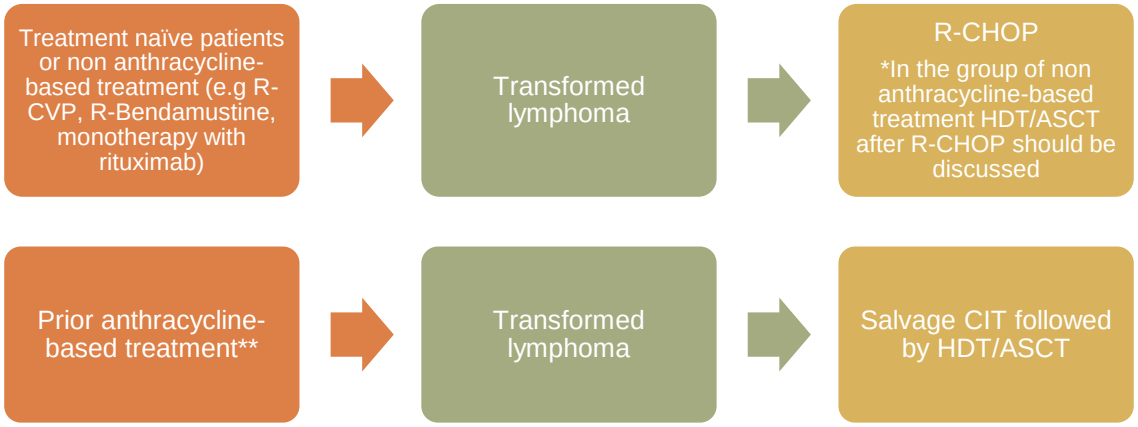


Figure 3. Treatment strategies on tFL depending of previous therapy regimens & lymphoma biology

1.6 Biology and Pathology

FL is composed of the malignant counterparts of normal germinal centre B cells (GCB) that reside within the follicles. These cells typically express B-cell surface antigens such as CD20, follicle centre B-cell markers like CD10 and Bcl-6 and, in contrast to normal GCB cells, they also express cytoplasmic Bcl-2 protein. Overexpression of the Bcl-2 protein is the result of the translocation t(14;18)(q32;q21), which brings the BCL2 gene on chromosome 18 near the Ig heavy chain gene on chromosome 14, which acts as a promoter.

Lymphomagenesis

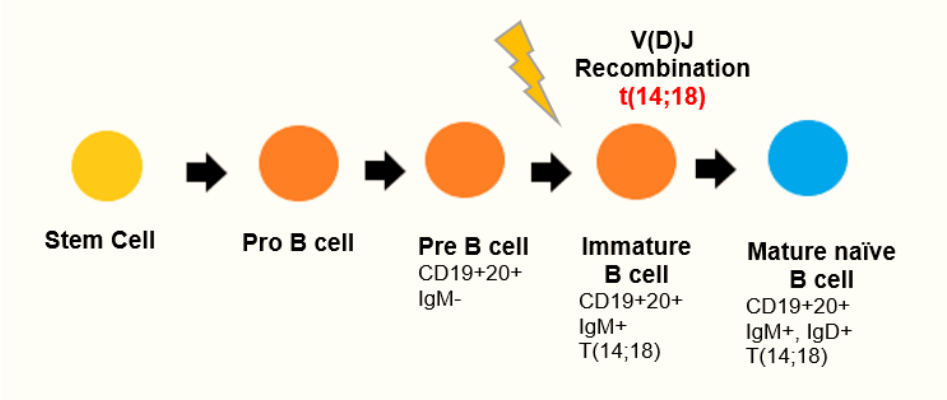


Figure 4. B cell development. Naive B cells in the bone marrow (BM) acquire the t(14;18) translocation due an error in V(D)J recombination.

During early B-cell devolpment in the bone marrow, progenitor B cells undergo V(D)J recombination processes to assemble the immunoglobulin heavy and light chain genes that encode the variable parts of antibody molecules.This process of recombination involves DNA double-strand breaks at specific sequences which are located at the ends of rearranging V, D and J genes. This proces continues in the dark zone of the germinal centre of the lymph node, where antigen-activated cells undergo a process of clonal expansion and somatic hypermutation to modify their variable (IgVH) genes. This allows for even more variability of antigen affinity.

Most cells acquire disadvantageous mutations and are destined to die by apoptosis whereas the fewer affinity-increasing mutations are positively selected by the T follicular helper (TFH) cells to become either plasma or memory cells.

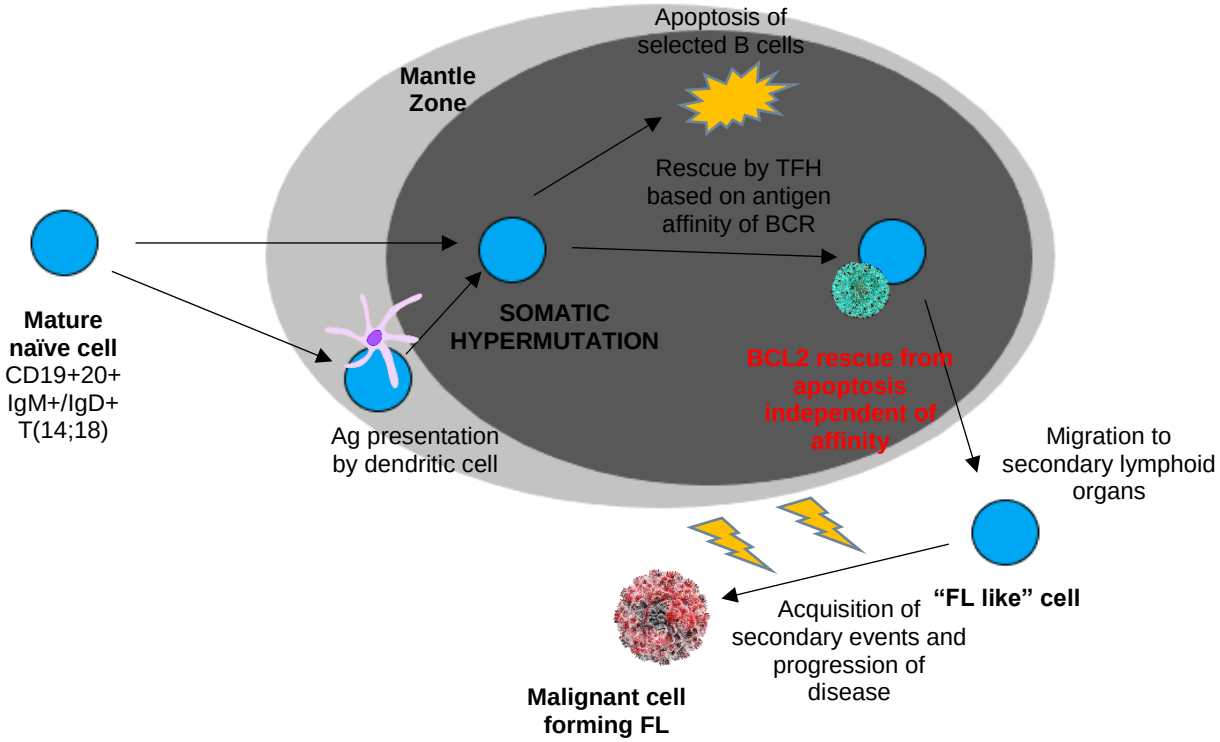


Figure 5. Model of FL pathogenesis. Naïve cells migrate from the bone marrow to lymph nodes where they undergo the GC reaction. In the dark zone of GC, B cells proliferate as centroblasts and undergo somatic hypermutation (SHM) and class switching of their BCRs. Centroblasts then become centrocytes and migrate to the light zone of the GC where they interact with follicular dendritic cells (FDCs) and are selected to either undergo apoptosis or be rescued by follicular helper T cells (TFH) based on Ag affinity of their BCRs. Overexpression of BCL2 provides cells with t(14;18) a way to avoid apoptosis, independent of BCR affinity. These FL-like B cells then enter the bloodstream where they traffic between follicles and/or the BM and acquire additional genetic alterations necessary for the development of FL.

Adapted from Brad S. Kahl, David T. Yang, Follicular Lymphoma: evolving therapeutic strategies. *Blood* (2016) 127 (17): 2055-2063.

In some cases, accidents happen during this attempt of diversification of the immunoglobin (Ig) genes and when the ends of a rearranging gene in one of the Ig loci are mistakenly joined to DNA breaks in another chromosome, a reciprocal translocation occurs. Such mechanism is likely to be the cause of the t(14;18) translocation (35).

Afterwards, naive B cells carrying this translocation exit the bone marrow and colonize secondary lymphoid tissue, where they are driven into a T-cell-dependent immune response and become a GCB cell.

As the overexpressed Bcl-2 protein exerts anti-apoptotic functions, the mutated cells lose their programmed death capacities, start to proliferate due to their survival advantage and acquire new genetic alterations that lead to the development of the lymphoma.

Although the t(14;18) aberration is considered to be the molecular hallmark of the disease, it is neither necessary or sufficient for its development, and other genetic alterations are required for the acquisition of FL.

The t(14;18) translocation has also been identified at low frequencies in the periperal blood of healthy people who will never develop a FL and furthermore, it is absent in 15% of FLs and present in about 20-30% of GCB-type DLBLCs (41,42,43). All of this evidence strongly argues to the fact that this genetic abnormality itself is not sufficient for malignant transformation, and that other genetic alterations are required for the development of FL.

