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Unmet clinical needs in indolent lymphomas: how to identify high risk patients and how to adapt therapy

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Abbreviations

ALC	Absolute lymphocyte count
ASCT	Autologous stem cell transplant
B2M	Beta2microglobuline
BM	Bone Marrow
cfDNA	Cell-free circulating DNA
CI	Confidence Interval
CMR	Complete Metabolic Response
CR	Complete Response
CR30	Complete Response at 30 months
CSS	Cause-specific survival
CT	Chemotherapy
CTC	Circulation Tumor cells
ddPCR	Droplet digital PCR
dissMZL	Disseminate Marginal Zone Lymphoma
DLBCL	Diffuse Large B Cell Lymphoma
DS	Deauville five-point Scale
ECOG	Eastern Cooperative Oncology Group
ENMZL	Extra-Nodal Marginal Zone Lymphoma
EOT	End of Induction
EOT	End of Treatment
FDG-PET	[18F] Fluorodeoxyglucose Positron Emission Tomography
FFR	Freedom from Recurrence
FIL	Fondazione Italiana Linfomi
FL	Follicular Lymphoma
FLIPI	Follicular Lymphoma International Prognostic Index
fPET	Final PET
G-	Obinutuzumab
Hb	Haemoglobin
HL	Hodgkin Lymphoma
HR	Hazard Ratio
ICT	Immunochemotherapy
IG	Immunoglobulin
IIL	Intergruppo Italiano Linfomi
INFL	Indolent Non-Follicular Lymphoma
iNHL	indolent Non-Hodgkin Lymphoma
iPET	Interim PET
ITT	Intention to Treat
LDH	Lactate dehydrogenase
LSS	Lymphoma specific survival
MALT	Mucosa- associated lymphoid tissue
MR	Metabolic Response
MRD	Minimal Residue Disease

MZL	Marginal Zone Lymphoma
NCCN	National Comprehensive Cancer Network
NCT	Number of Clinical Trial
NHL	Non-Hodgkin Lymphoma
NMZL	Nodal Marginal Zone Lymphoma
NPV	Negative Predictive Value
OR	Odds Ratio
OS	Overall Survival
PB	Peripheral Blood
PCR	Polymerase chain reaction
PET-	PET negative
PET+	Pet positive
PFS	Progression Free Survival
POD	Progression of Disease
POD24	Progression of disease with 24 months
PPV	Positive Predictive Value
PR	Partial Response
PRIMA-PI	Primary Rituximab and Maintenance Prognostic Index
PS	Performance Status
Pts	Patients
R-	Rituximab
R-Benda	Rituximab- Bendamustine
R-CHOP	Rituximab-cyclophosphamide, doxorubicin, vincristine, prednisolone
R-CVP	Rituximab- cyclophosphamide, vincristine, prednisone
R-DHAOX	Rituximab dexamethasone, Ara-C, Oxaliplatin
R-HDS	Rituximab- High Dose Therapy
R-ICE	Rituximab-ifosfamide carboplatin etoposide
R/P	Relapse/Progression
R/R	Relapse/Refractory
RT	Radiotherapy
SD	Stable Disease
SMZL	Splenic Marginal Zone Lymphoma
SPD	Sum of Products
SUVmax	Maximum Standardized Uptake Value
TLG	Total lesion glycolysis
TMTV	Total Metabolic Tumor Volume
W&w	Watch & Wait

Abstract (English Version)

Follicular Lymphoma (FL) is the most common subtype among indolent lymphomas accounting for about 20% of Non-Hodgkin Lymphoma (NHL). Second for frequency, marginal zone lymphomas (MZL) are rarer but not uncommon, representing about 10% of NHL.

Usually, as prototypes of indolent diseases, FL and MZL are characterized by slow growth, often do not require immediate treatment and, when therapy is needed, excellent response rates are achieved. However, most patients with indolent lymphomas relapse after initial or subsequent therapies, thus making both FL and MZL incurable diseases. In contrast with the majority of patients who have a true indolent disease, a small but significant portion of patients reflects the characteristics of a more aggressive disease experiencing an early progression, usually within two years from diagnosis (POD24), or a transformation into an aggressive lymphoma. These patients are often designated as “high-risk”, owing to less favorable outcomes compared with the cases who relapse beyond two years. The early definition of the individual risk profile is identified as an important step to support clinical and therapeutic decision.

Several prognostic factors and indexes, classified as baseline and post-induction features, have been studied during the last years. Moreover, the concept of duration of remission is gaining attention as an accurate tool to identify a subset of high-risk patients. However, there are still issues to be addressed related to earlier identification of these vulnerable cases, especially in the era of new drugs. The POD24 cannot be used to guide first-line treatment including consolidation/maintenance regimens in first remission, and by definition is unable to identify high-risk patients who do not fail first-line treatment within 24 months. In addition, no data are currently available to understand how a different definition of risk could be effectively translated into a useful decisional tool or risk-adapted therapeutic recommendation.

The key research priority of my Ph.D. project is to try to focus on the recognition and validation of robust prognostic tools, and on the translation of these tools into actionable predictive factors to guide the choice of treatment to improve personalized approach to patients.

The study populations analyzed comprised patients enrolled in the Fondazione Italiana Linfomi (FIL) Trials: FOLL05 (504 patients), FOLL12 (807 patients), PETRA (175 patients) for follicular lymphoma, and NF10 for MZL (785 patients).

The entire Ph.D. project has been conducted in three main fields:

First: to describe the current prognostic available indexes highlighting the most controversial aspects regarding their actual application and combining the evidence coming from the literature with the new results deriving from our research (FOLL05, FOLL12, NF10, and PIMENTO).
Second: to investigate the possibility to integrate novel prognostic tools such as molecular response (MRD) and metabolic response (MR) early in the therapeutic path to define risk adapted strategies (FOLL12, FOLL19).
Third: to evaluate the impact of the relapse or early recurrence on the outcome of indolent lymphomas (Petra study, NF10).
A continued effort in the front-line treatment of indolent NHL is early identification of high-risk patients before starting therapy and to determine risk adapted strategies. The results of this project will provide clarification as to when and how best use different prognostic and predictive tools in order to lead the basis for next prospective trials in which this subset of patients could be addressed to a risk-adapted induction therapy improving the outcome for the high-risk group.

Key words: indolent lymphomas, high-risk lymphoma, risk-adapted therapy

Abstract (Italian version)

Tra i linfomi indolenti, il linfoma follicolare è il sottotipo più comune e rappresenta circa il 20% dei linfomi non-Hodgkin (NHL). Secondi per frequenza, i linfomi della zona marginale (MZL) sono più rari ma non infrequenti, rappresentando circa il 10% dei NHL. Solitamente, come prototipi di malattie indolente, sono caratterizzati da una crescita lenta, spesso non richiedono un trattamento immediato e, quando è necessaria una terapia, si ottengono ottimi tassi di risposta e di sopravvivenza. Tuttavia, la maggior parte dei pazienti anche con linfoma indolente recidiva dopo terapie iniziali o successive e i linfomi follicolari e i linfomi della zona marginale sono tuttora considerati incurabili con un atteggiamento tipicamente remittente-recidivante. Inoltre, il comportamento clinico di una piccola ma significativa porzione di pazienti riflette le caratteristiche di una malattia più aggressiva presentando una progressione precoce della malattia, entro due anni dalla diagnosi (POD24), o una trasformazione in un linfoma aggressivo. Questi pazienti non si avvantaggiano della lunga storia naturale dei linfomi indolenti e sono spesso designati come “ad alto rischio” per gli esiti meno favorevoli rispetto ai casi di recidiva oltre i due anni.

Diversi sono i fattori prognostici e predittivi studiati negli ultimi anni, classificabili come fattori utilizzati al baseline o come post trattamento durante il follow-up. Inoltre, sta prendendo sempre più spazio il concetto di definizione del rischio basato sulla durata della risposta identificando i pazienti recidivati precoci come pazienti a più alto rischio. Nonostante l’evolversi delle nostre conoscenze, vi sono numerose questioni aperte e bisogni a cui dare risposte relative ad una più precoce identificazione del rischio applicabile anche nell’era dei nuovi farmaci. Inoltre, la POD24, come surrogato di risposta post trattamento per definizione non è in grado di definire il rischio fin dalla diagnosi o nei primi due anni. Infine, non vi sono attualmente disponibili dati per comprendere come una diversa definizione di rischio possa essere efficacemente tradotta in uno strumento decisionale clinicamente utile o in una raccomandazione terapeutica adattata al rischio.

La chiave del progetto di ricerca è cercare di risponde a questi quesiti irrisolti non solo focalizzandoci sul riconoscimento e validazione dei più robusti strumenti prognostici ma anche cercando di integrarli più precocemente nel percorso terapeutico e traducendoli in strumenti utili per una terapia personalizzata.

Le popolazioni di studio analizzate comprendono i pazienti arruolati negli studi della Fondazione Italiana Linfomi (FIL): FOLL05 (504 pazienti), FOLL12 (807), PETRA (175) per linfoma follicolare e NF10 per i MZL (785 pazienti).

L'intero progetto di dottorato è stato condotto in tre campi principali:

Primo: descrivere gli attuali strumenti a nostra disposizione mettendo in luce gli aspetti più controversi di ognuno riguardo le attuali applicazioni e combinando le evidenze della letteratura con i risultati derivanti dalle nostre ricerche (FOLL05, FOLL12, NF10 e PIMENTO).
Secondo: studiare la possibilità di integrare i nuovi modelli prognostici come la malattia minima residua (MRD) e la risposta metabolica (MR) precocemente nel percorso di cura per definire strategie adattate al rischio (FOLL12, FOLL19, PIMENTO).
Terzo: valutare l'impatto delle recidive sia tardive che precoci sulla sopravvivenza dei pazienti (PETRA e NF10).
Uno sforzo continuo già a partire dalla prima linea nei linfomi indolenti è l'identificazione precoce dei pazienti ad alto rischio prima di iniziare la terapia e la determinazione di strategie adattate al rischio. I risultati di questo progetto forniranno chiarimenti su quando e come utilizzare al meglio diversi strumenti prognostici e predittivi al fine di gettare le basi per i prossimi studi prospettici in cui questo sottogruppo di pazienti potrebbe essere indirizzato a una terapia di induzione adattata al rischio migliorando l'efficacia della terapia nei pazienti a rischio senza provocare tossicità non necessaria.

Parole Chiave: linfomi indolenti, linfoma ad alto rischio, terapia adattata al rischio

Chapter 1. Introduction

Indolent Non-Hodgkin Lymphomas (iNHLs) comprise a heterogeneous group of diseases sharing a typically slow tumor growth and a long natural history measured in decades, that in some cases is similar to that of an age-matched population (1–4)

Among iNHLs, Follicular Lymphoma (FL) is the most common subtype accounting for about 20% of NHLs; second for frequency, Marginal Zone Lymphomas (MZL) are rarer but not uncommon representing about 10% of NHL.(5,6)

As the prototype of indolent disease, most patients have an excellent prognosis, often do not require immediate treatment and, when needed, they experience excellent response.

An improvement of survival has been observed during the last years and was mainly attributed to the introduction of more active anti-lymphoma treatments and better insights into the clinical, biological and genetic landscape of the disease leading to improved accuracy in the development of treatment strategies over the course of patients' lifetime.

However, current available treatment algorithms define the initial approach to FL patients based only on the clinical stage, tumor burden, and symptoms. Patients are usually categorized into those with localized or with advanced stage; the latter group is further divided into low or high tumor burden based on the presence of specific criteria (Table 1, in p. 13). Several alternative options are available for these different groups, ranging from observation to radiotherapy or immunochemotherapy. Typically, treatment is indicated for patients with symptomatic or high tumor burden disease. The standard approach for advanced stage consists of an initial therapy with R-CHOP (Rituximab-cyclophosphamide, doxorubicin, vincristine, prednisolone) or R-bendamustine or Rituximab-CVP (cyclophosphamide, vincristine, prednisone) followed by two-year maintenance with R or Obinutuzumab (G-) as per monoclonal CD20 antibody. Recently, a novel strategy, combining the immunomodulatory agent Lenalidomide with Rituximab, showed similar results to standard immunochemotherapy approach and favorable tolerability, opening the scenario of a chemo-free approach in first-line setting for some patients especially those wishing to avoid hematologic toxicity (7–13) (Figure 1, p. 13).

Despite the improvement achieved, the clinical behavior of indolent lymphomas remains extremely heterogeneous and FL and MZL are still considered incurable diseases. Patients typically show a relapsing-remitting course of the disease, and even after having achieved a first remission, the risk of relapse is high, and it is increasing as patients are long survivors. That being said, patients could receive several lines of treatment to control the disease with

a shorter remission after any recurrences, impairing the quality of life. Moreover, about 20% of patients do not share the long natural history of indolent lymphomas and are often designated as “high-risk” owing to less favorable outcomes. These patients usually exhibit a refractory disease or experience an early progression, usually within two years from diagnosis, or undergo a transformation in aggressive lymphoma (14–18).

One of the main challenges in iNHL is the lack of accurate prognostic factors that can early identify patients at increased risk of poor outcome and to aid in treatment selection and sequences.

Unfortunately, none of the prognostic models currently available can be used to guide earlier treatment in daily practice, or have been validated in the era of new drugs or have been determined as tools to guide risk-adapted therapeutic recommendations.

Moreover, most clinical, biological, and metabolic prognostic factors are borrowed from FL whereas prognostic scores in MZL are not stringently validated and follow the same criteria of definition of tumor burden and disease activity of follicular lymphomas.

Finally, at the time of relapse, there is a variety of therapeutic strategies in the face of a paucity of tools for risk stratification and for therapeutic decision-making. Importantly assessment of the risk at the time of relapse may contribute to select optimal treatment also in this setting.