Follicular lymphoma has become a prototype of cancer that depends not only on genomic alterations but also on the epigenetic control of gene expression. Mutations in chromatin-modifying genes (CMGs) such as histone methyltransferases or histone linker proteins occur in high frequency. Evidence suggests that these mutations may act synergically rather than independently to promote cancerogenesis (37).

For instance, histone lysine methyltransferase *KMT2D* (also known as *MLL2*) inactivating mutations are observed in 60-70% of FL cases. Its loss results in decreased *H3K4* methylation levels and down-regulation of key genes involved in B cell differentiation and immune signaling (38,39).

Similarly, histone acetyltransferases *CREBBP/EP300* act as transcriptional co-activators in multiple pathways. About 40% of FLs present mutations that inactivate the histone acetyltransferase (HAD) domain of these genes. Their loss of function causes acetylation-mediated activation of the Bcl-6 oncogen and inactivation of *TP53* tumor-supressor (40,41).

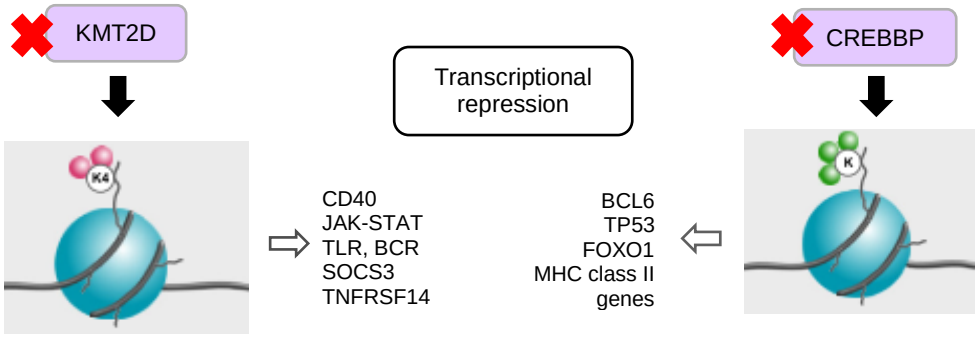


Figure 6. Alterations in epigenetic modifiers. Loss of function of histone methyltransferase KMT2D acts through decreased methylation of the enhancer H3K4 which in turn causes transcriptional supression of various genes. Inactivation of histone acetyltransferase CREBBP/EP300 causes altered acetylation of Bcl-6 and TP53, promoting lymphomagenesis. Adapted from Lackraj, T. Pathogensis of follicular lymphoma. *Best Practice and Research*. 2018. p. 2–14

All in all, the described genetic and epigenetic lesions contribute to a “freezing” of the FL cells at a germinal centre stage and prevent their further differentiation into plasma or memory cells (35).

Pathways of transformation (33,42–44)

Although the term “transformation” entails a switch from indolent to aggressive disease, the reality is that there is not a single moment in time where the so-called transformation occurs, but rather multiple changes in the biology of the disease accumulate during the life of any patient with this lymphoma.

Because of the evolutionary dynamics of transformation, there is not a single aberration sufficient to drive transformation on its own. Be that as it may, the recurrent alteration of genes related with the control of cell cycle progression and DNA damage responses suggest that a loss of genetic stability and deregulated proliferation are essential steps in the transformation of FL.

Studies of clonal evolution of FL and tFL have pointed at the existence of a common population of progenitors that give rise to all FL subclones, including those involved in transformation. This theory postulates that tFL derives from the divergent evolution of a primary common precursor cell (CPC) that acquires distinct mutations to become a FL or a transformed FL (42).

The most affected genes in both FL and tFL are those encoding for histone modification enzymes. Different epigenetic regulatory genes such as CREBBP, KMT2D (or MLL2) and EZH2 are affected, leading to the loss of normal B-cell phenotype and increased proliferation (42).

Whereas mutations in epigenetic modifiers and anti-apoptotic genes are introduced early in the common precursor population, transformation is specifically associated with alterations in classic tumor supression pathways such as TP53 and cell-cycle regulator CDKN2A/B, as well as

translocations and amplifications of oncogenes like MYC. One possible mechanism of acquisition of such alterations could be an aberrant somatic hypermutation (43).

It has to be taken into consideration that despite the great advances in ellucidating the pathways involved in transformation, these events alone can't explain the majority of transformed cases, leaving room for new discoveries of the genetics of transformation.

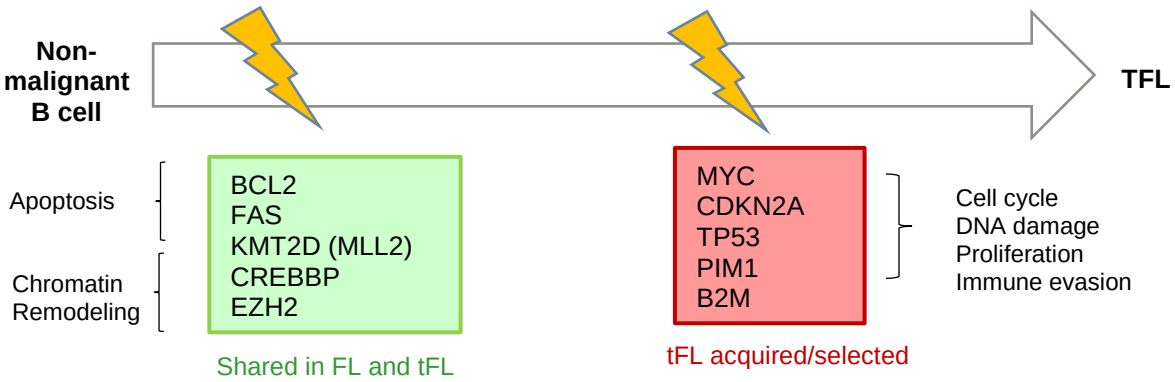


Figure 7. Reccurrently Mutated Genes in tFL. Adapted from Pasqualucci, L. Genetics of Follicular Lymphoma Transformation. *Cell Reports*. 2014;6(1):130–40.

1.6.1 Can we predict transformation?

As mentioned previously, the importance of histological transformation into a behaviourly more agressive lymphoma relies on the fact that it's the event that causes a greater downfall in patient's prognosis, including a decrease in life expectancy and survival rates.

To date, predicting the risk of subsequent transformation at the time of diagnosis remains a challenge. There are different clinical and biological features that have been studied in an attempt to find predictive markers of transformation but so far none of the reported factors can predict transformation with enough sensitivity and specificity to imply a change in patient's management.

Here, we review the reported factors associated to HT to reinforce the necessity of finding more discriminative biomarkers.

Clinical factors

Various clinical factors have been identified to be associated with a higher risk of HT over the years (*See Table 1).* Evidence regarding which of them could help predict transformation is not consistent, nonetheless, due to their simplicity, they remain to be the only routinely available method for identifying at risk patients.

For instance, in the follow-up of patients of the PRIMA trial, an ECOG performance status of ≥2 and low hemoglobin levels were found to be independent predictors of transformation in a multivariate analysis. The hazard ratio (HR) was of 5.6 (95% CI, 1.7 to 17.7) for ECOG PS and HR of 3.7 (95% CI, 1.4 to 9.7) for anemia (7). Still, ECOG PS of ≥2 and anemia <12g/dl failed to identify 85% and 60% of the transformed cases respectively.

Likewise, none of the other reported clinical factors (high FLIPI score, elevated LDH, high histological grade, more than 1 extranodal site affected, etc) could identify the majority of patients at risk of transformation, even if statistically significant results were present.

Biological factors

There are several gene alterations that have been recurrently identified in tFL samples. This alterations are *CDKN2A/B* deletions and/or loss of heterozygosity, translocations, gains, amplifications or mutations involving *MYC* and mutations, deletions and loss of heterozygosity affecting *TP53* (42).

Other genes (*SOCS1, GNA13, B2M, POU2AF1*) have been found to be more frequent in tFL samples than pre-tFL matched samples but the differences were not statistically significant (45).

It is not clear that this reported biological markers can actually predict transformation as the majority of them are not present in the initial FL clone, and thus are not useful to be determined in diagnostic biopsies. To date, none of the above-mentioned biomarkers have been validated and therefore are still not in use in clinical practice.

Table 1. Transformation rates and clinical risk factors reported in literature

	Rituximab era	Criteria for transformation	Median follow-up	Median time to HT	Clinical risk factors[1]	5-year risk	10-year risk	Median OS after HT
Bastion et a. (1997) (46) N: 220 tFL (n): 52	No	HT + clinical	9 years	NR	β-2 microglobulin	22%	31%	7 months
Giné et al. (2006) (9) N: 276 tFL (n): 30	No	HT + clinical	6,5 years	3,5 (0,6-20)	High risk FLIPI Histological grade 3A	NR	15%	1,2 years
Montoto et al. (2007) (8) N: 325 tFL (n): 88	No	H	15 years	3 (0,1-16,2)	Advanced stage High risk FLIPI and IPI	17%	28%	1,2 years
Al-Tourah et al. (2007) (5) N: 600 t(FL (n): 170	No	HT (63%) + clinical	9 years	3,3 (0,2-16)	Advanced stage	15%	30%	1,7 years
Conconi et al. (2012) (47) N: 281 tFL (n): 37	No	HT	10 years	2,75 (1,3-5)	Age <65 years Diagnosis before 1990	NR	15%	2,7 years
Link et al. (2013) (6) N: 631 t(FL (n): 60	Yes	HT (85%) + clinical	5 years	NR	Increased LDH	10,7%	NR	50 months OS at 5y 48%
Wagner-Johnson et al. (2014) (13) N: 2,652 tFL (n): 379	Yes	HT (39%) + clinical	6,8 years	NR	ECOG PS >1 More than 1 extranodal site Increased LDH B symptoms	12,8%	NR	4,9 years OS at 5y 75%
Alonso et al. (2014) (10) N: 1,734 tFL (n): 106	Yes	HT	6,2 years	2,5	High FLIPI score Non-response to first-line therapy	5%	8%	OS at 5y 26%
Federico et al. (2017) (48) N: 7,405 tFL (n): 439	Yes	HT	7,3 years	1,6 (0,2-9,2)	High FLIPI score	5,8%	7,7%	OS at 5y 43% and 10y 32%

tFL, transformed follicular lymphoma; HT, histologically confirmed; NR, not registered; OS, overall survival
[1]*Only statistically significant values measured by multivariate analysis are shown*

1.6.2 The paper of the NOTCH family in B cell malignancies (49,50)

The *NOTCH* signalling pathway activates different cellular processes including cell proliferation, differentiation and death. Considering its multiple roles in a great variety of organs and tissues, aberrations resulting in the loss or gain of function of *NOTCH* signalling components have been associated with the development of solid cancers and hematological malignancies.

Mutations specifically in *NOTCH1* and *NOTCH2* have been linked to various lymphoproliferative disorders of the B cell, namely chronic lymphocitic leukemia (CLL), mantle cell (MCL), splenic marginal zone (SMZL), diffuse large B cell (DLBCL), Hodgkin's (HL), Burkitt's (BL) and follicular lymphoma (FL).

NOTCH pathway components and mechanisms of signaling (50)

There are four NOTCH receptors (*NOTCH1-4*), each encoded by a different gene, that interact with five ligands. Out of the four receptors, *NOTCH1* and *NOTCH2* are the most widely expressed receptors, being present in many tissues both in the developmental stage and in adults.

NOTCH receptors are single-pass transmembrane proteins composed of extracellular (EC), transmembrane and intracellular domains (NICD). The IC portion of the receptor consists of a protein-binding RBPJk-associated molecule (RAM) and a domain that regulates protein stability and degradation, as it contains the substrate site that is recognized by E3 ubiquitin ligases (PEST domain).

Ligand binding of a neighboring cell to the EC domain leads to a conformational change of the receptor that ultimately triggers the proteolytic cleavage of the intracellular domain by a γ-secretase, releasing it from the membrane and allowing its translocation into the nucleus.

Once in the nucleus, the NICD forms a a transcriptional complex. It mediates the displacement of co-repressor molecules that are bound to a DNA-binding factor known as RBPJk and directly interacts with RBJk recruiting co-activators, finally inducing gene transcription (*See Figure 8)*. One of the genes under its control is *MYC*, which is an important mediator of the NOTCH effects in the transformation process.

The activity of NOTCH signaling is then shut down by the phosphorilation and ubiquitinylation of the PEST domain, driving its degradation by the proteasome.

The most common mutation detected in follicular lymphoma samples (p.P2514fs*4) produces a truncation in the PEST domain that results in the elimination of the degradation signals of NOTCH proteins. This leads to a decresed physiological turnover of the protein and consequently a persistently active NICD and high expression levels of *NOTCH1* target genes.

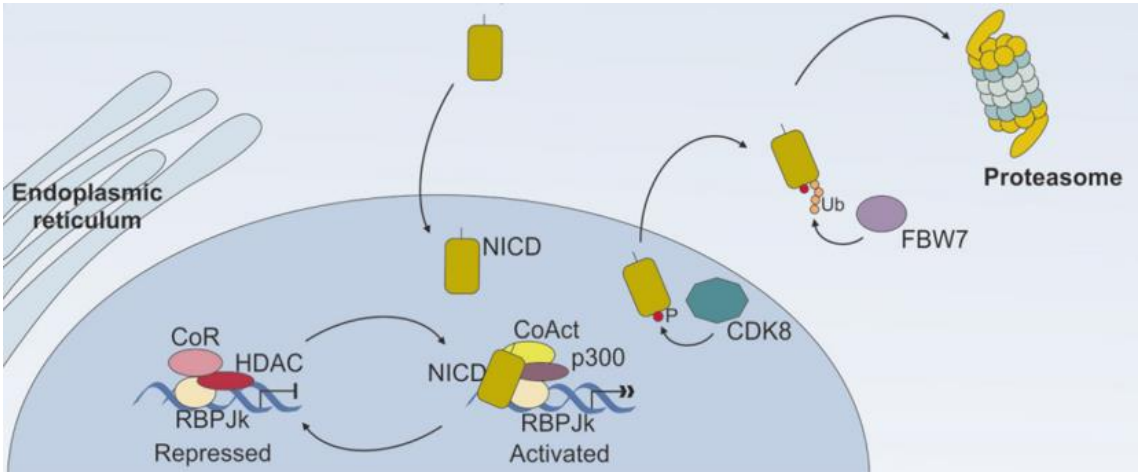


Figure 8. NOTCH signalling pathway. NICD translocates to the nucleus where it mediates the displacemet of co-repressors (CoR) and histone DeAcetylase Complex (HDAC) and interacts with RBJk recruiting co-activators (CoAct). These modifications shift the conformation of the transcriptional complex from a repressor to an activator of gene transcription. The signalling pathway is then terminated by the phosphorylation and ubiquitination of the PEST domain and subsequently degradated via proteosome. Adapted from Arruga F. The NOTCH pathway and its mutations in mature B cell malignancies. *Frontiers in Oncology*. 2018; 8:550.

1.6.3 Relationship between *NOTCH1* and transformation

A study of a small group of patients (49) analyzed the mutational status of *NOTCH1* in follicular lymphoma samples. Despite the small number of patients analyzed, there were relevant differences in the risk of transformation between *NOTCH1* mutated and *NOTCH1* wild-type FL samples (risk of transformation of 71% vs 23% respectively, p = 0.02). Unfortunately, the number of cases analyzed was very small and the follow-up time was short.

Although the detected frequency of *NOTCH1* mutations in this series was 6,3%, it identified a subset of cases with distinctive clinipathological characteristics such as lower frequency of t(14;18), higher incidence of splenic involvement, female predominance and association to transformation to DLBCL.

Additionally, the mutational analysis of *NOTCH1* in paired pre and post-transformation samples showed no differences, suggesting that the *NOTCH1* mutation may already be present in the initial FL component and may facilitate progression to DLBCL.

Further evidence pointing to the *NOTCH1* gene as a factor involved in the biology of transformation is the fact that mutations of this gene have been found in other indolent lymphomas acquiring an agressive biology. The paradigm of this matter is CLL's transformation to DLBCL, an event known as Richter syndrome.

Genetic lesions of DLBCL-type Richter syndrome recurrently target the *TP53, NOTCH1, MYC* and *CDKN2A* genes, indicating that lesions affecting regulators of apoptosis and proliferation are

shared among other transformed lymphomas. For this reason, we presume that *NOTCH1* mutations could play a role in driving transformation from indolent FL to DLBCL (51).

Furthermore, in a study characterising the molecular features of DLBCL (52), mutations in the *NOTCH* pathway, as well as *TP53/CDKN2A* genes, conferred a poorer prognosis independently of IPI score and cell of origin (GCB like DLBCL and ABC like DLBCL) adding up to the evidence that mutations in the *NOTCH* pathway are related to tumor agressiveness. Other papers (53,54) investigating the relation between *NOTCH1* or *NOTCH2* mutations and other mature B cell lymphomas (CLL and MCL) have also found that the mutated cases usually have a more agressive clinical behaviour in comparison to the wild-type cases.

Despite the evidence gathered so far, further analyses are needed to stablish the functional role of *NOTCH* mutation in FL lymphomagenesis and fine-tuning its potential role as a therapeutic target.

1.6.4 Can we prevent transformation?

Given the generally poor outcome of patients with tFL and the lack of response to therapy after transformation, prevention of the transformation itself appears to be an appealing strategy.

The first question to answer is wether the treatment of FL at diagnosis could prevent transformation or, at least, reduce its incidence.

There are different studies that have attempted to answer this question (6,13,32,47,48). Evidence argues strongly to the fact that treatment wih rituximab reduces rates of tFL over time (*See Table 2)*. This is true both for initially untreated patients who recieve rituximab versus observation and also for patients receiving rituximab maintenance after enduring immunochemotherapy treatment.

Table 2. Effect of immunotherapy in transformation risk

	Treatment	Effect of treatment
Link et al. (2013) (6) N: 631 t(FL (n): 60	Observation: 33% RTX mono: 12% Chemo (+/-RTX): 42%	Observation vs RTX: Increased risk **RTX-mono: Reduced risk** (14.4% vs 3.2% risk of HT at 5y)
Wagner-Johnson et al. (2014) (13) N: 2,652 tFL (n): 379	Observation: 21% RTX mono: 13% R-chemo: 48% Other: 19%	Observation vs RTX: Increased risk Anthracycline vs no athracycline chemo: No differences **RTX-mono: Reduced risk** (HR 0.58) **RTX (M) vs no RTX (M): Reduced risk** (HR 0.67)
Federico et al. (2017) (48) N: 7,405 tFL (n): 439	4468 pt recieved RTX	Observation vs RTX: Increased risk **RTX (I) vs RTX (I+M): Reduced risk** (5.9% vs 3.6% risk of HT at 10y)

RTX, rituximab; Mono, monotherapy; I, induction; M, maintenance; tFL, transformed follicular lymphoma; HT, histological transformation

The fact that immunotherapy reduces transformation rates and improves its outcomes highlights the importance of finding predictive biomarkers that allow us to identify which patients present a higher risk of transformation, as those will be the patients who will benefit the most from early treatment to prevent transformation.