With our research we try to answer some of the unsolved questions not only by focusing on the recognition and validation of robust prognostic models, but also by using them early in the diagnostic process and by translating them into tools to guide the choice of treatment for patients identified as at higher risk.

The population examined is representative of the patients enrolled in the Fondazione Italiana Linfomi clinical trials. Follicular lymphoma patients were retrieved from the FOLL05, FOLL12 and PETRA trial. The first two studies were mainly focused on prospectively identifying the best first-line strategy for advanced stage high tumor burden disease. The FOLL05 study was the starting point for recognizing early relapse as an event that deeply impacts the overall survival and for detecting high risk clinical features at the time of diagnosis. The FOLL12 trial investigated the possibility of risk-adapted therapy assuming that patients with different risk profiles, defined by metabolic and molecular assessment at the end of induction, could benefit from adapted interventions.

Going forward, in the PETRA trial we retrospectively collected data on patients who experienced a relapse after a first response to treatment.

Finally, in the observational NF10 study, we prospectively collected and analyzed the clinical characteristics, treatment patterns, prognostic factors and outcomes data for marginal zone lymphoma patients. The study was designed with the aim to develop a more accurate prognostic assessment for non-follicular low-grade B-cell lymphomas.

A description of the current prognostic available indices, both for follicular lymphoma and marginal zone lymphoma, opens each chapter of this thesis. Such description aims to highlight the most controversial aspects related to the actual application of these tools and to combine the evidence coming from the literature with the new results deriving from our research.

Chapter 2. Follicular Lymphoma Overview

Follicular lymphomas generally have an indolent behavior, and most patients could be considered long survivors experiencing an excellent control of the disease when standard chemoimmunotherapy is applied. However, 20% of the patients have a poor prognosis with an overall survival (OS) rate at 5 years of only 50% (19).

The concept of high-risk FL recently emerged from data showing an increased risk of death for patients who experience an early recurrence, usually within 2 years from diagnosis (POD24) or 30 months (CR30), or are refractory to alkylator-based therapy or anti-CD20 antibody-based therapy, or in patients who undergo a transformation in aggressive lymphoma (14,20–22).

These events are suggestive of the presence of an unfavorable outcome despite the standard chemoimmunotherapy approach and irrespective of initial prognostic stratification.

The concept of POD24 was initially described by Casulo et al as a robust predictor and surrogate endpoint of survival in a large cohort of 588 high tumor burden FL within the National LymphoCare Study Cohort treated with R-CHOP (19). Outcome results showed an inferior OS for early progressor patients compared with patients who never relapse or have an event beyond two years (5-year OS 50% vs 90%) (19). The relevance of POD24 for prognosis was confirmed in other studies and in real life settings (23).

Most notably, although POD24 has been shown to be a powerful predictor of poor outcome, it is not clear if it can become a standard surrogate endpoint to evaluate the efficacy of investigational treatments. The major limitation is that, as a post-treatment marker, POD24 cannot guide first-line treatments including consolidation/maintenance regimens, and by definition is unable to assess patients who die within 24 months without prior documented POD or to identify high-risk patients who do not fail first-line treatment within 24 months.

Another point to focus on is that current treatment strategies are based on a “one size fits all” approach bringing out the unmet clinical need to early identify high-risk patients with risk models that integrate established clinical risk factors with disease specific biomarkers.

Table 1. Definition of tumor burden according GELF, BNLI, and National Comprehensive Cancer Network criteria

Groupe d'Étude des Lymphomes Folliculaires GELF (24)	British National Lymphoma Investigation (BNLI) (25)	(NCCN) (26)
Diameter of tumor (>7 cm)	B-symptoms or pruritus	Candidate for clinical trial
> 3 nodal sites > 3 cm	Rapid generalized disease progression	B-symptoms
Systemic symptoms	Marrow compromise (Hb<100 g/l; WBC<3.0x10^9;PLT <100x10^9/l)	Threatened end-organ function
Substantial splenomegaly	Life-threating organ involvement	Cytopenia secondary to lymphoma
Vital organ impairment	Renal infiltration	Bulky disease
Serum effusion	Bone lesions	Steady progression
Lymphocyte count > 5.0 x 10^9/L		
Cytopenia (ANC < 1.0 x 10^9/L o platelets count < 100 x 10^9/L)		

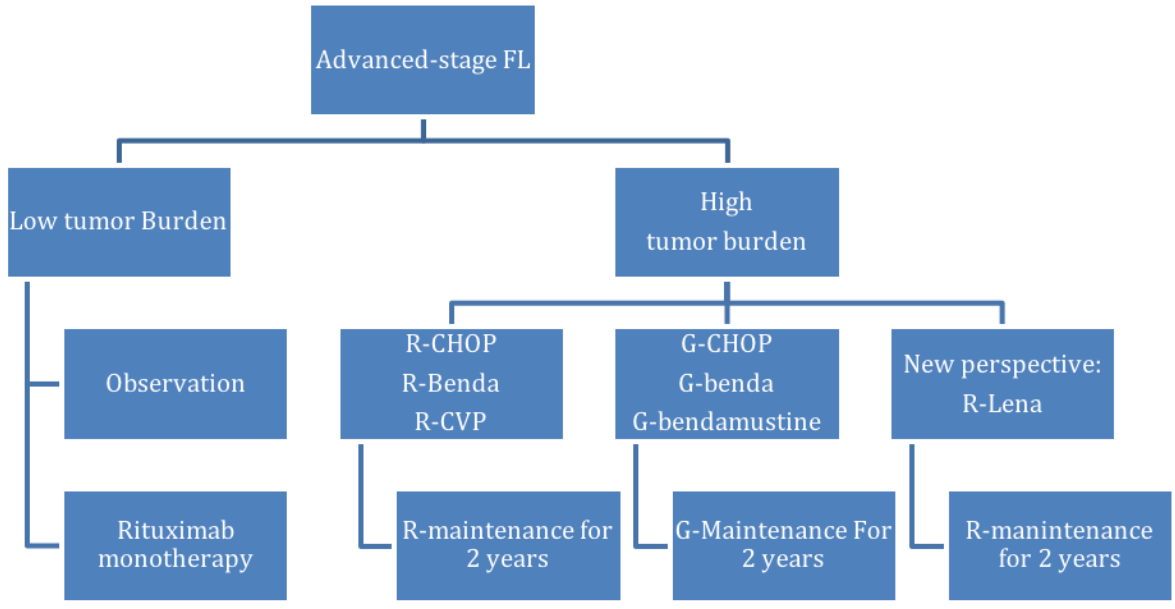

Figure 1. Treatment strategy for advanced-stage follicular lymphoma

Prognostic Indices

Prognostic studies in FL can be classified into two main groups: those based on baseline features and those based on post-treatment assessment.
The current prognostic indices in use are described below, combining the evidence coming from the literature with the new results deriving from our research.

Baseline Prognostic tools

In FL the most well-established prognostic factors include clinical, biological, or metabolic features such as Follicular Lymphoma International Prognostic Index (FLIPI), FLIPI-2, the baseline study of the Total Metabolic Tumor Volume (TMTV), and the biological indexes namely m7FLIPI, and the 23 gene predictor score.

FLIPI and FLIPI-2

FLIPI is the most common score based on patient's clinical characteristics used to predict the risk of disease progression or death. The index, based on a retrospective analysis of patients treated before the Rituximab era, incorporates 5 clinical variables that could influence OS (age >60 years, stage III-IV, hemoglobin <12 g/dl, number of nodal areas >4, and elevated level of lactate dehydrogenase (LDH). It recognizes 3 different categories of risk classified as low/good risk (0-1 adverse factors), intermediate-risk (2 factors), and high/poor risk (>3 factors). This latter group shows a 5-year OS rate of only 52.5% (27).
Although designed before the standard immunochemotherapy era, in a recent analysis of Sarkozy et al it has been noted that high-risk FLIPI score preserves its prognostic value also in Rituximab treated patients where the cumulative incidence of lymphoma related mortality is increased by 27% compared with low and intermediate score (4% and 10% respectively) (14).
FLIPI-2 is an updated version of FLIPI prospectively developed in a cohort of patients receiving Rituximab-based therapy having as primary endpoint correlation with progression free survival (PFS) instead of OS (28). Indeed, although OS should be the optimal endpoint, building an index with that endpoint is not informative given the natural indolent course of FL. It led to a critical need to identify surrogate endpoints that are measured earlier.
FLIPI-2 includes bone marrow infiltration and measurement of the largest mass of more than 6 cm in lieu of stage and nodal regions, and beta2microglobuline (B2M) instead of LDH.

Also in this case, patients were considered at high-risk if they had 3 to 5 risk factors and, similarly to FLIPI, high-risk patients accounted 27% and their 5-year PFS rate was only 18.8% (28).

In recent years, the ability of FLIPI and FLIPI-2 to discriminate patients at higher risk according to POD24 criteria has been questioned. Recently, a study by Jurinovich et al revealed some limitations of FLIPI-2 and heterogeneity among the POD24 group (29). Indeed, according the authors, FLIPI-2 seemed to overestimate the number of patients with poor outcome and inadequately captures early progressor patients reporting a sensitivity between 70% and 78% and a specificity of 56-58%. This heterogeneity is probably related to the proportion of patients considered at high-risk that did not present progression at 24 months (29). An analysis of correlation between FLIPI, FLIPI-2 and POD24 is one object of my Ph.D. thesis as below described (chapter 4, p.27)

Primary Rituximab and Maintenance Prognostic Index (PRIMA-PI)

Another clinical index, PRIMA-PI, estimated risk stratification in patients treated with chemoimmunotherapy, with or without Rituximab maintenance, and enrolled in the PRIMA study (30,31). It is a simplified model incorporating only bone marrow involvement and B2M level. Patients were considered at high risk of progression or death if they had B2M level higher than 3 mg/l reporting a 5-year PFS of only 37%.

Of note, an important limitation of its use in daily clinical practice is that the PRIMA-PI was predictive only in patients treated with R-CHOP or R-CVP and not considered bendamustine regime, today commonly used. Moreover, in contrast with data of PRIMA-PI, from GALLIUM Trial (that included patients receiving also bendamustine), it emerged that bone marrow biopsy was not predictive of outcome (32).

Finally, the role of PRIMA-PI was recently explored in the context of a chemo-free approach for patients enrolled in the RELEVANCE trial (Rituximab and Lenalidomide regimen). Despite during the construction of the model the authors did not explicitly evaluate the PRIMA-PI to assess POD24 , they found improved risk stratification for this index compared to FLIPI, especially in patients older than 60 years (33).

Follicular Lymphoma Evaluation Index (FLEX*)*

The introduction of new therapeutic strategies such as the combination of Obinutuzumab and Bendamustine (G-B) imposes the necessity to evaluate the role of prognostic tools also in patients receiving this combination. The novel model, called FLEX, was developed to

identify high-risk patients enrolled in the GALLIUM study, where patients received also G-B, and to compare this newer tool to FLIPI, FLIPI-2, and PRIMA-PI indexes. It includes 9 variables: male sex, sum of products (SPD) in highest quartile, grade 3A, >2 extranodal sites, Eastern Cooperative Oncology Group (ECOG PS) Performance Status >1, hemoglobin <12 g/dL, B2M higher than normal, peripheral blood natural killer cell count <100/μl and increased LDH. A high score (3-9 factors) was predictive of poor PFS (3-year PFS of 86% for low-risk vs 68% for high-risk), poor OS and increased risk of early progression (sensitivity of 60% vs 53% of FLIPI and FLIPI-2 and 69% for PRIMA- PI and specificity for POD24 of 68% with FLEX vs 59% for FLIPI and FLIPI-2 and 47% for PRIMA PI) (34).

Of note, FLEX is not used in clinical practice and further studies are desirable.

Biologic and genetic variables

M7- FLIPI

In the context of a precision medicine approach, Pastore et al made a first attempt to integrate clinical and biological data to create a clinic-genetic risk model and stratify more accurately high tumor burden patients receiving standard front-line R-CHOP (35). They used DNA deep sequencing to retrospectively analyze the mutation status of 74 genes and distilled them into 7 genes that subsequently combined with high-risk FLIPI status and ECOG performance status. These included mutations associated with shorter failure-free survival such as EP300, FOXO1, CARD11 and CREBBP genes, and those including EZH2, MEF2B and ARID1A that were associated with longer failure-free survival. The model, called m7-FLIPI identified a high-risk group of 28% of cases with 5-year failure-free survival of 38% vs 77% in a low-risk group. The score improved risk stratification by re-classifying patients previously defined at high-risk alone with FLIPI into the low group according to m7-FLIPI (35).

Moreover, to evaluate the sensitivity and specificity of m7-FLIPI in early progressors, Jurinovich et al developed a specific model known as POD24-PI (29). It includes FLIPI, ECOG performance status and the mutational status of only 3 genes, EP300, FOX01 and EZH2. They started from two independent series of patients with FL (GLSG 151 pts; BCCA 71 pts) and showed that the m7-FLIPI, had the highest accuracy to predict POD24 (76% and 77%, respectively in the two series). High-risk m7-FLIPI patients were significantly more likely to develop POD24 with an odds ratio (OR) of 5.82 and 4.76 in GLSG and BCCA

patients. Compared to FLIPI, the specificity of the m7-FLIPI to identify POD24 increased from 56% to 79%, and 58% to 86%, respectively (29).

It's important to note that in a recent analysis of GALLIUM study, although the m7-FLIPI maintains its prognostic role in CHOP/CVP patients and outperformed the FLIPI, it lost its significance with different chemotherapy backbones (36).

23-Gene Expression Profiling Model

The investigators of the French Lysa Group performed a gene expression profiling using nanostring technology from 160 untreated high tumor burden patients enrolled in the PRIMA trial (37). The authors identified 23 out of 395 genes related to tumor microenvironment and B cell biology and associated them with a risk of progression.