Evidence suggests that at least in some cases, genetic alterations in the transformation of FL are early events that already exist at the time of clinical FL diagnosis (42,55), albeit at minuscule proportions.

This finding opens the door to the possibility of identifying very early pre-clinical transformed events (perhaps through techniques such as circulating tumour DNA (55) and targeting the transformed subclone through precision therapy, provided that the biology of such subclone is understood well enough.

With the current clinical and histological definitions of HT along with the diagnostic methods, it is clear that we have insufficient tools for adequately identifying at risk of transformation patients. For this reason, clinical practice at this time is limited to nonspecifically supressing the FL cell pool to minimize the odds of a cell acquiring the necessary transformed biology, instead of targeting specific transforming clones.

2. JUSTIFICATION

Follicular lymphoma is the second most common NHL in the Western world, accounting for 20% of all lymphomas (1). It is generally an indolent though incurable disease, with a pattern of relapses and increasingly long survival rates in the immunotherapy era. However, a minority of patients suffer histological transformation into an agressive lymphoma, a compelling event that overshadows clinical course and life expectancy.

In the past decades, extensive research has led to the identification of genetic and epigenetic alterations involved in the pathogenesis of transformation. Despite the substantial progress accomplished elucidating the contributing factors leading to transformation, no predictive biomarkers have been validated for clinical use.

Likewise, adverse clinical factors have been associated with a higher risk of transformation, but evidence is not consistent and still, these clinical features alone can't adequately predict the majority of patients who will transform in the future.

This lack of knowledge is relevant to the prognosis of patients with FL because typically the majority of patients who are asymptomatic and present a low tumour burden are not treated from the beginning but rather managed following a "watch and wait" strategy.

Several papers suggest that early treatment with immunotherapy can reduce transformation rates (15-y risk of transformation of 30% in the pre-rituximab era to 8% in the most recent series) as well as improve the prognosis of patients with a transformed biology. Accordingly, finding predictive biomarkers that allow us to detect which patients present a higher risk of HT would be useful to determine which patients would benefit the most from early treatment to prevent transformation.

Although *NOTCH1* mutations are a rare event in FL patients (6,3% according to literature research), one paper studying a small group of patients with FL (49) analyzed the mutational status of this gene and found that this mutation identified a subset of patients with distinctive clinicopathological characteristics; amongst them being association to transformation to diffuse large B cell lymphoma. The same study found evidence suggesting that *NOTCH1* gene mutations might be acquired before the transformation occurs, which implies that they could be used as a predictive biomarker for transformation before its occurrence.

Further evidence pointing to *NOTCH1* as a factor involved in the biology of transformation is the fact that previous research has implicated mutations in this gene as an important driver of other indolent lymphomas acquiring an agressive biology, as is the case of CLL's Richter syndrome (51).

Analysis of NOTCH1 mutation as predictive biomarker for transformation in follicular lymphoma patients

Mutations in the NOTCH family have been assessed in previous papers on FL samples. However, the functional role of this molecule in the FL pathogenesis and its influence on transformation have not been well determined.

The aim of this study is to characterise the frequency of the *NOTCH1* mutations in non-transformed FL patients and evaluate its potential role as a biomarker for transformation at the time of diagnosis.

3. HYPOTHESIS AND OBJECTIVES

Hypothesis

Aberrant activation of the NOTCH signalling pathway via gain-of-function mutation of the *NOTCH1* gene could predict a higher risk of transformation from indolent follicular lymphoma to DLBCL at the time of diagnosis.

Objectives

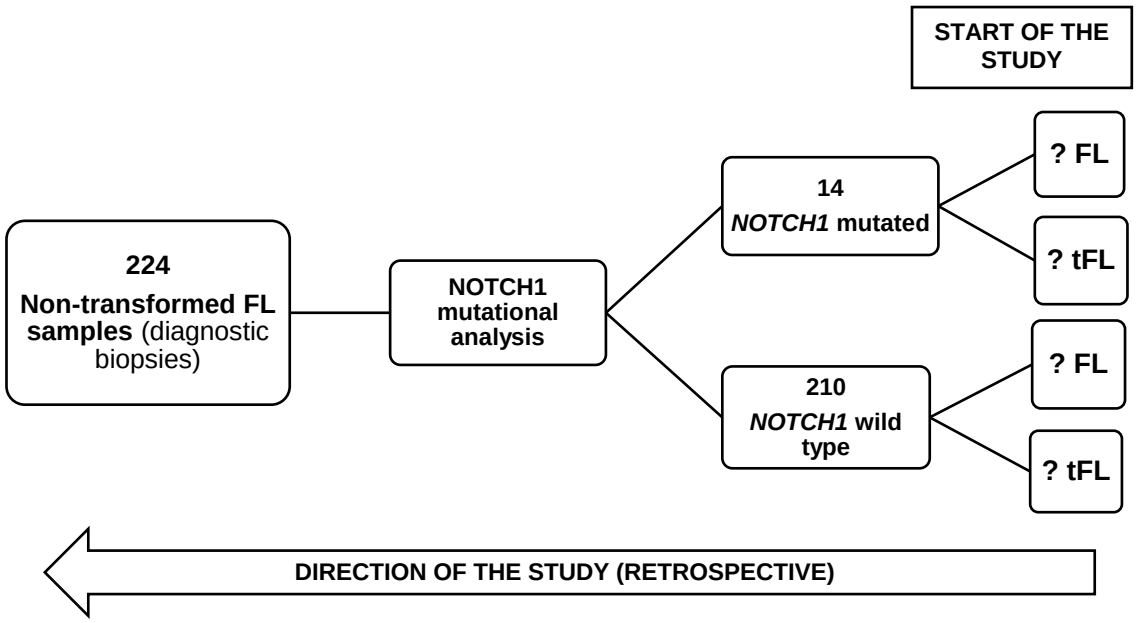
To analize the relationship between the mutational status of the *NOTCH1* gene at diagnosis and the risk of transformation in a cohort of patients with non-transformed follicular lymphoma.

4. PATIENTS AND METHODS

4.1 Study Design

This study will be designed as a 10-year retrospective observational cohort study. Frozen samples of patients with FL registred from 2009 to 2019 will be collected from the pathology files of the *Institut Català d'Oncologia* (ICO) from *Hospital Universitari Dr. Josep Trueta* (Girona, Spain).

Mutational analysis of *NOTCH1* gene by PCR will be performed using the frozen samples. Posteriorly we will compare the incidence of *NOTCH1* mutations in patients who have undergone transformation and those who have not to assess if there are statistically significant differences.



4.2 Study Population

The eligible population will include patients with non-transformed follicular lymphoma at diagnosis from 2009 to 2019 registered in Hospital Dr. Josep Trueta (Girona). All patients were treated in the rituximab era.

Inclusion Criteria

1. Histologically documented diagnosis of follicular non-Hodgkin's lymphoma according to the current WHO criteria
2. Follicular lymphoma of grade 1, 2 or 3A

Exclusion criteria

1. Evidence of transformed follicular lymphoma (clinical and/or histological)
2. Specific FL subtypes including pediatric, primary cutaneous, and primary duodenum FL

4.3 Sample Selection

All patients with a clinical suspicion of lymphoma undergo a biopsy of a lymph node as a part of the diagnostic procedure to characterise its histological features and stablish a diagnosis. These biopsy samples are cryopreserved in the *Biobanc HUB-ICO-IDIBELL* [3] pathology files for conservation and future research use.

Because this study is based in a retorspective cohort, the biopsies of the eligible patients were already performed at the time of diagnosis and their samples were kept in the *Biobanc HUB-ICO-IDIBELL.* Therefore, the selection of our sample was accomplished during the years of recruitment (2009-2019), using a consecutive non-probabilistic method.

4.4 Sample Size

Sample size was calculated using Sample Size and Power Calculator GRANMO, using the POISSON approximation.

Accepting an alpha risk of 0,05 and a beta risk of 0,2 in a two-sided test, 14 exposed subjects (*NOTCH1* mutated) and 210 non-exposed subjects (*NOTCH1* wild-type) are necessary to recognize as statistically significant a relative risk greater or equal than 2.4.

Incidence of *NOTCH1* mutations in patients with follicular lymphoma has been estimated to be 6% according to previous studies (49). Transformation rates have been estimated to be 2-3% annually according to literature research. A drop-out rate of 0,05 has been anticipated.

As the *Hospital Universitari Dr. Josep Trueta* recieves approximately 30 patients of follicular lymphoma each year, we will need to look at the data from the last 10 years to achieve the proposed sample size.

[3] Hospital Universitari de Bellvitge, Institut Català d'Oncologia i Institut d'Investigació Biomèdica de Bellvitge

4.5 Variables and measurement methods

i. Dependent variable: Transformation

Transformation will be defined as refractory or recurrent disease with either clinical or pathological evidence of transformed follicular lymphoma. Medical records and clinical pathology will be reviewed to verify all transformations.

Pathologically defined transformation will consist of a biopsy with a confirmed subtype of 3B FL, DLBCL, unclassifiable B-cell lymphoma with characteristics of Burkitt's and DLBCL or high grade B cell lymphoma, by an experienced pathologist's report.