In a multivariate Cox model for progression-free survival also after adjustment for Rituximab maintenance and FLIPI, this index identified a group with a higher risk of progression (accounted for 21-35% of patients) with a 5-year PFS of 26% (95% CI, 16–43) vs 73% (95% CI, 64–83) in the low-risk group (HR= 3.68).

In the combined validation cohort, the proportion of patients with POD24 was 19% (95% CI 15–24%) in patients with a low predictor score (low-risk group), but 38% (29–46%) in patients with a high predictor score (high-risk group), showing the model's ability to predict early relapse (37).

Similar to observations made for m7-FLIPI, a recent study by Bolen et al demonstrated that the prognostic information obtained from the 23 GEP model varied according to chemotherapy administered (38). In the Gallium study, the nanostring technology model identified a high-risk group with a dismal outcome in CHOP/CVP chemotherapy backbone and an opposite result in bendamustine treated patients (38).

Generally, despite both biological models, m7FLIPI and 23GEP, reveal important features of disease biology, we highlight some limitations mainly due to the reproducibility of results with different chemotherapy backbone and lack of clinical validation in the context of prospective studies and in different subgroups of patients (i.e. low tumor burden cases and patients treated with novel drugs). Additionally, they still cannot be used to drive target therapies.

In summary, although all these baseline prognostic factors are important steps towards the goal of personalized care, they also raise new questions that should be addressed. Indeed, all of them are restricted to baseline measurements, are usually applied to patients with a high tumor burden and have not yet been validated as robust tools to select or adapt therapy to overcome the bad outcome associated with early progression. Furthermore, the

introduction of new therapeutic strategies, such as Obinutuzumab and bendamustine, increasingly in use, or a chemo-free therapeutic approach, requires the validation of all these models in the current clinical practice.

The Table 2 describes the most relevant clinical baseline prognostic models used and their correlation with early progression.

Table 2. Clinical and biological variables associated with High-Risk FL patients in Pre e Rituximab era.

Risk Model	FLIPI(27) Pre-Rituximab Era	FLIPI-2(28) (50% Rituximab)	PRIMA-PI(30) Rituximab Era	FLEX(34) Rituximab era	POD24-PI(29)
Adv. Risks Factors	-Age >60 y -Stage III-IV -Hb < 12 g/dl -LDH > UNL ->4 nodal sites	-Age > 60 y -BM involvement -Hb< 12 g/dl -B2M>UNL -Nodes >6 cm	-BM involvement -B2M >3 mg/l	-Male sex -SPD in the highest quartile -Histologic grade 3A ->2 extranodal sites -ECOG >1 -Hb < 12 g/dl -Elevated B2M -NK cell count > 100/micr -Elevated LDH	ECOG>1 -High-FLIPI -EP300,EZH2, FOX01 mutations
High Risk definition	3-5 Risk Factors	3-5 Risk factors	B2M >3 mg/l	3-9 factors	High FLIPI and mutational status
5-y PFS for High Risk group	44%	41%	37%	3-year PFS 68%	38%
Sensitivity for POD24 Specificity for POD24	66% 60%	58% 68%	43% 80%	60% 68%	76% 79%

Postinduction prognostic factors

During the last years, metabolic assessment of FL has become the standard for the response assessment following first-line chemoimmunotherapy. Moreover, it is well established that the achieving of a complete metabolic response on a Deauville five-point scale (DS) with 18-F-fluorodeoxyglucose (FDG)-positron emission tomography (PET) is

highly prognostic of disease progression or death (39–42). Along with that, also molecular evaluation through Minimal Residual disease (MRD) has been suggested as an important prognostic tool. MRD evaluation and FDG-PET are both identified as potential factors to guide personalized treatment as explored in our FOLL12 trial (chapter 4, p. 33).

PET- based markers

End of Induction FDG-PET

FDG-PET is a functional imaging technique that plays a relevant role in the management of lymphomas in the baseline staging, evaluation of metabolic response to treatment, and for the identification of disease recurrences (43).

The role of end of induction (EOT) FDG-PET and its impact on FL outcome were explored by Trotman et al in a large pooled analysis of 246 patients retrospectively retrieved from PRIMA, FOLL05 trial, and from one prospective study by the Lysa group (42,44,45).

Overall, 17% of patients were classified as non-responders using the Deauville Score 4 as cut off to define PET positivity. PET+ patients had a significantly lower PFS of 23% compared to 63% for those who achieved a complete metabolic response (HR= 3.9). Similar results were found also for OS with 4-year OS of 87% versus 97%, respectively (HR= 6.7). Estimates of the differences between groups were determined using a stratified Cox proportional hazards analysis and expressed as HRs (41). Data were validated by an additional study (46).

PET response and Total Metabolic Tumor Volume (TMVT)

Very recently, the total metabolic tumor volume (TMTV) emerged as a novel metabolic parameter reflecting the tumor burden of the disease. Its measurement is obtained from PET images processed with a specific software calculating the sum of the metabolic volume of each lesion. A threshold of 41% of the maximum signal intensity was used to delineate MTV as recommended by the European Association of Nuclear Medicine (47). Interestingly, in newly diagnosed FL, it has been shown that TMTV correlated with unfavorable clinical outcomes. Meignan et al showed that the cut-off of 510 cm^3 or greater could strongly predict the clinical outcome (48).

Cottereau et al combined metabolic response and TMTV in 159 patients with advanced stage FL (49). In the univariate analysis both high TMTV (>510cm3) and positive EOT-PET were independent, significant risk factors for PFS. Their combination stratified the population

into three risk groups: 5-year PFS was 67%, 33% and 23% for patients without risk factors, for those with one adverse feature and for patients with both adverse factors respectively; 10%, 39% and 54% were POD24 in the three groups, respectively. This model enhanced the prognostic value of PET staging and response assessment and allowed the identification of a small subset of patients with a very high risk of progression within 24 months (50).
The analysis of TMVT's role in patients enrolled in the FOLL12 trial is currently ongoing.

Molecular Response

The molecular hallmark of FL is the chromosomal translocation (t) 14;18, resulting in the rearrangement that juxtaposes the BCL2 gene (normally located on chromosome 18) to the enhancer of the immunoglobulin heavy chain variable (IgH) gene (located on chromosome 14), thus leading to a constitutive expression of the BCL2 protein.
The spread of high sensitivity techniques to detect this translocation in peripheral blood and bone marrow sample makes it feasible to support diagnosis and to work on the concept of molecular tumor burden and molecular response (51,52).
The assessment of MRD through quantitative polymerase chain reaction (PCR) analysis for t(14;18) and IG gene rearrangement was the basis for a prospective study on 128 FL patients treated with sequential CHOP and Rituximab therapy conducted by Rambaldi et al (53). Molecular response (PCR negativity) was achieved in 32% of cases after CHOP and rose to 57% and 75% after Rituximab and during follow-up, respectively. For patients with a durable PCR-negative status, a better clinical outcome was also observed since Freedom from Recurrence (FFR) was 57% compared to 20% of the patients who never achieved or lost the molecular negativity. In a second paper, Ladetto et al studied the concept of molecular response in a randomized trial for untreated high-risk FL patients that compared standard R-CHOP with High-Dose therapy implemented with Rituximab (R-HDS). Molecular remission was achieved in 44% of R-CHOP and 80% of R-HDS patients and was the strongest independent outcome predictor suggesting that achieving molecular remission is critical for an effective disease control, regardless of the treatment used (54).
An analysis of the role of molecular tumor burden and response was also conducted in patients enrolled in the Italian FOLL05 trial. At the time of diagnosis, the t(14;18) was detected in the bone marrow of 53% of cases. Patients without molecular marker or with a low molecular tumor burden (<1×10−4 copies) showed a higher complete remission rate and longer PFS compared to MRD+. High BCL2/IGH level were documented in patients with high FLIPI and FLIPI-2 scores. Moreover, PFS was significantly conditioned by the PCR

status at 12 and 24 months, with 3-year PFS of 66% for MRD− cases versus 41% for those MRD+ at 12 months (p=0.015), and 84% versus 50% at 24 months (p=0.014) independently from the randomization treatment arm (55). Survival curves were calculated using the Kaplan–Meier method, and statistical comparisons between curves were made using the log-rank test.

Recently, an analysis of molecular marker (MRD analysis) was also conducted in the Gallium trial, where it was detected in 1101 patients, a higher number of patients compared to other studies (56). This result could be explained by the use of consensus PCR to screen MRD. The analysis revealed a higher rate of negative bone marrow and peripheral blood MRD in R-chemotherapy at EOT compared with other studies and further increased in G-chemotherapy arm independently from chemotherapy backbone. After a median follow-up of 57 months, the prognostic role of MRD negativity was confirmed by better PFS in MRD-group compared to MRD+. Moreover, a better OS was also observed (56).

More recently the prognostic role of MRD was also evaluated in 444 patients enrolled in the Relevance trial (chemo-free approach). MRD was quantified for the first time by droplet digital PCR (ddPCR). In the study, molecular tumor burden at diagnosis was significantly associated with MRD status at the end of therapy and correlated with PFS (3-year PFS 84% vs 55% for MRD- and positive, respectively)(57).

However, there are still conflicting data regarding the use of MRD in clinical practice, principally limited by lack of consensus and standardization of MRD techniques and timing and by the lack of this molecular marker in all patients with FL; the rate of patients without a measurable marker is around 30% when MRD is studied with t(14;18)t, and this rate can be only partially improved with better methods and technologies (VDJ region analysis or rarer breakpoint regions of BCL2/IGH chromosomal translocation). It is hoped that technical advancements currently under investigation will overcome these limitations.

During the last years, the concept that tumor undergoing apoptosis or necrosis cells release cell-free circulating DNA (cfDNA) into the blood enabled the use of whole exome sequencing (“next-generation sequencing technologies” – NGS) to detect tumor presence from blood samples. Moreover, cfDNA can characterize, at diagnosis or during treatment, mutations that may contribute to the choice of an optimal targeted therapy, or detect the emergence of resistance to therapies.

Real-time follow-up of cfDNA levels during therapy in several lymphoma subtypes has been explored: preliminary studies have demonstrated that this monitoring technique can predict

clinical outcomes and that this approach may complement the information provided by metabolic imaging assessments (58,59).

Recently, Delfau et al showed an association between cfDNA and circulating tumor cells (CTC) with TMTV >510 cm^3 in newly diagnosed follicular lymphoma (60). Lower CTC was associated with better PFS (86% vs 58%) emphasizing the impact of cfDNA levels and TMTV in risk stratification of FL patients.

Combined models: PET and MRD response

Analyzing the cohort of patients enrolled in FOLL05 trial, Luminari et al combined EOT-FDG evaluation and molecular response in a small group of 41 patients for whom both MRD analysis and central review of post-induction PET were available. PET/MRD concordance was 76%, with Kappa=0.249, suggesting that PET and MRD are not strongly correlated at the end of induction. Overall, at the end of induction, MRD positivity rate was 27% whereas PET positivity rate was 11%. When combining information on PET and MRD into 2 groups (PET−/MRD−vs. PET+ or MRD+), the achievement of both PET and MRD negativity (32% of cases) was associated to a better outcome, with a 5-year PFS of 75% and 35% for PET/MRD −/− and PET+ or MRD+, respectively.

Cohen's kappa statistic was used to verify the agreement between PET and MRD results.

Although conducted on a small series of patients, this study showed that combining both EOT-PET and MRD may improve our ability to predict the risk of progression (61).

A combination of prognostic factors, both clinical, metabolic, and molecular, despite able to identify only a small percentage of patients, could represent the starting point for integrated models of more detailed and sensitive risk definition.

Chapter 3. Marginal zone Lymphoma Overview

Marginal zone lymphomas are a group of Indolent Non-follicular B-cell lymphomas (INFL) comprising approximately 10% of all NHL.

The WHO classification includes three distinct clinical-pathological diseases comprising, in order of incidence, extranodal marginal zone lymphoma (ENMZL), about 70% of cases, nodal marginal zone lymphomas (NMZL) representing 20% of all, and finally, splenic marginal zone lymphomas (SMZL) accounting less than 10% (62,63).

The three clinical entities have distinct diagnostic criteria, specific biological characteristics, a different and heterogeneous clinical behavior, and alternative therapeutic implications (64).

The majority of patients usually have an indolent attitude and benefit from a long progression free survival and overall survival measured in decades. However, similar to FL, a small but significant group of patients present a high risk of relapse owing to a dismal prognosis (65–69).

Low-grade non-follicular lymphomas displayed many other common characteristics with FL as indolent behavior, treatment management and long-lasting survival. Moreover, several demographic, clinical and biological prognostic factors had the same relevance both for MZL and FL when evaluated in different retrospective series (70,71). An exception is represented by gastric marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT), which emerged as a very peculiar entity with well-defined pathogenesis and specific therapy, whose prognostic stratification could be considered aside (72).

It is important to note that, while many efforts have been made to better define the prognosis of follicular lymphoma patients, a widely accepted prognostic tool for clinical usage for all indolent non-follicular B-cell lymphomas is still lacking. Indeed, only few studies have investigated the prognostic features of the remaining group of indolent B-cell lymphomas. Furthermore, the studies available mainly focused on defining prognostic factors in single lymphoma subtypes, with the development of specific-entity scores based on retrospective series, for example splenic marginal zone lymphoma score.

Given the rarity of the disease, the definition of homogeneous and prospectively defined criteria that can early identify patients at increased risk of poor outcome is still a challenge (66,67,69). Only recently, the length of duration of response as POD24 has emerged as an important prognostic tool (18,73).

Here, a brief description of the main characteristics, prognostic indexes and treatment of marginal zone lymphoma subtypes is reported.

Extranodal Marginal Zone Lymphoma (ENMZL) is the most frequent MZL subtype. Known also as MALT, it is arisen from mucosal sites and often involved an extranodal site (stomach, lung, and the ocular adnexa) presenting a localized stage. The Limited stage usually carries a good prognosis with a 5-year PFS and OS of 74% and 92%, respectively (74).

A prognostic model for MALT lymphoma (MALT-IPI) was developed with the aim to identify patients with a more aggressive disease at diagnosis. The score was derived from analysis of clinical characteristics of 401 patients enrolled in the IELSG-19 trial and subsequently validated in a separate cohort of 633 cases (67). It incorporates three clinical factors: age>70-year, elevated LDH, advanced stage, and it defines three different categories of risk: low (0 factors), intermediate (1 factor), and high-risk (> 2 factors). Each category has a 5-year progression risk equals to 70%, 56 and 29%, respectively. EMZL localized outside of the gastrointestinal tract seems to be associated with a shorter progression free survival but, generally, the prognostic value of the primary anatomical site is still controversial (75,76).

Recently, Alderuccio et al observed a difference in PFS and OS based on the primary localization in stage I ENMZL treated with radiotherapy. In addition, the authors underlined that these differences may be attributed to therapies used (77).

Other clinical factors correlating with a worse outcome were: the dissemination of disease and the involvement of multiple sites defined by the presence of disease in two or more different anatomical organs independent of spleen and BM involvement. Alderuccio et al found a significant shorter PFS (1.7 vs 13.2 years) and OS (10-years OS 40.5% vs 81.1%) in patients with a disseminated disease compared to localized one (78).

Greater incidence of transformation in aggressive lymphoma was also observed for multiple site disease with a 5-year cumulative incidence of 13% vs 26% (78).

The treatment strategy comprises several options. For patients with gastrointestinal localized disease or for ocular adnexal MALT, often correlated with an infectious disease, the eradication therapy for Helicobacter Pylori or Chlamydia Psittaci can lead to regression of lymphoma in 75% of cases (79,80). In the cases not related to an infection or in which eradication therapy had failed, other treatment options include involved site radiotherapy, Rituximab monotherapy and chemoimmunotherapy such as R-CVP or R-bendamustine for advanced stage and symptomatic disease (81–84).

Nodal Marginal Zone Lymphomas (NMZLs)

The clinical outcome for this subgroup is very similar to other indolent lymphomas despite historically, due to their advanced stage and bone marrow infiltration, NMZLs were thought to have a less indolent course in comparison to ENMZL. However, very few studies evaluated prognostic factors and indolent behavior in NMZL (85,86). In different retrospective studies, 5-year PFS ranged from 55% to 96% (86–88). As well as for follicular lymphoma, FLIPI score was found to be predictive of OS (89).

For NMZLs, treatment strategies are mostly extrapolated from FL. Patients with an asymptomatic low tumor burden are suitable for a watch and wait strategy, whereas incorporation of chemoimmunotherapy may be reserved for patients with high tumor burden and symptomatic disease.

Splenic Marginal Zone lymphoma (SMZL) is an uncommon type of indolent non-follicular lymphoma typically involving the spleen, splenic hilar lymph nodes, bone marrow, and peripheral blood**.** Usually, SMZL patients benefit from a long survivor. However, about 30% have a worse outcome with a 5-10% of the risk for transformation in aggressive lymphoma (90,91).

Two scores have been proposed to identify SMZL patients at risk for shorter survival (66,70). The HPLL score (hemoglobin level, platelet count, elevated LDH and extra-hilar lymphadenopathy) was developed based on an international analysis of 593 patients. The index discriminates three risk groups with different lymphoma-specific survival (LSS) of 94%, 78% and 69%, respectively for low, intermediate and high-risk group (66,92).

The other score system was developed by the Intergruppo Italiano Linfomi (IIL), which identified in a large series of 309 patients hemoglobin <12 g/dL, elevated LDH, and albumin <3.5 g/dL as independent variables associated with poor outcome (70). The 5-year cause-specific survival rates (CSS) were 88% for the low-risk group, 73% for the intermediate-risk group, and 50% for the high-risk group.

A recent SEER database analysis found that age >60 years, Hispanic ethnicity, presence of B-symptoms, histologic transformation, and treatment with non-Rituximab containing chemotherapy were associated with shorter LSS (93).

While asymptomatic patients can be managed with observation, the presence of symptomatic splenomegaly, cytopenia for bone marrow involvement and/or hypersplenism or autoimmune destruction, mass effect or effusion or local compressive effect, B-symptoms or bulky lymphadenopathy are usually indicative for treatment. When therapy is needed,

frontline options include Rituximab monotherapy or chemoimmunotherapy. Splenectomy in the modern era is a treatment option beyond the second line setting (94–96).

Summing up, in MZL poor-risk clinical features have been elucidated in recent years. However, if compared with FL, factors associated with adverse outcome or risk of transformation remain scarce. Existing studies generally include diseases with different presentations, data on treatment are often extrapolated from trials involving patients with indolent B cell lymphomas not specifically MZL or have been retrieved from retrospective series. It is important to note that retrospective evaluation of some study parameters, for example clinical response, cannot be easily defined and all derived endpoints, such as failure or progression-free survival, may be biased. Finally, the results of a retrospective analysis aiming at the evaluation of survival are dependent on the type of administered treatment, and with the introduction of therapies such as monoclonal antibodies and purine analogues, the role of some established prognostic factors may have changed. Finally, these indexes currently cannot guide treatment decisions at diagnosis or relapse.

Consequently, as well as for follicular lymphoma, there is a place for improvement and development of novel clinical indexes that can better identify patients with short survival and that may need different therapeutic approaches also for marginal zone lymphoma patients.

Chapter 4. From literature to our contribution: thesis results for follicular lymphoma studies

Analysis of POD24 in FOLL05 Trial

As previously described, strong predictors of survival are now available in FL, including both baseline and post-induction tolls. Beside the well-established prognostic indices, the use of molecular-based predictors, time of progression and diagnostic imaging are gaining momentum as more sensitive methods to refine risk with the promise of a more personalized approach to treatment on the concept of precision medicine (i.e. one patient, one treatment). The first part of my Ph.D. project was devoted to validating the role of POD24 in our series of patients with advanced stage FL prospectively enrolled in the FOLL05 trial (ClinicalTrial.gov Identifier: NCT00774826) and to exploring the power of the prognostic indices available in clinical practice to early predict progression.

FOLL05 Trial

In 2005, the Fondazione Italiana Linfomi launched the FOLL05 study, a multicenter prospective randomized trial, conducted to compare the efficacy of the anthracycline-containing regimen, CHOP, with that of the purine analogue fludarabine combined with mitoxantrone (FM), with a reference treatment with CVP, all combined with Rituximab. 534 patients were enrolled (7,97).

In 2013, we published the primary analysis of the study for the 534 patients enrolled, with a median follow-up of 34 months, showing the superiority of R-CHOP and R-FM over R-CVP in terms of time to treatment failure and PFS, but also revealed a better toxicity profile of R-CHOP compared with R-FM. Overall, these data were interpreted to suggest R-CHOP as the standard ICT for the treatment of advanced-stage FL patients.(97)

In 2018 we performed an update of the data, with a median follow-up for the entire group of patients of 84 months (7).

With the mature follow-up, the most relevant prognostic features and indexes, were reassessed, including FLIPI-2, PET response and MRD analysis.

Moreover, among the promising prognostic parameters, new data on POD24 were analyzed and correlated with other available indexes.

This analysis aimed to validate the prognostic role of POD24 in patients who received first line chemoimmunotherapy in FOLL05 cohort, and to verify their power as surrogate endpoints when combined with FLIPI-2 score to improve the identification of patients at high risk of progression and worse outcome.

Patients and Method

The FOLL05 trial included patients aged 18 to 75 years, with histologically confirmed diagnosis of grade 1, 2, or 3a FL according to WHO classification, Ann Arbor stage II to IV, Eastern Cooperative Oncology Group performance status of 0 to 2, and active disease according to the Italian Society of Hematology guidelines. Patients ineligible for enrollment included those with grade 3b FL, Ann Arbor stage. This study was conducted in compliance with the Declaration of Helsinki and in accordance with Good Clinical Practice rules and was approved by a research ethics committee. All enrolled patients provided written informed consent.

Patients were randomly assigned to receive a first-line treatment comparing R-CVP, R-CHOP and R-FM.

Follow-up updates were actively conducted among participating institutions. Relapses or progressions were based on clinical or radiological assessment. For the analysis of PET and MRD, patients should have data available on the end of treatment PET, performed up to 3 months after the last dose of induction and have been assessed for the BCL2/IGH translocation at diagnosis and the end of therapy within 2 months from last dose.

PET was centrally reviewed by three independent nuclear medicine physicians applying the Deauville scale. Positive scans (PET+) were defined by residual FDG uptake ≥ score 4 (i.e. moderately increased uptake > liver uptake). The final result was selected by agreement between at least two of three reviewers.

Endpoint definition

The primary endpoints of this analysis were POD24 and OS according to POD24 data. The POD24 was defined as the progression of disease within 2 years after diagnosis. OS was calculated from the time of POD until the date of last follow-up or death for any cause for early progressors, and from two years after diagnosis for the group without POD or who experienced POD after 24 months until the date of last follow up or death for any cause.

Statistical Analysis

Regarding the baseline data analysis, categorical variables were reported as absolute frequencies and percentages and were compared through the use of Fisher's exact test.

When not otherwise indicated, $p < 0.05$ was considered statistically significant.

When the binary variable POD24 was used as surrogate endpoint, the association between the prognostic factors and POD24 was estimated using the logistic regression, and the effect due to the regressor was expressed as odds ratio (OR) with 95CI. The OR is the probability

of occurrence of the early progression given the exposure to a prognostic factor, compared to the probability of the early progression without exposure to that prognostic factor.

OS were calculated using the Kaplan-Meier method with 95% confidence interval (95CI) based on the Greenwood's formula of the variance, and statistical comparisons between curves were performed using the log-rank test.

All statistical analyses were performed using STATA statistical software (release 10.1; College Station, TX).

Results

From March 2006 to September 2010, 534 patients were recruited into the FOLL05 trial, by 58 Italian Institutions (list of centers is shown in the appendix). A total of 502 patients enrolled in the FOLL05 trials met the required selection criteria for the POD24.

Overall, out of the 502 patients evaluable for the analysis, 141 (28%) experienced a relapse/progression of lymphoma within 24 months after the diagnosis. The remaining 361 patients had no relapse or death during the first 24 months and were defined as the reference group. For the POD24 group median age was 57 years (range 35-74); 77 (54.6%) were male. The early progressor patients were equally distributed among the three treatment arms.

In our analysis, high FLIPI and FLIPI-2 were equally accurate to predict POD24 as shown through an analysis of ROC area of 0.593 for FLIPI 2 and 0.567 for FLIPI, p=0.010 for FLIPI and 0.001 for FLIPI-2 (Table 3). The FLIPI-2 tended to be more discriminative.

Table 3. Distribution and association of patient's characteristic by POD 24 in FOLL05 study.

Factor		**POD24, n (% by row)**		
		No	**Yes**	**p-value**
Age	Median (range)	55 (29-75)	57 (35-74)	0.087
Treatment	R-CVP	111 (66)	56 (34)	0.139
	R-CHOP	121 (73)	44 (27)	
	R-FM	129 (76)	41 (24)	
Age	≤60	246 (73)	90 (27)	0.399
	>60	115 (69)	51 (31)	
Gender	M	185 (71)	77 (29)	0.551
	F	176 (73)	64 (27)	
FLIPI	0-1	80 (73)	29 (27)	0.010
	2	160 (78)	45 (22)	
	3	121 (64)	67 (36)	
FLIPI-2	0	75 (86)	12 (14)	0.001
	1-2	200 (71)	80 (29)	
	3-5	86 (64)	49 (36)	

The association between early progression and clinical prognostic features was analyzed by means of multivariable logistic regression with the effect expressed as odds ratio with 95% CI (data not published)

We analyzed the association between early progression and clinical prognostic features using multivariable logistic regression, with the effect expressed as odds ratio with 95% CI. FLIPI-2 >2 resulted in a powerful predictor of progression of disease for POD24, p<0.001. Moreover, in our experience, we evaluated the association between EOT-PET and early progression. From this analysis, we found that metabolic response was significantly associated with POD24. For patients with PET negative at the end of therapy, the risk of early progression was 18% compared with 38% for patients with PET + (p=0.009) (Table 4).

Table 4. Metabolic Response at the end of treatment and its correlation with early progression of disease.

POD24	**PET, n (%)**		
	-	**+**	**p-value**
Achieve	128 (82)	30 (62)	0.009
Fail	28 (18)	18 (38)	
ROC Area	*0.601*		
Sensitivity	*39%*		
Specificity	*81%*		
PPV	*38%*		
NPV	*82%*		

In addition, we also conducted an analysis aiming to associate MRD status with POD24. Interestingly, the MRD negativity was associated with a lower risk of early progression of 19%, while for MRD+ patients the risk was 38% (p=0.007) (Table 5).

Table 5. Multivariable logistic regression analysis of molecular response assessment and early progression in the POD24 Cohort enrolled in FOLL05 trial

POD24	**MRD, n (%)**		
	-	+	
Achieve	135 (81)	31 (62)	0.007
Fail	31 (19)	19 (38)	
ROC Area	*0.597*		
Sensitivity	*38%*		
Specificity	*81%*		
PPV	*38%*		
NPV	*81%*		

Finally, after a median follow-up of 84 months, a significant difference in OS was observed between the early progressors and the reference group. Indeed, the 7-year OS was 59% (95% CI, 48-68) and 91% (95% CI, 87-94) for patients with or without POD24, respectively (p<0.001) (Figure 2).