Clinical transformation will be defined by sudden changes in the clinical course such as an increase in LDH over 2 to 3 times normal values, rapid nodal growth, novel involvement of extranodal sites, new B symptoms, hypercalcemia, high uptake values on a PET scan or according to the better judgement of an experienced hematologist.

ii. Independent variable: *NOTCH1* mutational status

The mutational analysis of *NOTCH1* will be performed by PCR and Sanger sequencing. Mutations reported for *NOTCH1* in FL mainly affect the PEST domain, with the P2514fs∗4 truncating mutation being the most frequently detected in previous reports (49,50). This mutation is a 2-bp deletion in exon 34 that shifts the reading frame and generates a premature stop codon.

Two forward primers (specific for the mutant allele p.P2514fs*4, 5'-TTCCTCACCCCGTCCCGA-3'; for wild-type alleles, 5'-TTCCTCACCCCGTCCCCT-3') and common reverse primer (5′-AACATGTGTTTTAAAAAGGCTCCTC-3′) will be used.

Amplification by PCR will be performed using a DNA Polymerase Kit (Life Technologies, Madrid) with 50ng DNA. The reaction mix contains 1 PCR buffer, 200µM dNTP mix (mixture of four nucleotides (dATP, dCTP, dGTP, dTTP), 100 nM forward and reverse primers, 1.5 $MgCl_2$ and 1.25 U Taq DNA polymerase in a final reaction volume of 50µL.

The exact thermal protocol will vary depending on the instrument used. An example of the thermal protocol for the TaqMan Fast Advanced Master Mix (StepOnePlus™) is shown in Table 4.

Table 3. PCR Thermal Protocol Example

Step	Temperature	Time	Cycles
Enzyme activation	95º	20 seconds (up to 2 minutes)	1
Denature	95º	1 seconds	40
Anneal/Extend	60º	20 seconds	

For sequencing, PCR products will be cleaned using an enzymatic clean-up (ExoSAP-IT, Life Technologies). The resulting amplicon will be sequenced using ABI Prism BigDye terminator v 3.2 (Life Technologies).

Sequencing reactions will be run on an ABI-3730 automated sequencer (Life Technologies). All sequences will be examined using Mutations Surveyor DNA Variant Analysis software (Soft Genetics).

iii. Covariables

In order to prevent confusional variables to interfere with the results of the study, other known prognostic factors will be evaluated.

- **Gender**

- **Time to transformation:** Categories will vary between early transformation (HT before 2 years after indolent FL diagnosis) and late transformation (HT after 2 years of indolent FL diagnosis). Date of transformation corresponds to the date in which the biopsy was performed and/or the date of the clinical report certifying transformation. This variable is relevant to the outcome of patients because early transformation has been shown to confer a poorer prognosis.

- **Ann Arbor Stage**: Stages will vary between localised stage (stages I-II) and advanced stage (stages III-IV). Clinical staging will be based on medical history and physical exam, CT scan and bone marrow biopsy.

- **FLIPI Score**: Scores are divided according to the risk category. FLIPI scores of 0-1 represent low risk, scores of 2 represent intermediate risk and scores 3 and above represent high risk. For the purpose of this study we will categorise patients in the low risk (<3 score) and high risk (≥3 score) categories. This index is calculated with age, Ann Arbor stage, number of nodal areas affected, complete blood count and LDH measurement. *See Table 2 in Annex I.*

- **M7-FLIPI Score**: Outcomes will be low and high risk. It will be assessed using the mutational status of *EZH2, ARID1A, MEF2B, EP300, FOXO1, CREBBP* and *CARD11* (mutated or non-mutated), FLIPI score and ECOG performance status (*See Table 4 in Annex)*. We will use the calculator facilitated by the German Low Grade Lymphoma Study Group (GLSG), available at http://www.glsg.de/m7-flipi/. *See Annex III for gene-specific primer sequences used in the PCR analysis.*

- **T(14;18) rearrangement:** Outcomes will be positivity or negativity. FISH studies will be performed using formalin-fixed, paraffin-embeded (FFPE) samples and using break-apart rearrangement probes.

- **Immunohistochemistry of BCL2, CD10, BCL6:** Outcomes will be positivity or negativity. Immunohistochemical analysis will be performed using the FFPE samples and following a panel of antibodies (CD10, BCL2, BCL6).

- **Treatment received**: Categories will vary between observation vs. treatment. Treatment will be subdivided in the following groups: Rituximab induction monotherapy, R-chemotherapy (with or without anthracyclines) and "other categories".

4.5 Data Collection

The clinical data used for analysis in this study is part of the routine management of follicular lymphoma patients and is registered in the ICO patient's database. As this is a retrospective study, all clinical data has already been acquired by medical practitioners during the years of follow-up and will be accessed by the main researcher.

The evaluation of the dependent variable (transformation from indolent FL to aggresive lymphoma) has occurred already and will have been reported on the patient's medical history. The percentage of the transformation cases that were histologically confirmed at the time will be specified.

The only measure that will have to be determined for the purpose of this study is the analysis of the mutational status of the following genes by PCR. Specific primer sequences used for the analysis of these genes can be found in the Annex III.

- **Genes composing the m7-FLIPI score:** *EZH2, ARID1A, MEF2B, EP300, FOXO1, CREBBP* and *CARD11*
- **Idependent variable**: *NOTCH1* mutational status

The mutational analysis will be carried out using the frozen material of the diagnostic biopsy samples.

5. STATISTICAL ANALYSIS

All statistical analysis will be executed with the Statistical Package for the Social Sciences (SPSS) version 25 (IBM, Armonk, NY, US) for Windows®. In all cases, a confidence interval of 95% will be assumed and a $p<0.05$ will be considered statistically significant.

A descriptive analysis of all the baseline characteristics will be performed. All variables except for time to transformation are categorical and will be summarized as percentages and proportions. Time to transformation is the only quantitative variable and will be summarized as median +/- interquartile range.

Any variable having a significant univariate test will be selected as a candidate for the multivariate analysis. To analyze the contribution between variables that are taken into account in the study and the risk of transformation, a multivariate analysis will be performed using a logistic regression model. Categorical variables will be compared using a χ^2 test (chi square test).

Cumulative incidence of transformation will be estimated by using the Kaplan-Meier method compared by the log-rank test. Multivariate analysis will be performed for transformation using the Cox proportional hazards regression model. The optimal cut-off point will be determined using a ROC curve analysis.

6. WORK PLAN AND CHRONOGRAM

The whole study will last approximately 12 months. All activities will be organized following the phases detailed below:

1) Preparation and coordination phase (3 months)

- Study set-up. In this first period the principal investigator (PI) and co-investigators will review the relevant literature, edit the protocol of the study and present the final manuscript
- Evaluation of the protocol by the Hospital's *Comitè d'Ètica d'Investigació Clínica (CEIC)*
- Modifications of the protocol and approval by the *CEIC*
- Informative meeting. With the purpose of homogenizing a standarized method, a meeting will be scheduled before the beginning of the study. All the participant professionals will be trained on how to acquire and register information.

2) Data extraction and organization (3 months)

- Access to clinical information and obtainment of data
- Creation of a unified database. The entrance of information and quality controls of this database will be performed exclusively by the PI. To manage computerized data, Microsoft Office Excel and Access will be used.
- Simultaneously during this time, the PI along with the lab technicians will perform the PCR analysis of the selected genes and register the results in the created database

3) Statistical analysis of results (1 month)

- A qualified statistician will analyze all data using the SPPS package. Results will be presented in tables and figures to illustrate the findings

4) Interpretation of results and article redaction (2 months)

- The research team will meet to analyze, interpret and discuss the obtained results to stablish definitive conclusions.
- Final manuscript redaction declaring the relevant findings of the study

5) Publication and dissemination of the results (4 months)

- Submission of article for publication to selected scientific journals
- Dissemination of results to conferences, workshops and meetings, among others (National and International Congress in 2020/202