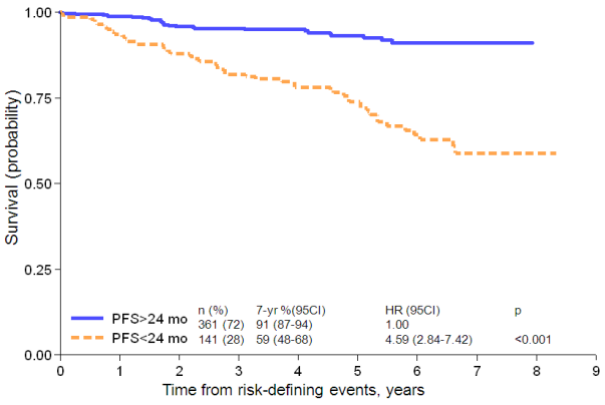

Figure 2. OS by POD24 (pts 502)

Discussion

Despite the improved effectiveness of chemoimmunotherapy, and the indolent course for most follicular lymphoma patients, high heterogeneity exists in the clinical behavior. The awareness of the importance of risk stratification in FL is rapidly growing and becoming essential for the approach to patients. The identification of patients who present an aggressive clinical course and have inferior survival with a standard therapeutic approach is a crucial step to modify the natural history of the disease. In our analysis, in patients with advanced stage FL who received standard ICT, we provided an accurate evaluation of predictive factors associated with POD24 and confirmed their prognostic role and validated the use of both as surrogate endpoints for the early assessment of patient outcome. In details, we sought to evaluate whether baseline clinical features can predict POD24 in patients with FL. In multivariable regression analysis, factors that were significantly predictive of POD24 were high FLIPI and FLIPI-2.

We confirmed this finding not only for patients treated with R-CHOP, as in the original report by Casulo et al (19), but also for patients treated with R-CVP or R-FM regimen.

Moreover, an exploratory analysis of EOT-PET and MRD was performed demonstrating their role as useful tools in predicting the early progression. Nevertheless, when FOLL05

was designed, PET was not acknowledged as a recommended procedure for staging and response assessment in FL, thus it was not included among the planned study procedures; however, it was performed at physician discretion in a substantial proportion of cases. Both PET+ and persistence of molecular marker at the end of induction were associated with a higher risk of early recurrence.

All these observations are crucial as they suggest that different objectives should be defined for patients with FL; higher chances of cure, likely achieved with innovative therapies, should be offered to patients experiencing early events, while careful preservation of quality of life, without unwanted side effects, should be the goal of patients with late progressions.

These results emphasize the usefulness of EOT-PET and the MRD analysis as decision-making tools to modulate the treatment of patients. As a consequence, this finding reinforces the scientific rationale of the FOLL12 study, which represented a first and original study of personalized medicine for the treatment of FL.

Risk and response adapted therapy for follicular lymphoma patients: the FOLL12 trial

Introduction

Although results of randomized trials confirmed that the standard chemoimmunotherapy followed by a maintenance approach made a step forward in the management of patients and prolonged survival, one important question that can be raised is if this treatment is really needed or adequate for all patients with FL, or if some of them could benefit from a reduced intensity treatment, or if there is room for intensified therapy for high-risk patients achieving the same results in terms of outcome and survival.

The therapeutic decision taken in newly diagnosed FL patients could be associated with the best results in terms of response rates and time to progression, but might also act on subsequent risks (i.e., histologic transformation, late toxicity, chemo-resistance). Most importantly, in a significant proportion of patients, first-line therapy could be the only treatment that patients receive in their lifetime. Taking into account that among the available treatment options none has ever been associated with an OS advantage, it is very important to understand the pros and cons of available alternatives for the initial management to optimize medical intervention.

Even if all the currently available prognostic models have contributed to a better definition of the risk, some limitations and many open questions emerged. POD24 resulted in a robust prediction of outcome, but it was useless to upfront identify high-risk patients as a postinduction factor. Other baseline models are still inaccurate and unstable with different therapeutic strategies. Moreover, there are still many open questions regarding their applicability in clinical practice and, especially, their use to guide the best therapeutic choice in the perspective of personalized medicine. On the other side, more recent data demonstrated that the outcome of patients can be further predicted by evaluating the quality of response to therapy, through the combination of the well-established prognostic factors making the prognostic definition of the risk more accurately.

In this context, an important question is whether chemoimmunotherapy followed by maintenance could be modulated based on current prognostic criteria.

For all these reasons, based on previous studies on prognostic models, and driven by the fact that the concept of treatment adaptation has not yet been extensively studied in FL, the Fondazione Italiana Linfomi designed the randomized FOLL12 trial to investigate the efficacy of a response-adapted strategy in advanced stage FL patients (ClinicalTrials.gov Identifier: NCT02063685). Our trial was the first to use a simple feature like the quality of response to induction therapy (based on FDG- PET and MRD response assessment) as a predictive factor.

The study aimed at evaluating whether a PET and MRD response-based therapy were non-inferior when compared to standard Rituximab maintenance therapy in terms of PFS.

The preliminary results of the study were object of my specialization thesis. Final results were recently published in the Journal of Clinical Oncology ("Response-Adapted Postinduction Strategy in Patients with Advanced-Stage Follicular Lymphoma: The FOLL12 Study" Luminari et al doi:10.1200/JCO.21.01234).

The design of the study is shown in Figure 3.

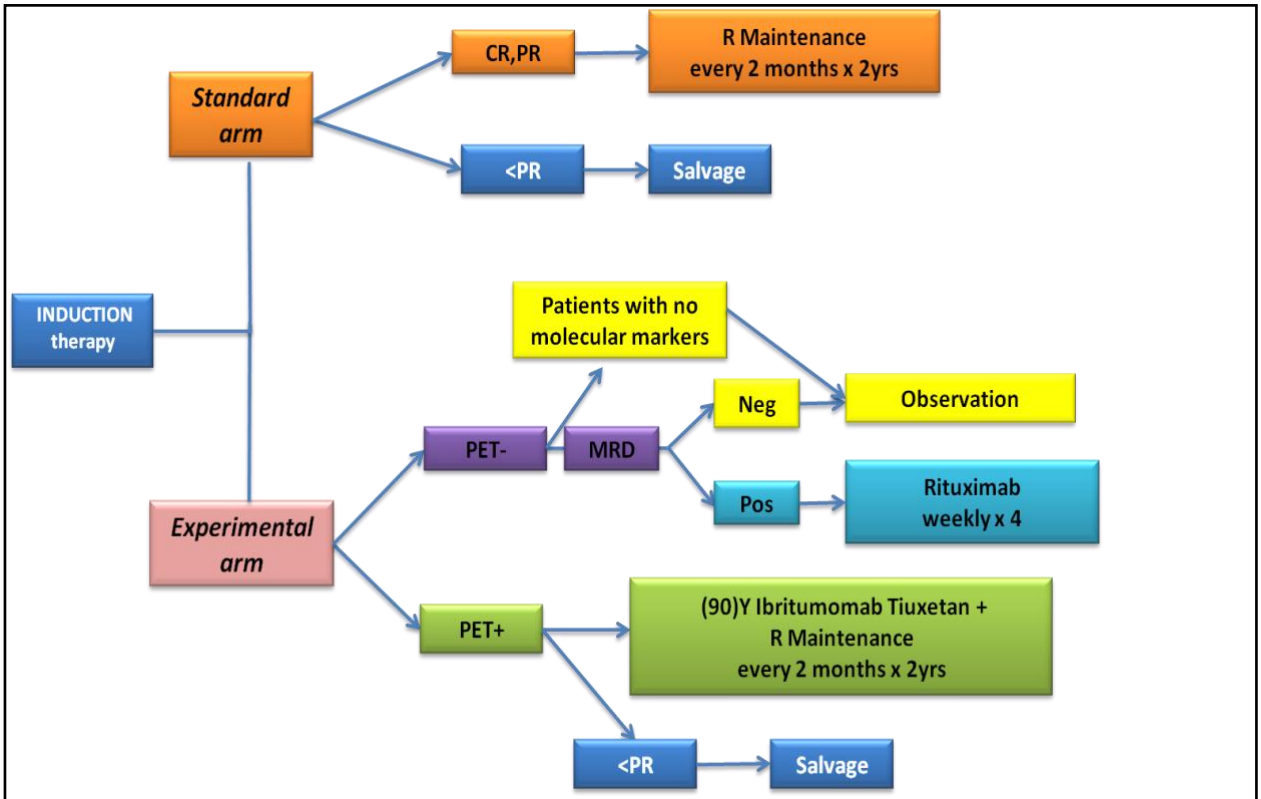

Figure 3. FOLL12 Study design

Methods

Foll12 is a multicenter, phase III, randomized study in which 807 patients were enrolled. Eligible patients had a histological confirmed diagnosis of FL from grade 1 to 3a, according to the WHO classification (62), with FLIPI-2 more than 0 , stage II-IV, ECOG 0-2. They required therapeutic intervention according to GELF criteria (Table 1, p. 13) (24). They were randomly assigned to either standard or experimental response-driven therapy. After a common induction treatment consisting of 6 cycles of R-CHOP or R-bendamustine followed by 2 additional doses of Rituximab, responding patients in the standard arm received Rituximab maintenance therapy (every 2 months for 2 years), while responding patients in the experimental arm were assigned to different post-induction treatments based on PET and MRD results.

FDG-PET was centrally assessed by three expert blinded reviewers and was defined applying the 5-point Deauville scale (DS). Complete metabolic response (CMR) was defined by DS scores 1 to 3; while positive EOI FDG-PET was defined by a Deauville score of 4 or 5. PET and MRD negative patients underwent observation, PET-negative but MRD-positive patients received pre-emptive Rituximab therapy (4 weekly doses for a maximum of 3 courses until negativizing of MRD) and PET-positive patients, regardless of their MRD status, received consolidation with (90)Y Ibritumomab Tiuxetan prior to starting conventional Rituximab maintenance. The study was conducted in compliance with the Declaration of Helsinki, was accepted by the appropriate Research Ethics Committee, and required each patient to give written informed consent prior to registration and randomization.

Statistical consideration

The primary endpoint was PFS, defined as the time from the date of study entry to the last follow-up, or to one of the following events: disease progression or relapse confirmed at CT scan or to the date of death from any cause.

Additional study endpoints were OS, response, and toxicity. OS was defined as the time from study entry until the date of death. Patients who had not died at the time of end of the whole study, and patients who were lost to follow-up, were censored at the date of the last contact. The response was defined according to international criteria.

All statistical analyses were performed using the Stata Statistical Software, Release 14.2. Survival curves were calculated using the Kaplan-Meier method, and statistical comparisons between curves were made using the log-rank test. Post-hoc comparisons between PFS curves, adjusted by potentially confounding factors, were obtained using the Cox

proportional hazard (PH) regression method. The Chi-squared test, Fisher exact test and Kruskal-Wallis test were used to compare variables when appropriate. All statistical comparisons were two-sided.

Results

Between December 2012 and March 2018, 807 patients were randomized by 50 Italian institutions (list of centers is shown in the appendix).

Seven hundred and forty-four patients (368 in the reference and 376 in the experimental arm, respectively) completed the induction therapy and were assessed for response. Clinical characteristics at baseline was reported in Table 6 (p.37)

PFS data

After a median follow-up of 53 months (range 1-92 months), 197 events for PFS were recorded, including 186 disease progressions and 11 deaths for causes unrelated to lymphoma progression. Overall, the 3-year PFS was 79% (95%CI, 76-82), being 86% (95%CI, 82–89) for the reference and 72% (95%CI, 67–76) for the experimental arm (Figure 4, p.37). The risk of progression was significantly higher for the experimental arm (HR 1.92, 95%CI, 1.43–2.569), also when adjusted by FLIPI-2 and induction treatment, P<0.001. Details on metabolic response at the EOT therapy were available in 691/712 patients: complete metabolic response (CMR) was confirmed in 628 (90%), while 65 patients had positive EOT FDG-PET (9%), and 2 patients had indefinite results. Overall, the 3-year PFS was 81% (95%CI, 77-84%) and 60% (95%CI, 47-71%) for patients with and without CMR, respectively (p<0.001). Among patients with CMR, the 3-year PFS was 90% (95%CI, 86-93%) and 72% (95%CI, 67-77%) in the reference and experimental arms, respectively (p<0.001) (Figure 5A, p. 37).

Among the 65 patients who did not achieve a CMR, the 3-year PFS was 50% (95%CI, 32-66%) and 70% (95%CI, 51-82%) for the 31 cases in the reference arm and for the 34 in the experimental arm (p=0.274; Fisher exact test), respectively (Figure 5C).

Baseline molecular evaluation was available for 615/628 cases that achieved CMR at EOT. In the group of 299 patients who became MRD- at EOT, the 3-year PFS was 92% (95%CI 78-91%) and 78% (95%CI, 61-77%), in the reference and experimental arm, respectively (p=0.0008) (Figure 5B).

During follow-up, 30/299 (10%) patients changed their MRD status from negative to positive at a median time from EOT of 7 months (range 5 to 30 months). In the experimental arm, 37 out of 44 MRD+ patients received weekly Rituximab according to protocol, 17 after EOT

assessment and 20 for a late MRD+. After receiving the weekly R, 22 out of 37 patients developed a molecular response. In the reference arm, 9 out of 32 MRD+ patients developed a molecular response despite the R maintenance every 2 months. Overall, the use of weekly R in the experimental arm was still associated with inferior 3-year PFS compared to that of MRD+ patients in the reference arm (p<0.001) (Figure 5D).

OS data

At the time of the last follow-up update, 30 deaths were recorded, of which 15 were associated with disease progression or recurrence (50%). The three-year OS resulted in 98% (95%CI, 96-99) and 97% (95%CI, 95-99) in the reference and experimental arm, respectively (p=0.238) (Figure 4).