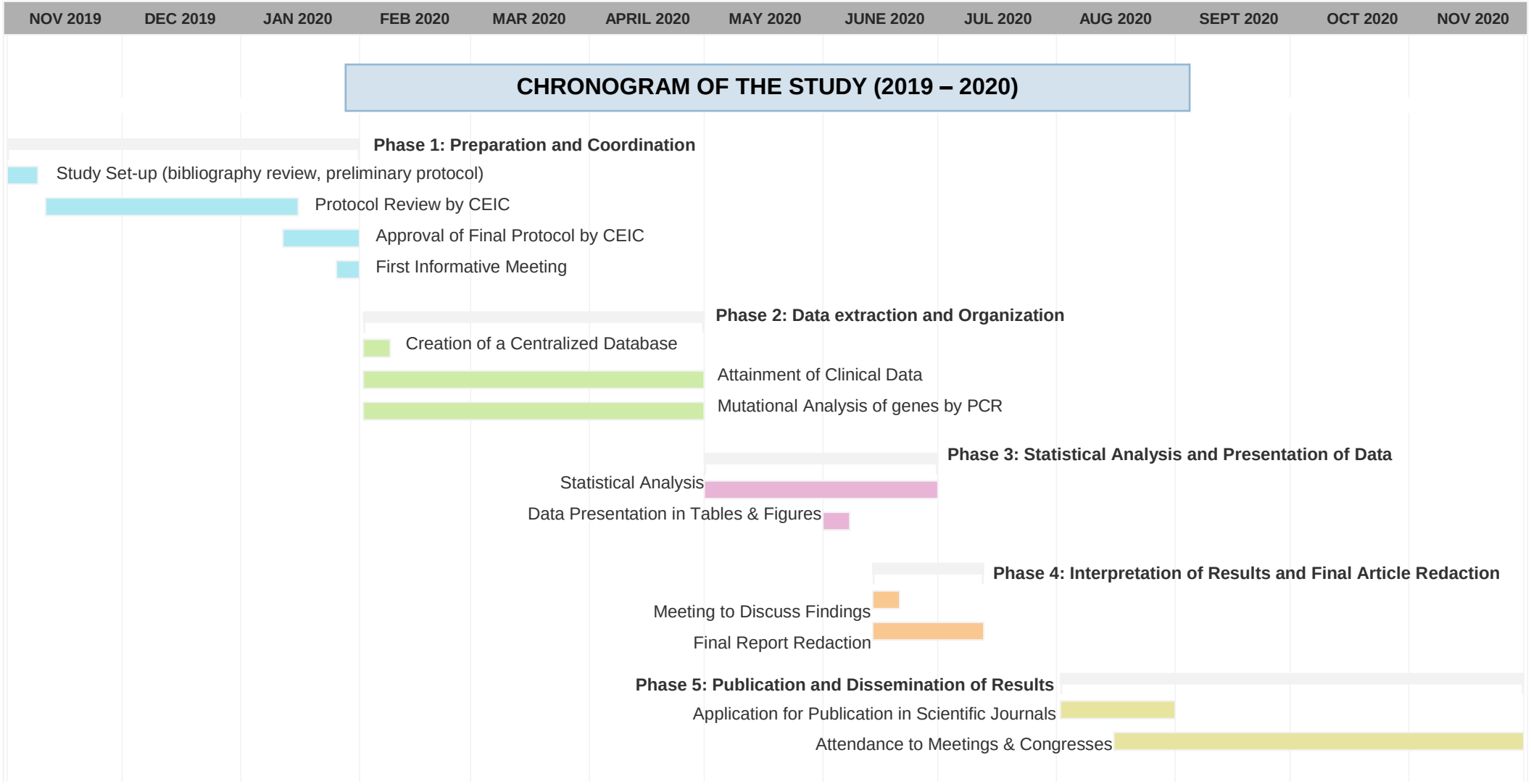


Figure 9. Chronogram of the study. This timeline has been created with the online TeamGantt® tool. Available at www.teamgantt.com

7. ETHICAL ASPECTS

This protocol will be submitted for evaluation to the *Comitè d'Ètica d'Investigació Clínica (CEIC)* of *Hospital Universitari Dr. Josep Trueta*.

The main researcher and collaborators guarantee that the present study will be conducted in accordance to the human rights and ethical considerations gathered in the *World Medical Association Declaration of Helsinki of Ethical Principles for Medical Research Involving Human Subjects,* revised in the 64th General Assembly, in Fortaleza, Brazil, October 2013.

This study includes the analysis of human tissue samples. Therefore it is under the legal framework of *Ley 14/2007, del 3 de Julio, de investigación biomédica; Real Decreto 1716/2011, de 18 de noviembre, por el que se establecen los requisitos básicos de autorización y funcionamiento de los biobancos con fines de investigación biomédica y el tratamiento de las muestras biológicas de origen humano, y se regula el funcionamiento y organización del Registro Nacional de Biobancos para investigación biomédica.*

The present study will be designed to ensure the privacy of all participans as well as confidentiality, according to *Ley Orgánica 3/2018, de 5 de diciembre, de Protección de Datos Personales y Garantía de los derechos digitales.* All personal and clinical information will be used exclusively for the purpose of research and will be treated anonymously. To maintain confidentiality, a randomised identification number will be used instead of the patient's name during the processing and analysis of data. Any data will only be accessible for the responsible researchers of the project.

A standarized consent form by the *Biobanc HUB-ICO-IDIBELL* was signed voluntarily prior to realization of the biopsy in all participants of the study. Patients have at disposition an information sheet explaining the purpose and details of the investigation. Both documents can be found in the Annexes IV and V. The consent form firmed by any participant of the study can be revoked at any time contacting to the personnel of the *Biobanc HUB-ICO-IDIBELL.*

By voluntarily signing the consent form, we ensure that the principle of autonomy is respected, and the fulfilment of the *Ley 41/2002, de 14 de noviembre, básica reguladora de la autonomía del paciente y de derechos y obligaciones en materia de información y documentación clínica.*

The authors declare that they do not have any conflict of interest.

8. STRENGHTS AND LIMITATIONS OF THE STUDY

The present study is designed as an observational retrospective cohort to take advantage of the large number of samples that are already available at the *Biobanc HUB-ICO-IDIBELL* as well as minimizing the costs and duration of the study. We decided to only analyze diagnostic non-transformed FL samples to stablish the predictive value of the *NOTCH1* gene at the time of diagnosis and its actual relationship to the transformation risk.

Transformation was defined not only by pathological criteria but also by clinical criteria because transformation can not always be biopsy proven in a typical clinical setting. The reason of this matter is that if the clinical suspicion is strong, performing and analyzing the results of a biopsy delays the initiation of treatment, which usually calls for urgent measures.

If this study had been designed as a prospective cohort, we could have narrowed the definition of transformation to only histologically proven biopsy samples, to be able to establish clonality on the base of molecular studies and ensure that all cases actually endured transformation. However, considering the study is based on a retrospective cohort, this decision would have meant the rejection of many patients and subsequently the need to perform a lengthier study or to involve another centre to achieve the proposed sample size.

Transformation rates have been reported to be 2-3% annually for at least 15 years after diagnosis so by the time the study starts some patients might not have transformed yet but will in the future. Besides the need to look at 10 years worth of data due to the required sample size, a shorter cohort wouldn't have allowed us to detect as many transformed cases, which is another reason why we chose to focus on a single institution as opposed to several institutions and shorter follow-up time.

Because transformation is a heterogenous disease with complex evolutionary pathways and many intervening factors, studying the contributing effect of a single gene alteration is a factitious construction of reality. To control possible confusion we have chosen the most relevant gene mutations and clinical risk factors to perform a multivariate analysis. Still, we acknowledge there could be other uncontrolled factors that could be influencing the risk of transformation and that could interfere with the results.

To avoid information bias, all personnel participating in the study will be informed and trained to obtain and process the samples correctly. A standard manufacturer's protocol will be followed for the PCR analysis and all instruments will be calibrated and validated before the beginning of the study.

9. BUDGET

The research team participating in this project is already employed by the *Hospital Universitari Dr. Josep Trueta*, so their work has not been contemplated in the budget breakdown. We will hire a qualified statistician to perform the the statistical analysis so we budgeted the estimated amount of work hours required.

The main costs of the study are those regarding the analysis of the mutational status of 8 different genes. Seven of those genes are part of the m7-FLIPI prognostic index (*EZH2*, *ARID1A, MEF2B, EP300, FOXO1, CREBBP* and *CARD11)* and the other is the gene of interest of the study, the *NOTCH1* gene.

We also considered the expenses related to the publication of the article in a scientific journal and the attendance to congresses to divulgate the results of the study. The expected congresses that will be attended are the National Congress of the *Sociedad Española de Hematología y Hemoterapia* (SEHH) in 2020/2021 (dates not confirmed) and the *International Conference on Malignant Lymphoma* (Lugano, Switzerland) in 2021.

Table 4. Budget Breakdown			
STAFF			
Statistician	35€/hour	40 hours	**1.400 €**
MEDICAL RESOURCES: Analysis of mutational status of 11 genes[1]			
DNA extraction	5 x 96 preps	1 set	**1.400 €**
AmpliTaq Gold DNA Polymerase	1.000 units	2 sets	**812 €**
Primers	8€ each primer (64€ per patient (8 genes)	224 patients	**14.336 €**
Enzymatic clean-up	2.000 reactions (1.356€)	2 sets	**2.712 €**
Additional material (tubes, tips with filters, racks, other)			**1.600€**
PUBLICATION AND DISSEMINATION OF FINDINGS[2]			
Article publication	1,500 €	1 journal	**1.500€**
Attendance to National Congress	Inscription – 400€ Transport – 100€ Meals & Accommodation – 150€		**650€**
Attendance to International Congress	Inscription – 500€ Transport – 300€ Meals & Accommodation – 300€		**1.100€**
		ESTIMATED TOTAL	**25.520 €**

[1]All the necessary supplies to perform the mutational analysis by PCR will be provided by ThermoFisher Scientific (Life Technologies). The cost of each item was obtained from ThermoFisher's online catalog at https://www.thermofisher.com/.

[2]The cost of the inscription fee is an estimation of the fee requested in the previous years. The expenses of transport, meals and accommodation are an estimation and might vary depending on the location of the congress.

10. FEASIBILITY

This study is designed as a 10-year observational retrospective cohort to be carried out in a single institution (*Hospital Universitari Dr. Josep Trueta).* Because of its characteristics, no costs regarding an intervention, patient follow-up and coordination between different institutions apply.

As the hospital recieves approximately 30 patients diagnosed with follicular lymphoma each year, it will be necessary to look at 10 years worth of patients to achieve the proposed sample size.

The biopsy samples needed to perform the genetic mutational analysis and all relevant clinical variables have already been acquired during the years of follow-up of the patients included in this study, so no further investigation besides collecting all of the necessary information is required.

The hospital is already equipped with the technology needed to perform the PCR analysis and the laboratory technicians are familiarised with this technique, as it is part of routine diagnostic procedures. However, to ensure maximum eficiency, an informative meeting with the research team will take place before the beginning of the study.

Computer devices and software to eleborate the database and carry out the statistical analysis will be provided.