Table 6 Baseline patients' characteristics and response with full induction treatment (n=744)

Variable		Arm A	Arm B	Total
Age	>60	176 (48)	192 (51)	368 (49)
Gender	F	191 (52)	195 (52)	386 (52)
B2M	>UNL	199 (54)	200 (53)	399 (54)
BM	+	202 (55)	213 (57)	415 (56)
LoDLIN	>6 cm	203 (55)	212 (56)	415 (56)
Hemoglobin	<12 g/dL	61 (17)	49 (13)	110 (15)
Nodal Sites	>4	157 (43)	146 (39)	303 (41)
Ann Arbor Stage	III-IV	324 (88)	335 (89)	659 (89)
LDH	>UNL	79 (22)	81 (22)	160 (22)
FLIPI -2	1-2	221 (60)	227 (60)	448 (60)
	3-5	147 (40)	149 (40)	296 (40)
FLIPI	0-1	90 (25)	89 (24)	179 (25)
	2	142 (40)	152 (42)	294 (41)
	3-5	122 (34)	123 (34)	245 (34)
Response EoI	CR	301 (82)	300 (80)	301 (81)
	PR	50 (14)	61 (16)	111 (15)
	ORR	351 (96)	361 (96)	712 (96)

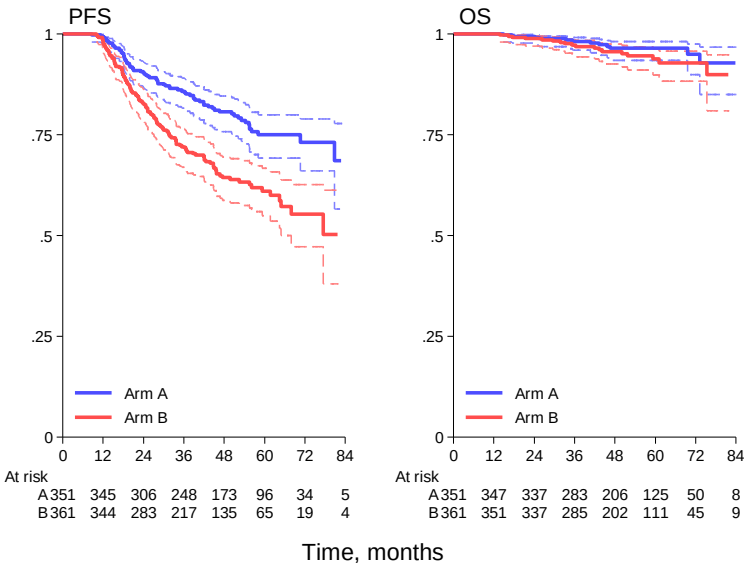

Figure 4. Progression-free survival and Overall Survival in the reference (Arm A) and experimental arm (Arm B) (n=712). Survival curves were calculated using Kaplan-Meier, and statistical comparisons between curves were made using the log-rank test

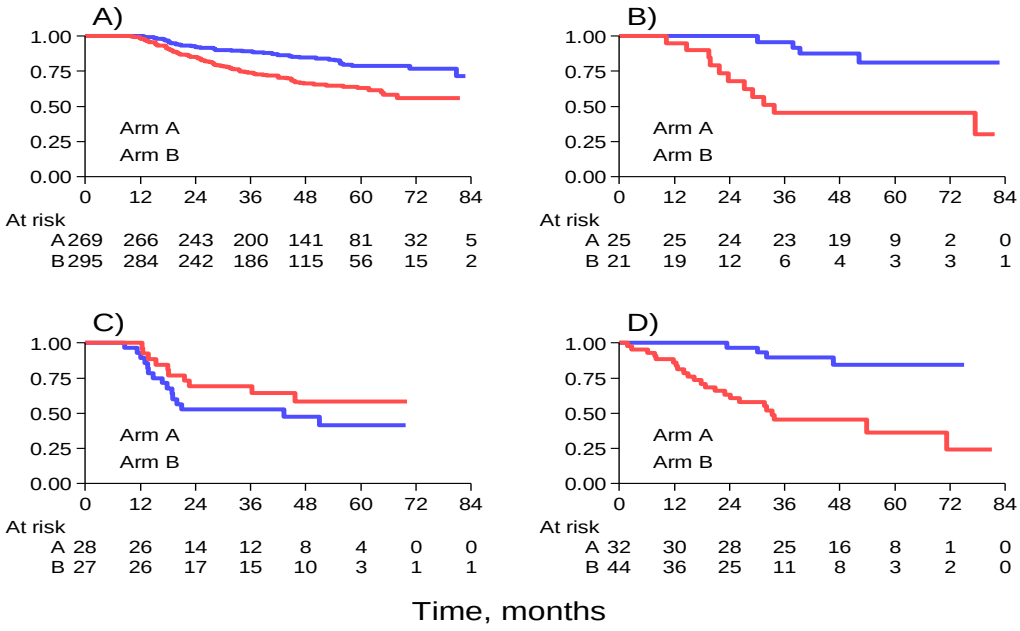

Figure 5. PFS for patients according PET, MRD status and arms at the end of treatment. A) EOT PET/MRD -/- (P<0.001), B) EoT PET/MRD -/+ (P=0.001), C) EoT PET/MRD +/- (P=0.274); D) PFS for patients PET negative after EoT with MRD+ at EoT or MTD+ during follow-up (P<0.001).

Discussion

Briefly, the study demonstrated that the response adapted therapy resulted significantly inferior in term of 3-year PFS (86% vs 72%), with an HR of 1.92 of the risk of progression for the experimental arm compared to the patients treated with standard Rituximab maintenance in the reference arm. The inferiority of the response adapted arm was found in most sub-groups and in particular in patients with the highest quality of response defined by both CMR and MRD negativity.

Based on these results, we concluded that patients responding to induction ICT should be addressed for 2 years of RM to guarantee the lowest risk of lymphoma progression.

From FOLL12, CMR emerged as the main factor to define the subsequent risk of progression and to define post induction management. MRD analysis was identified as an additional factor to identify patients at higher risk of progression among those with CMR.

Ladetto et al recently analyzed data on MRD in peripheral blood and bone marrow samples centralized at four Italian Euro-MRD certified laboratories at different time points: baseline, end of induction and every six months thereafter till month 36th from diagnosis. MRD was assessed by both nested and RQ-PCR.

MRD positive was associated with an increased risk of relapse in the subsequent six-month interval (HR for PFS 2.82, 95%CI 1.84-4.34, p<0.001), independently from the randomization arm (HR 0.330), treatment received (HR 0.859) and FLIPI-2 (HR 0.302). Moreover, BM allows a better prediction at the early time points but, starting from month 12ve after EOT, PB is superimposable to BM, allowing effective and reliable long-term non-invasive MRD monitoring (98).

Another important result of the FOLL12 is represented by the very high CMR rate, which was 90% in our series, consistently with what reported by other recent trials. Moreover, the observation of the poorer survival of non-CMR vs CMR patients confirmed that, even if reduced to 1 out of 10 patients, a high-risk group exists for whom more active therapeutic options are warranted.

Based on the above observations, our study confirms that even if treated with standard therapy, FL show heterogeneous outcomes, confirming the need for treatment personalization. The main aspect of risk adapted therapy is related to the choice of accurate predictors. A better definition of risk could come from the combination of metabolic response with other prognostic biomarkers or from anticipation of response assessment as done for other lymphomas.

Early metabolic response in Follicular lymphoma: a subset analysis of the FOLL12 trial by the FONDAZIONE ITALIANA LINFOMI (FIL)[1]

Introduction

Despite metabolic early response assessment has been extensively studied in Hodgkin Lymphoma (HL) and Diffuse Large B cell Lymphoma (DLBCL) allowing the definition of response adapted therapies for these subtypes (99–102), so far, only few data are available to define the role of an earlier assessment of MR during the initial ICT in FL. A first attempt to define the role of an early FDG-PET assessment of response in FL has been reported in one published study on 121 patients prospectively treated with 6 cycles of R-CHOP plus 2 cycles of Rituximab, without maintenance. In this study 5 point Deauville criteria were used by a central review panel of expert nuclear physicians to define the response. EOT FDG-PET was negative in 78% of patients and was predictive for both a better PFS (2-year PFS

[1] S. Luminari, R. Durmo, S. Chauvie, S. Peano, A. Franceschetto, F. Fallanca, V. Tarantino, A. Pinto, C. Ghiggi, A. Pulsoni, M Merli, L. Farina, M. Tani, C. Boccomini, G. Musuraca, B. Falini, F. Ballerini, P. M. Stefani, S. Bolis, G. Pietrantuono, M. Manni, L. Marcheselli, M. Federico, A. Versari.

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86% vs 51% for non-responders) and a better OS. An early assessment on FDG-PET was also performed after 4 courses of immunochemotherapy and a negative scan was found in 76% of patients. This result was associated with a better PFS compared to non-responders (2-year PFS 86% vs 61%). The authors concluded that an interim-PET (iPET) was strongly predictive of outcome like the EOT-PET, and they endorsed further evaluation of therapeutic intervention based on early PET results (45).

Considering the lack of solid data on the early metabolic response and its correlation with survival, FOLL12 represents a resource to try to answer this unsolved question.

Methods

Originally the FOLL12 trial was not designed to assess the role of interim PET, however early metabolic evaluation after 4 cycles of ICT was allowed. iPET results were based on the local report and were also centrally reviewed by applying standard DS.

The primary endpoint was 3-year progression free survival.

Statistical Considerations

The Log-rank test was used to compare different groups and effect estimated as HR with 95%CI from Cox PH regression. Survival curves were calculated using Kaplan-Meier.

Fisher exact test was used to compare variables.

Results

In our study iPET was performed in 211/807 patients and a local report was available in 186 cases. Forty-eight percent of patients were older than 60 years, 37% had a high-risk FLIPI-2, 44% received R-Benda as induction ICT. Based on a local report iPET was considered positive in 38/186 patients (20%).

A comparison of clinical characteristics of patients in which iPET was performed or not is shown in Table 7. iPET and PET at end of treatment (fPET) were both available for comparison in 168 cases and showed a concordance rate of 82%: 131 out of 140 iPET- confirmed their CMR at fPET (94%). Regarding the 31 iPET+, a fPET- was achieved in 23 cases (68%) (Table 7 and 8).

Considering both iPET and fPET, a positive iPET was associated with an increased risk of progression also if a negative PET was achieved at the end of induction (HR 2.09: 95% CI

3.22-19.5) (Figure 6). iPET was also associated with a different 3-year OS rate (99% vs 89% for iPET – vs +; p=0.035) (Figure 7, p. 43).

In univariable analysis the 3-year PFS was lower for the iPET+ patients compared to the iPET- (52% vs 87%) (HR of 2.73 95%CI, 1.51- 4.95). In multivariable analysis the prognostic role of iPET for PFS was confirmed (HR 2.60) and was independent from FLIPI-2 (0-2 vs 3-5 HR 1.88) and for ICT (R-Benda vs R-CHOP HR 1.39) (Figure 6 and Table 9, p. 43)

Table 7. Patient's Characteristics and comparison between iPET yes or not

Variable		**Measured PET, n (%)**		
		Yes	**No**	**p-value**
Age	>60	102 (48)	*271 (49)*	0.809
Gender	F	115 (54)	*282 (51)*	0.466
Beta2microglobulin	>ULN	116 (55)	*293 (53)*	0.745
Bone marrow biopsy	Positive	125 (59)	*300 (55)*	0.289
Hemoglobin	<12 g/dL	34 (16)	*83 (15)*	0.737
Largest LN diameter	>6 cm	111 (53)	*312 (57)*	0.328
LDH	>ULN	40 (19)	*124 (23)*	0.279
Number of nodal areas	>4	88 (43)	*218 (40)*	0.507
Stage	III-IV	182 (87)	*493 (90)*	0.244
FLIPI-2	3-5	79 (37)	*224 (41)*	0.409
FLIPI	3-5	69 (34)	*179 (34)*	0.418
Bcl2 qualitative or quantitative	Positive	121 (58)	*309 (57)*	1.00
Induction treatment	R-Benda	87 (44)	*236 (43)*	0.683
Response after induction	CR	165 (78)	*433 (79)*	0.844
3-year PFS		77 (70-82)	*76 (72-79)*	0.719

Table 8. Concordance Rate between iPET and fPET

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fPET/local	**iPET, n (%)**		**Total, n (%)**
	-	+	
-	**131 (96)**	**21 (68)**	**152 (90)**
+	**6 (4)**	**10 (32)**	**16 (10)**
Total (% by row)	**137 (82)**	**31 (18)**	**168**

i/f PET	N (%)	3-y PFS (95CI)	HR (95CI)
-/-	131 (75)	90 (83-94)	1.00
-/+	9 (5)	50 (14-76)	7.99 (3.22-19.5)
+/-	23 (13)	64 (41-80)	2.06 (0.92-4.60)
+/+	11 (6)	36 (11-63)	6.24 (2.67-14.5)

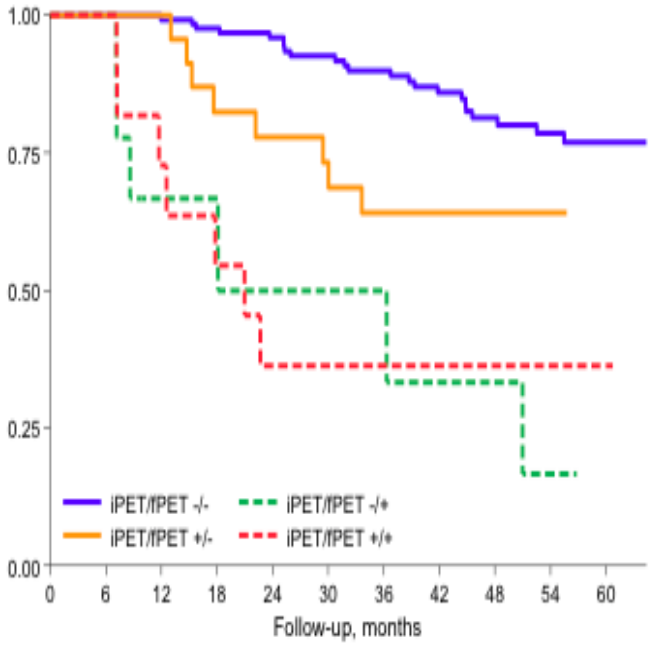
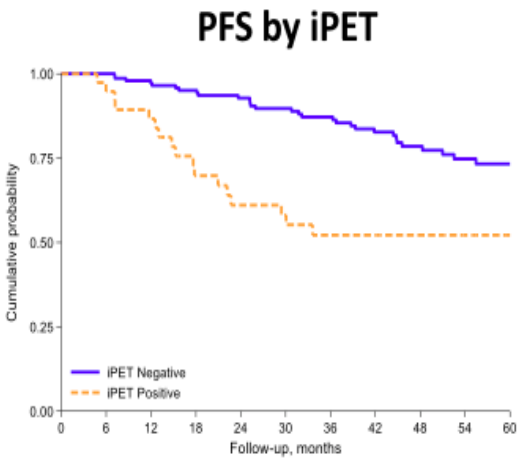
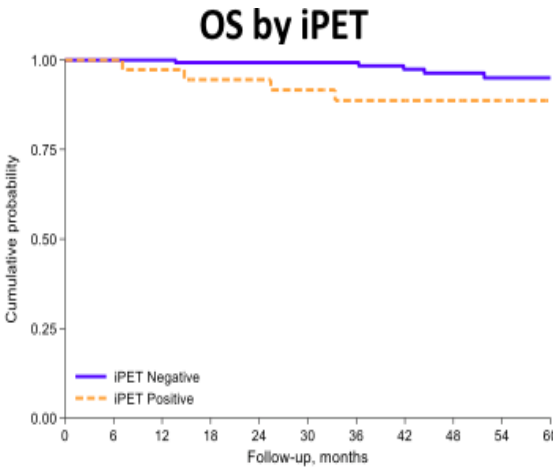


Figure 6. PFS analysis according to iPET and fPET





		3-yr PFS% (95CI)			
Status (n=186)	n	%	95CI	HR (95CI)	p
iPET Negative	148	87	80-92	1.00	
iPET Positive	38	52	35-67	2.73 (1.51-4.95)	<0.001

		3-yr OS% (95CI)		
Status (n=186)	n	%	95CI	p
iPET Negative	148	99	95-100	
iPET Positive	38	89	73-95	0.035

Figure 7. 3-year PFS and OS according to iPET status

Table 9. Multivariable analysis for prognostic role of iPET for PFS

Multivariable Cox PH Regression (n=186)

Factor	HR (95CI)	P value
iPET-	1.00	
iPET+	2.60 (1.41-4.79)	0.002
FLIPI2 3/5	1.88 (1.05-3.35)	0.031
Treat, R-B	1.39 (0.77-2.51)	0.281

Discussion

In our study**,** the interim metabolic response is confirmed with a strong prognostic role for PFS in patients treated with standard ICT. Considering the higher rates of iPET+ cases compared to fPET, iPET may better contribute to anticipate the identification of FL patients at different risk of progression and might be used to support the development of a novel generation of response adapted trials (FOLL19 platform, p. 46).

Compared to the end of induction assessment, early evaluation allows enriching the group of non-responding high-risk patients making it feasible to design intensified treatment programs. Conversely, early responding patients are identified early during immunochemotherapy, thus making it possible to act on the remaining course of chemotherapy to try to de-intensify treatment without affecting the anti-CD20 part of therapy and without negatively affecting the patient's outcome.

Future perspectives for Follicular Lymphoma first-line approach: design of FOLL19 trial

The design of the FOLL19 trial (NCT05058404) is based on two main pillars. The first one is based on the experience that the Italian groups have accumulated with the administration of shortened chemotherapy programs combined with a full dosage of Rituximab. This approach has been employed particularly in elderly patients using both fludarabine and bendamustine containing regimens (103,104). The second pillar of the study is represented by the very promising data that correlate the quality of response to the efficacy of the treatment (FOLL12 results).

In the present study, the evaluation of response to the short immunochemotherapy program, using up-to-date criteria, will be used to avoid the risk of undertreatment in patients who, based on an incomplete response, will be identified at higher risk of experiencing treatment failure and that could be eligible for parallel intensified studies. Conversely, responding patients could be identified early during the immunochemotherapy course, thus making it possible to act on residual treatment by trying to de-intensify the backbone chemotherapy without changing survival.

An exploratory analysis will be performed with the aim to complement heterogeneity information provided through radiomics analysis and TMTV and to evaluate the correlation of TMTV/radiomics at baseline with response and prognosis.

Study Design

This is an open-label, multicenter, randomized phase III trial. Once randomized, each patient will start immunochemotherapy with one of the approved regimens (R-CHOP, R-Bendamustine, G-CHOP, G-Bendamustine, G-CVP) chosen by the physician before randomization.

Patients randomized to Arm A will receive an induction immunochemotherapy at full doses (standard schedule). After cycle 4, patients will be assessed for response and will complete their planned therapy if at least a stable disease is confirmed. Patients randomized to Arm B (experimental arm) will start their induction treatment with 4 cycles of the immunochemotherapy standard dose. After cycle 4, patients will be assessed for response and will proceed with subsequent treatment based on the quality of their response. Specifically:

- Patients achieving a complete response (CR) will receive a shortened treatment: in detail, they won't receive any further chemotherapy but will complete induction with 4 additional cycles of only the monoclonal antibody given during the first four cycles (in the case of G-bendamustine, 2 additional cycles of obinutuzumab);
- In case of response less than CR (partial response or stable disease), patients will complete treatment as planned for patients in Arm A.

Design of the study is shown in Figure 8.

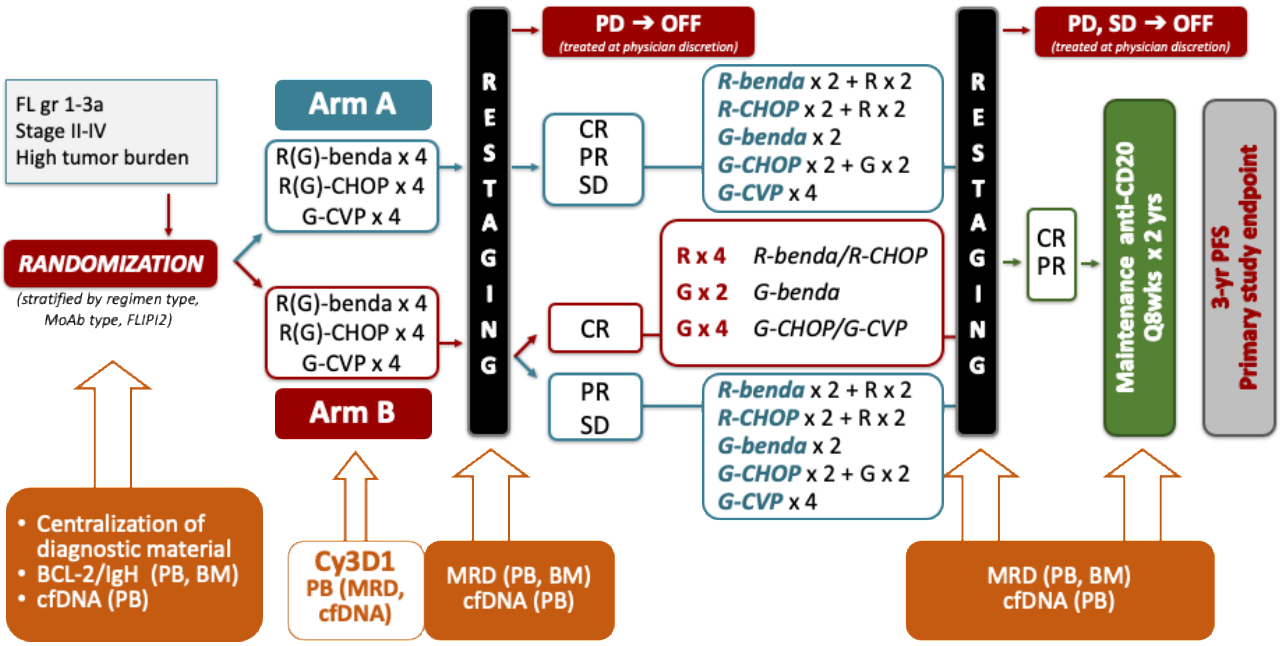


Figure 8. FOLL19 Study Flow Chart

Methods

Inclusion criteria

To be included patients must have:

- Histologically documented diagnosis of CD20+ Follicular Lymphoma grade 1-2 or 3a, as defined WHO classification;
- Age ≥ 18 years;
- ECOG performance status 0-2;
- No previous immunochemotherapy for the lymphoma (localized radiotherapy or Rituximab monotherapy with a max of 4 doses are allowed);
- Ann Arbor stage II-IV;

- High tumor burden as per GELF criteria defined;
- At least one site of measurable nodal disease at baseline ≥ 1.5 cm in the longest transverse diameter as determined by CT scan;
- Adequate organ functions;
- An informed consent form signed approved by an Independent Ethics Committee (IEC) prior to the initiation of any screening or study-specific procedures.

Response Assessment

• Response assessment after cycle 4 and at the end of induction therapy is per International Lugano 2014 criteria with PET;

• PET interpretation is per Deauville Criteria 5-points scale.

In order to achieve the best reliability of PET , all the FDG-PET exams performed (including possible exams performed in addition to those prescribed by the protocol) will be collected, but only the baseline and after 4 cycles studies will be centrally reviewed by blind independent central review;

The independent review facility consists of validated experts in the field of PET/CT scanning in FL. A timely review (within 72 working hours) will be provided for interim PET/CT assessment. An agreement between 2 reviewers is necessary to achieve the central panel judgment. In case of discordance, the scan must be read by further reviewers until there is agreement where the number of ‘positive’ reports exceeds the number of ‘negative’ reports by >1 (or viceversa).

This study will be conducted according to the Declaration of Helsinki Ethical Principles for Medical Research Involving Human Patients. The review of this protocol by the IEC and the performance of all aspects of the study, including the methods used for obtaining informed consent, must also be in accordance with principles enunciated in the declaration. Before implementing this study, the protocol, the proposed informed consent form and other information to patients, must be reviewed by a properly constituted IEC. A signed and dated statement that the protocol and informed consent have been approved by the IEC must be given to FIL before the study initiation.

Statistical Considerations

The non-inferiority design has been considered to demonstrate that a shortened exposure to chemotherapy in patients responding to the first four cycles of immunochemotherapy is not detrimental in terms of PFS compared to full dose standard treatment.

Based on recent literature data we took into account a 3-year PFS of 80% (GALLIUM trial and preliminary results of FOLL12) for patients receiving maintenance and a 3-year PFS of 65% for those who didn't receive maintenance (FOLL05, PRIMA with no maintenance), corresponding to a hazard ratio of 1.93 (95% CI 1.64-2.18) between patients who weren't given and those who were given maintenance.

We planned 48 months of uniform accrual and a minimum observation period of 56 months from the last patient enrolled, for a total study duration of 104 months (about 8.7 years).

The primary endpoint analysis (PFS) will be performed using a Cox proportional hazard regression. The survival curves and the HR with their confidence intervals at 90% (according to a 5% one-sided alpha error) will be also checked graphically by using the Kaplan estimator.

Study Objectives

Primary Objective

The purpose of this trial is to demonstrate that, in patients with a newly diagnosed advanced stage high tumor burden Follicular Lymphoma according to the GELF criteria, a treatment strategy that reduces the number of chemotherapy cycles in case of early response to immunochemotherapy is not inferior compared to standard therapy at full dose in terms of progression-free survival.

Secondary Objectives

• to compare the response rates between the Standard and Experimental treatment;

• to compare the rate of adverse events between the Standard and Experimental treatment;

• to compare a shortened vs full dose program in terms of change in quality of life (QoL) measured through the Patients Reported Outcomes (PROs) by means of the FACT-Lym-questionnaire;

• to recognize patients 'characteristics and biomarkers that help in identifying patients suitable for shortened chemotherapy treatment;

• to assess the role of minimal residual disease in predicting patient outcome;

• to assess the role of cell-free tumor DNA analysis in predicting patient outcome;

• to assess whether cfDNA analysis could be used to monitor residual disease;

• to correlate response and survival with clinical and biologic prognostic factors;
• to assess long-term outcome of the patients;
• to complement heterogeneity information provided through radiomics analysis to TMTV to correlate it to the prognosis and evaluate the correlation of TMTV/radiomics at baseline with response;
• to evaluate and to compare Lugano classification and TMTV/radiomics analysis results obtained from PET studies

The study has recently started the enrollment.

Follicular Lymphoma beyond the first-line.
Clinical characteristics, metabolic features and outcomes of follicular lymphoma patients after first relapse: an Italian report from PETRA FIL trial [2]

Introduction

Despite its prolonged natural history, Follicular Lymphoma is still considered an incurable disease. Relapse is inevitable for most patients in the form of recurrence or progression. However, the disease is highly treatable with several treatment options to maintain disease control. The improvement in the management of patients should be attributed to the whole therapies used rather than to one single line of therapy only. However, survival analysis based on different lines of treatment remains poorly described in the post-Rituximab era. Recently a study from the LymphoCare group reported an analysis of progression-free survival for patients who experienced relapse after first-line therapy and beyond. However, no data on OS was reported (105).

Moreover, unfortunately, there is no universal agreement on standard approach to treatment in relapsed/refractory settings and, currently, there is also a paucity of risk stratifying tools to inform treatment decisions. The best current surrogate markers to stratify the risk of patients remain clinical features such as depth and duration of response following front-line chemoimmunotherapy.