11. IMPACT ON THE HEALTHCARE SYSTEM

Given that histological transformation into a behaviourly agressive lymphoma is the major cause of morbidity and mortality of patients with follicular lymphoma, allocating resources to help understand the biology of this event is essential for the improvement of the prognosis of patients with this disease.

Several studies have showed that early treatment with immunotherapy reduces transformation rates vs. a “watch and wait” strategy. As mentioned previously, the standard clinical practice at this time is to only treat patients when they become symptomatic and/or present a high tumour burden. This is in part due to the fact that there are no clinical or biological markers that can detect a high risk of transformation with enough specificity and sensibility to imply a change in patient’s management.

If the hypothesis of this study were confirmed by the results, it would be reasonable to contemplate *NOTCH1* mutations as potential predictors of transformation at the time of diagnosis, although further independent validation cohorts should be performed to corroborate this results.

As stated before, transformation doesn’t occur through a single gene alteration, it is rather a multi-step process in which acquisition of several genetic and epigenetic alterations co-occur in such a manner that lead to an agressive biology. For this reason, it is unlikely that any single biomarker will be able to predict a higher risk of transformation on its own.

Indeed, several alterations have been reported in literature to be associated with transformation but have not been sufficiently validated to justify their assessment outside of the research setting. Future studies should focus on addressing this unmet need.

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Annexes

Annex I. Staging and Prognostic Indexes

Table 1	**Ann Arbor staging system** (17)
Stage I	One lymph node station involved or extranodal site (IE)
Stage II	Multiple lymph node stations on one side of the diaphgram or extranodal and one or more lymph nodes on the same side of thediaphragm (IIE)
Stage III	Multiple lymph node stations on both sides of the diaphragm
Stage IV	Presence of disseminated involvement of one or more extranodal organs

Table 2	**FLIPI Prognostic Model** (each factor confers 1 pt) (56)
Age >60 years	
Serum LDH > ULN	
Hemoglobin <12 g/dl	
Stage III or IV	
Number of nodal sites >4	

Table 3	**FLIPI2 Prognostic Model** (each factor confers 1 pt) (16)
Age >60 years	
Bone marrow involvement	
Hemoglobin <12 g/dl	
Greatest diameter of the largest involved node >6 cm	
Serum β2-microglobulin > ULN	

Table 4	**Eastern Cooperative Oncology Group (ECOG) Scale** (57)
Grade	**Description**
0	Fully active, able to carry on all pre-dsease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.
5	Dead

Annex II. GELF criteria

Table 5	**Definition of "high tumor burden" according to GELF criteria** (24)
Any nodal or extranodal site >7cm	
Involvement of 3 or more sites greater than 3 cm	
Presence of B symptoms	
Splenomegaly below umbilical line	
Any compressive symptoms (reteral, orbital, GI, etc)	
Pleural or peritoneal effusions	
Leucemic phase (over 5 x 10^9/L tumor cells	
Cytopenias (platelet count <100.000/mm^3 or absolute neutrophil count <1000/mm^3)	

Annex III. Gene-Specific Primer Sequences

Primers used for PCR analysis of the *NOTCH1* and m7-FLIPI genes[1]		
Gene	**Forward Primer**	**Reverse Primer**
NOTCH1	5'-TTCCTCACCCCGTCCCGA-3'	5'-AACATGTGTTTTAAAAAGGCTCCTC-3'
EZH2	5'-GACCTCTGTCTTACTTGTGGAGC-3'	5'-CGTCAGATGGTGCCAGCAATAG-3'
ARID1A	5'-TTCCTCAGTGACCGAAAGAAC-3'	5'-GCCGATACTGCCCTTCTG-3'
MEF2B	5'-ATGGACCGTGTGCTGCTGAAGT-3'	5'-TCCGAAACTTCTCTCCTGGCTC-3'
EP300	5' TCCGAGACATCTTGAGACGACAG 3'	5' GGGTTGCTGGAACTGGTTATGG 3'
FOXO1	5′-TGGACATGCTCAGCAGACATC-3'	5′-TTGGGTCAGGCGGTTCA-3′
CREBBP	5'-CACTGGGAGTTCTCCTTGCG-3'	5'-ACAGGATGCTTCGTCAGACC-3'
CARD11	5′-CAGGGTGCCTGCCTCATAG-3′	5′-TATAGGGAGAAGCAAGGCAGGG-3′

[1]These primers were obtained from the Supporting Appendix available from Kridel R et al. Histological Transformation and Progression in Follicular Lymphoma: A Clonal Evolution Study. *PLoS Med*. 2016 Dec 1;13(12) (58).

Annexes IV and V. Information Sheet and Informed Consent Form of *Biobanc HUB-ICO-IDIBELL*

Information Sheet and Informed Consent Form of *Biobanc HUB-ICO-IDIBELL* are attached in the following pages.

These documents can be requested at any time by participants of the study. The informed consent form can be revoked at any point by contacting to the personnel of *Biobanc HUB-ICO-IDIBELL* and fulfilling the corresponding form. The revocation of the consent form will not affect negatively on the clinical assistance revieved by the donor, and their clinical information and biological material will be excluded from the *Biobanc*.

The present documents and further information can be found at the *Biobanc HUB-ICO-IDIBELL* website http://www.idibell.cat/es/servicios/plataformas/biobanco-hub-ico-idibell

Full d'informació per al pacient

UTILITZACIÓ DE MATERIAL BIOLÒGIC EXCEDENT I DADES CLÍNIQUES OBTINGUTS EN EL PROCÉS ASISTENCIAL PER A INVESTIGACIÓ BIOMÈDICA I LA SEVA CONSERVACIÓ EN EL BIOBANC HUB-ICO-IDIBELL

En l'Hospital Universitari de Bellvitge (HUB), en l'Institut Català d'Oncologia (ICO) i en altres centres adscrits, a més a més, de l'assistència als pacients, es realitza investigació biomèdica. La finalitat d'aquesta investigació és progressar en el coneixement de las malalties, en la seva prevenció, diagnòstic, pronòstic i tractament. Aquesta investigació biomèdica requereix recollir dades clíniques i mostres biològiques dels pacients o donants per analitzar-los i obtenir conclusions amb l'objectiu de conèixer millor i avançar en el diagnòstic i/o tractament de les malalties.

En aquest sentit, les mostres obtingudes per al diagnòstic o control de les malalties, una vegada utilitzades amb aquesta finalitat, resulten també útils i necessàries per a investigació. De fet, molts dels avenços científics obtinguts en els últims anys en medicina són fruit de l'ús d'aquest tipus de mostres.

D'acord amb les normes bioètiques i la legislació vigent, sol·licitem la seva autorització per a utilitzar en investigació el material biològic sobrant i la informació clínica associada de les proves que, com part del procés assistencial, se li han realitzat o se li realitzaran en el Hospital Universitari de Bellvitge (HUB), en el ICO i/o d'altres centres hospitalaris adscrits.

Seguint l'establert per la Llei 14/2007, d'Investigació Biomèdica i el Reglament (UE) 2016/679 del Parlament Europeu i el Consell, del 27 d'abril de 2016, relatiu a la protecció de les persones físiques en el que respecta al tractament de dades personals i a la lliure circulació d'aquestes dades, li sol·licitem que llegeixi detingudament aquest document d'informació i el consentiment informat que se li adjunta al final per a la seva signatura.

FINALITAT DE LA INVESTIGACIÓ: progressar en el coneixement de les malalties

La finalitat de la investigació es millorar el nostre coneixement de les malalties. Per aquest motiu, les mostres biolò- giques humanes i les dades clíniques i analítiques associades són d'especial rellevància per a la investigació.

Amb aquest material es poden realitzar estudis científics que millorin la informació que disposem actualment de moltes malalties i que contribueixin al progrés i al coneixement d'aquestes repercutint positivament en la salut del conjunt de la societat en el futur.

MOSTRES BIOLÒGIQUES I INFORMACIÓ ASSOCIADA: la seva custòdia i conservació es realitzarà en el Biobanc HUB-ICO-IDIBELL fins la seva extinció

El material biològic sobrant que se li extregui durant el procés assistencial (mostres de sang, líquids biològics i/o teixits), una vegada finalitzat el diagnòstic, es guardarà i disposarà en un futur per a realitzar estudis d'investigació biomèdica, sense que aquest fet li causi molèsties addicionals. La donació de mostres excedents d'aquest procés assistencial no impedirà que vostè o la seva família puguin reclamar-les quan sigui necessari per motius de salut.

Les mostres i la informació associada a aquestes es custodiaran i conservaran en el Biobanc (banc de mostres bio- lògiques) HUB-ICO-IDIBELL.

El BIOBANC HUB-ICO-IDIBELL és un establiment legalment autoritzat, sense ànim de lucre, que acull col·leccions organitzades de mostres biològiques i informació associada en les condicions i garanties de qualitat i de seguretat que exigeix la legislació vigent i els codis de conducta aprovats pels Comitès d'Ètica.

Aquestes mostres i la seva informació associada estan disponibles per aquells centres o institucions d'investigació nacionals o internacionals que ho sol·licitin oficialment al Biobanc.

Qualssevol estudi d'investigació pel qual es sol·liciti l'ús d'aquestes dades o mostres haurà de disposar sempre de l'aprovació del Comitè d'Ètica d'Investigació (CEI) competent, que velarà per a que els investigadors desenvolupin els seus estudis seguint sempre les més estrictes normes ètiques i legals, així com de l'aprovació d'un Comitè Cien- tífic que garanteixi l'excel·lència i utilitat científica.

A partir de les mostres donades, si la investigació ho requereix, es podrien realitzar estudis genètics que compor- tin l'obtenció d'informació referent a la seva salut i dels seus familiars. Sempre s'actuarà vetllant per a la protecció d'aquesta informació (veure apartat de protecció de dades i confidencialitat).

PROTECCIÓ DE DADES I CONFIDENCIALITAT: les mostres es conservaran codificades

Les dades que es recullin seran conservades d'acord amb les exigències normatives i/o fins a la finalització de les finalitats que hagin motivat la seva captació, i/o la prescripció de les accions legals que se'n pogueren derivar. Les dades personals captades al present formulari i la informació facilitada podran ser comunicades als investigadors acreditats, així com per obligació legal a les Administracions competents. La manca d'autorització per al tractament de les dades i el consentiment per a la realització del

procediment descrit comportarà la impossibilitat de realitzar les tasques descrites. La identificació de les mostres biològiques del Biobanc serà sotmesa a un procés d'anonimització. A cada mostra se li assigna un codi d'identificació que serà l'utilitzat pels investigadors. Únicament el personal au- toritzat pel Biobanc podrà relacionar la seva identitat amb els codis. Mitjançant aquest procés els investigadors que sol·liciten mostres al Biobanc no podran conèixer cap dada que reveli la seva identitat. Així mateix, encara que els resultats obtinguts de l'investigació realitzada amb les seves mostres es publiquin en revistes científiques, la seva identitat no serà facilitada. Aquestes dades seran tractades i cedides amb la única i exclusiva finalitat de portar a terme investigació biomèdica.

Si ho desitja pot contactar amb el nostre Delegat de Protecció de Dades (DPD), revocar el consentiment facilitat i/o exercir els seus drets d'accés, rectificació, supressió, oposició, limitació del tractament i/o portabilitat de les dades mitjançant escrit adreçat en la següent adreça: *Hospital Duran i Reynals, 3a planta / Gran Via de l'Hospitalet, 199, 08908, Hospitalet de Llobregat* o en la següent adreça de correu electrònic: dpo@idibell.cat. En cas de que consideri que s'han vulnerat els seus drets també li assisteix el dret a presentar una reclamació front a l'autoritat de control competent: Autoritat Catalana de Protecció de Dades.

CARÀCTER ALTRUISTA DE LA DONACIÓ: la cessió de mostres biològiques que vostè realitza al BIO- BANC HUB-ICO-IDIBELL es gratuïta

Vostè no obtindrà cap benefici econòmic directe per la cessió de la mostra i les seves dades associades ni per la seva participació en els estudis d'investigació. Tampoc tindrà drets sobre possibles beneficis comercials dels des- cobriments que puguin derivar de l'investigació biomèdica.

PARTICIPACIÓ VOLUNTÀRIA: la seva negativa no repercutirà en la seva assistència mèdica, present o futura

La seva participació és totalment voluntària. Pot negar-se a participar o retirar el seu consentiment en qualssevol moment posterior a la signatura sense haver de donar els motius. En cap cas aquest fet repercutirà negativament en la seva assistència mèdica, present i futura.

REVOCACIÓ DEL CONSENTIMENT: si vostè decideix signar aquest consentiment, podrà també cancel·lar-lo lliurement. Això comportarà la destrucció de la mostra.

Si en un futur vostè decideix anular el seu consentiment, les seves mostres biològiques seran destruïdes i les da- des associades seran retirades del Biobanc. També podria sol·licitar l'anonimització de les mostres, en aquest cas s'eliminarà la relació entre el seu material biològic i les seves dades clíniques i personals. Els efectes d'aquesta cancel·lació o anonimització no es podran extendre a l'investigació que ja s'hagués portat a terme. Si desitgés anul·lar el seu consentiment, haurà de sol·licitar-ho per escrit a la Direcció científica del Biobanc, en l'adreça a dalt indicada.

INFORMACIÓ SOBRE ELS RESULTATS DE L'INVESTIGACIÓ: se li proporcionarà informació si vostè desitja rebre-la

En el cas que vostè ho sol·liciti expressament, el Biobanc podrà proporcionar-li informació de les investigacions en que s'ha fet ús de les seves mostres i els resultats globals d'aquestes investigacions, excepte en els casos de cancel·lació i anonimització.

Els mètodes emprats en investigació biomèdica solen ser diferents dels aprovats per a la pràctica clínica, per tant no han de ser considerats amb valor clínic per vostè. Tanmateix, en el cas que aquestes investigacions proporcionin dades que poguessin ser clínicament o genèticament rellevants per a vostè o la seva família, li seran comunicats si ho considera oportú. També es podria obtenir informació rellevant per a la seva família. Li correspondrà a vostè decidir si vol o no comunicar-se-la. Si vostè vol que se li comuniqui aquesta informació rellevant ha de consignar-ho en la casella del consentiment informat.

Si vostè no desitja rebre aquesta informació, ha de tenir en compte que la llei estableix que, quan la informació obtinguda sigui necessària per a evitar un gran prejudici per la seva salut o dels seus familiars biològics, un comitè d'experts estudiarà el cas i haurà de decidir si és convenient informar als afectats o als representants legals.

Per favor, pregunti al personal sanitari que l'ha comunicat aquesta informació sobre qualssevol dubte que pugui tenir, ara o en el futur, en relació amb aquest consentiment. Així mateix, pot comentar els seus dubtes amb el seu metge, qui li posarà en contacte amb el personal sanitari autoritzat.

Moltes gràcies per la seva col·laboració.

BIOBANC HUB-ICO-IDIBELL

Bellvitge Hospital Universitari

ICO Institut Català d'Oncologia

IDIBELL Institut d'Investigació Biomèdica de Bellvitge

Consentiment informat
Consentimiento informado

NHC / Núm. SAP:
Unitat / Servei:

Utilització de mostres biològiques i dades clíniques obtingudes durant el procés assistencial per investigació biomèdica i conservació al Biobanc HUB-ICO-IDIBELL	***Utilización de muestras biológicas y datos clínicos obtenidos durante el proceso asistencial para investigación biomédica y conservación en el Biobanco HUB-ICO-IDIBELL***
Després d'haver rebut el full d'informació adjunt i de comprendre'n el contingut, signo aquest document i autoritzo l'Hospital Universitari de Bellvitge (HUB), l'Institut Català d'Oncologia (ICO) i altres centres hospitalaris adscrits a conservar al Biobanc HUB-ICO-IDIBELL: • les mostres biològiques sobrants de les proves que m'han realitzat o em realitzaran • i la informació clínica i assistencial associada amb la finalitat de prosseguir amb nous projectes de recerca biomèdica, sempre que aquests tinguin l'obligada aprovació del Comitè d'Ètica d'Investigació competent.	*Después de haber recibido la hoja de información adjunta y comprendido su contenido, firmo este documento y autorizo al Hospital Universitari de Bellvitge (HUB), al Institut Català d'Oncologia (ICO) y a otros centros hospitalarios adscritos a conservar en el Biobanco HUB-ICO-IDIBELL:* • *las muestras biológicas sobrantes de las pruebas que me han realizado o me van a realizar* • *y la información clínica y asistencial asociada* *con la finalidad de llevar a cabo proyectos de investigación biomédica, siempre que éstos cuenten con la obligada aprobación del Comité de Ética de Investigación competente.*

1. Autoritzo que les mostres biològiques sobrants de les proves diagnòstiques i la informació clínica associada s'utilitzin per a investigació, en els termes recollits en el full d'informació	☐ SÍ ☐ NO	*1. Autorizo que las muestras biológicas sobrantes de las pruebas diagnósticas y la información clínica asociada se utilicen para investigación, en los términos recogidos en la hoja de información*
2. Desitjo que se'm comuniqui la informació derivada de la recerca que realment sigui rellevant i aplicable per a la meva salut o la de la meva família	☐ SÍ ☐ NO	*2. Deseo que se me comunique la información derivada de la investigación que realmente sea relevante y aplicable para mi salud o la de mi familia*
3. Autoritzo que se'm contacti en cas de necessitar més informació o mostres biològiques addicionals	☐ SÍ ☐ NO	*3. Autorizo a ser contactado en el caso de necesitar más información o muestras biológicas adicionales*
4. Desitjo que es respectin les següents excepcions o restriccions:		*4. Deseo que se respeten las siguientes excepciones o restricciones*

Informació RGPD en el dorso de la còpia del document.

Espai per escriure / *Espacio para escribir*

DONANT / *DONANTE*		PERSONA QUE INFORMA
Nom / *Nombre*		Nom / *Nombre*
Cognoms / *Apellidos*		Cognoms / *Apellidos*
DNI	Edat / *Edad*	DNI
Signatura / *Firma* *Data / Fecha*		Signatura / *Firma* *Data / Fecha*

REPRESENTANT: *només en els casos (1) (2) (3)* / *REPRESENTANTE: sólo en los casos (1) (2) (3)*		
Nom / *Nombre*	Relació amb el donant / *Relación con el donante:*	
Cognoms / *Apellidos*		
DNI	Tipus de representant / *Tipo de representante:*	
Signatura / *Firma* *Data / Fecha*	(1) Autoritzat pel donant ☐	(1) *Autorizado por el donante*
	(2) Legalment autoritzat ☐	(2) *Legalmente autorizado*
	(3) Autoritzat per la família ☐	(3) *Autorizado por la familia*
	(1) (2) En cas d'incapacitat del donant / *En caso de incapacidad del donante* (3) En cas de donació post mortem / *En caso de donación post mortem*	