To address this gap, we retrospective analyzed treatment patterns and clinical outcomes of FL patients after their first relapse enrolled in FIL PETRA trial.

Patients and Methods

PETRA trial is a retrospective multicenter Italian study that included patients with a diagnosis of FL grade 1-3a who experienced a relapse/refractory event after first-line standard ICT. Relapses after watch and wait (W&w) or radiotherapy were excluded. Moreover, patients with a transformation at first relapses were not included in the analysis. PET availability as response assessment after first-line treatment was optional but recommended for metabolic evaluation, whereas clinical and radiological evidence of FL relapse or progression were mandatory. FDG-PET CTs at relapse were analyzed considering local report. For each PET

[2] V. Tarantino, M. Casali, , A. Versari, M. Manni, A. Anastasia , C. Rusconi, S. Sacchi, G. Mansueto, F. Zaja, M. Zanni, O. Annibali, M. Cimminiello, N. Di Renzo, F. Cavallo, A. Fragasso, D. Vallisa, L. Marcheselli, F. Merli, G. Rossi , S. Luminari

Submitted as abstract at 64th ASH Annual Meeting and Exposition, Atlanta December 2021.

scan SUV max was identified and correlated with clinical features and survival. PET interpretation was per Deauville Criteria 5-points scale.

Histological confirmation of relapse was strongly recommended.

The aim of the study was to analyze clinical and metabolic features, and outcome of FL patients after first relapse. The primary study endpoints were PFS and OS from the time of relapse/progression.

The study was approved by local ethic committees at any active center and a signed consent form was mandatory for all enrolled patients.

Statistical Considerations

Patient outcome was analyzed by OS after relapse and PFS after relapse. OS was calculated either from the time of relapse/progression or from the time of treatment commencement, until the last follow-up or death. PFS for sequential lines of therapy was calculated from treatment commencement until qualifying event (progression, change of treatment, or death). A subgroup analysis was performed to investigate the ability of FDG-PET at the time of the first relapse to predict patient outcome and to correlate it with the risk of progression and transformation.

Covariates were described in terms of frequencies and frequencies converted into percentage of the number of patients examined. Groups were compared using Chi2 test or Fischer's exact test. Continuous variables were reported in tables with sample size, median and dispersion from 2.5 to 97.5 percentiles. Mann-Whitney test and Kruskal-Wallis test were used to compare continuous variables among 2 or more than 2 groups.

Time to event variable was presented as Kaplan-Meier estimate with confidence intervals at 95%. Comparison between groups was tested using the log-rank test and the effect size was estimated using the Cox proportional hazard model, with CI 95. All tests were two-sided.

Results

From 2002 to 2017, 175 patients have been recorded in the PETRA trial. Overall 156 patients fully met the inclusion criteria and were eligible for the analysis. Of these, 127 (81%) and 29 (19%) were relapsed and progressed respectively. Median age at diagnosis was 57 years (30-82). 141 had an advanced stage; a high FLIPI and FLIPI-2 were reported in 68 and 42 patients, respectively: 73 patients had histology of FL grade 2, and 25 a FL grade 3a. All patients received an ICT followed by maintenance in 59 patients (38%).

After a median time to relapse of 33 months (7-105), 47 patients (30%) experienced an early event within one year from diagnosis. At the time of relapse/progression (R/P), median age

was 62 (21-80), 27% presented an elevated LDH and 67% had an advanced stage. Clinical characteristics at the time of R/P were shown in Table 10 (p. 52).

Going forward, for 26 patients a W&w strategy has been chosen. 129 patients received an active anti-lymphoma salvage treatment: a total of 47 (37%) were addressed to autologous transplant, 66 patients (46%) received a second line Rituximab maintenance after salvage treatment. In details, 8 patients received RCHOP-Like therapy, 38 R-Bendamustine, 25 RDHAOX/RDHAP, 10 underwent R-ICE. After salvage treatment, a complete response was achieved in 67% of the cases, a PR in 20% while 9% of the patients presented a stable disease or a progression. Overall 20 patients (12%) experienced a transformation into aggressive lymphoma.

For the 82 patients with available FDG-PET at the time of R/P, Maximum Standardized Uptake Value (SUV_{max}) was not associated with the risk of transformation or with survival (Figure 9, p. 53).

With a total of 63 events reported, the 5-year PFS after relapse was 52% (95CI, 41-61%) After a median follow-up of 48 months (range 1-137) 15 deaths were recorded resulting in a 5-year OS of 88% (95 CI 80-93%) (Figure 10-11, p. 54). Six patients died due to the progression of the disease, 3 for second cancer, 2 for infection, 1 for treatment toxicity, 1 for heart failure and 2 for not specified causes. In a univariate analysis time to relapse > 2 years correlate with PFS after relapse ($p \leq 0.001$).

Discussion

In our study, we aimed to capture the clinical behavior of the patients from frontline treatment to subsequent relapse analyzing the influence of second progressions on survival.

From our entire cohort 30% of the patients were early progressors, which defined a high-risk population, and, as expected, they had high tumor burden features. Moreover, 80% of the patients received subsequent lines of therapy including about 40% for whom a high dose therapy followed by autologous transplant was chosen.

Despite our dataset confirmed the improved outcome with an OS exceeding 5 years even after a first relapse, heterogeneity in clinical behavior emerged also in relapse setting. In our series, 29% failed to achieve a complete response after salvage therapy and 12% experienced a transformation into an aggressive lymphoma. The analysis FDG-PET seemed to fail the ability to detect high-risk population, however, considering the small numbers, further studies are needed.

Moreover, it is important to note that several recurrences-remitting events impact life expectancy even after excluding transformation at first relapse. In particular, events

recorded within one year significantly marked the clinical course with a shorter progression free survival after every recurrence.

Similar to our analysis, recently, other studies highlighted how the necessity of subsequent lines of therapies decreases survival in follicular lymphoma patients.

Batlevi et al performed a single institution study looking at their patients with follicular lymphoma showing how their survival has changed after they received different lines of treatment (17). They clearly demonstrated that the response rates were lower and their progression-free survival was shorter after every recurrence, making the interval in between each treatment progressively shorter and shorter. So, that suggests and confirms that patients that need multiple therapies over the course of their lifetime tend to have a worse outcome.

Recently, Ghione et al conducted the Scholar-5 trial, an international, retrospective analysis of 184 patients with relapsed or refractory follicular lymphoma (106). Data were sourced from 7 institutions considering patients treated with a third-line or later regimen, creating a cohort whose treatments are highly heterogeneous. Among the whole cohort, 31% of the patients were defined at high-risk for POD24, 43% had a refractory disease.

The overall response rate decreased after each line of treatment (only 42% of the patients had a complete response after 3 lines). As well as, OS and PFS after 3 lines were 84% and 35% respectively but decreased to 40% and 3% in ≥5 lines. In subgroup analysis, the POD24 group had a much lower median PFS compared with the non-POD24 group.

Altogether, these results impose the need for early identification of high-risk of relapse patients and the adoption of a risk-adapted personalized therapy also in the R/R setting.

Table 10. Clinical characteristics at time of progression/relapse of FL pts enrolled in Petra Trial (156 pts)

Variable		**Diagnosis**	
		Median (min-max)	
Age		60 (32-81)	
		n (%)	**missing**
Age	>60	73 (50)	-
Stage	I-II	48(33)	4
	III-IV	104 (67)	
LDH	>ULN	34 (24)	8
B2M	>ULN	51 (46)	36
Histology	NA	22 (21)	41
	1-2	56 (38)	
	3 a	20 (19)	
	3 b	2 (2)	

	DLBCL	6 (6)	
Treatment	W&W	27 (18)	1
	Treated	119 (82)	
Hb g/dL	<12	13 (9)	7

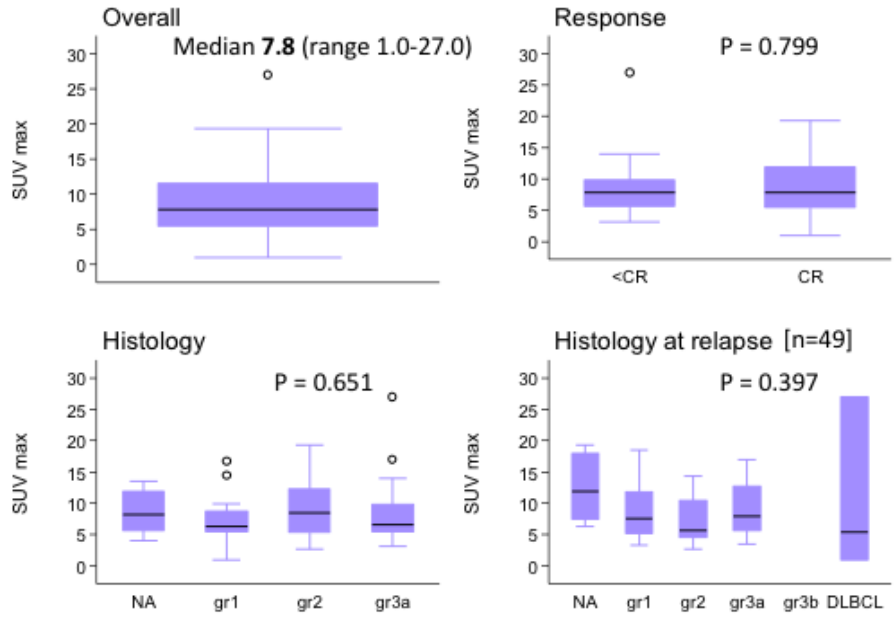


Figure 9. SUV Max analysis for relapse/progression patients (82 pts).
Logistic regression analysis of clinical factors did not allow to identify any predictive factor of elevated SUVmax. Based on local assessment, SUVmax was not associated with different prognosis in terms of PFS and OS. Regarding the risk of transformation, we did not find any correlation with higher SUV max values.

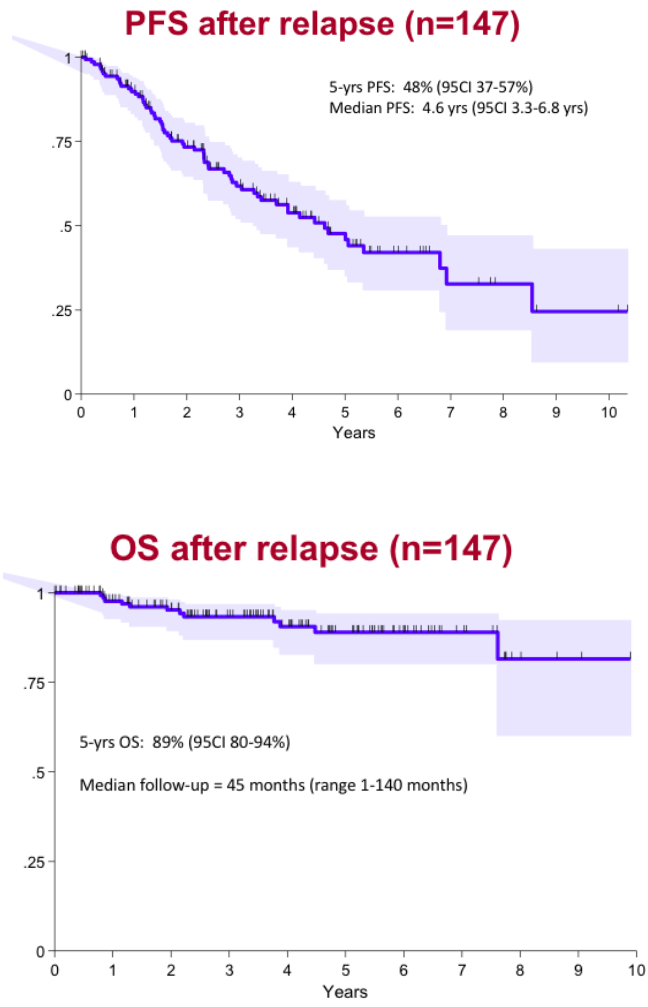


Figure 10-11. Survival after relapse (n=147).

PFS and OS curves were calculated using Kaplan-Meier. A statistical comparisons between curves were made using the log-rank test

Chapter 5. From literature review to our research: thesis results for marginal zone lymphoma

Clinical characteristics, prognostic factors and outcome of a large cohort of marginal zone lymphoma patients enrolled in the NF10 FIL study[3]

Introduction

Marginal zone lymphoma is the second most common indolent lymphoma representing about 10% of NHL.

As well as FL, MZL is characterized by slow growth, often does not require immediate therapy and, when treatment is addressed, an excellent response rate is achieved.

The overall prognosis is actually quite favorable, since overall survival at 10 years is about 80%. However, considering the three distinct pathological entities, ENMZL, SMZL and NMZL, there is a significant heterogeneity among the diseases, both in diagnostic and prognostic criteria, biological characteristics, clinical behavior and therapeutic implications.

Moreover, a small portion of patients displays a pattern of the disease that reflects the clinical characteristics of more aggressive lymphoma.

In MZL, poor risk clinical features have been elucidated in recent years (chapter 3). However, if compared with other indolent lymphomas such as follicular lymphomas, features associated with adverse outcome or risk of transformation remain scarce. Given the rarer entity, a widely accepted prognostic tool for clinical usage for all indolent non-follicular B-cell lymphomas is still lacking. Moreover, most clinical, biological, and metabolic prognostic factors are borrowed from FL, whereas prognostic scores in MZL are not stringently validated. Indeed, existing studies generally included diseases with different presentations, involving patients with indolent B cell lymphomas not specifically MZL, or have been retrieved from retrospective series. Furthermore, the studies available mainly focused on defining prognostic factors in single lymphoma subtypes, with the development of specific-entity scores, for example splenic marginal zone lymphoma score (chapter 3, p. 25). Consequently, also for MZL patients there is room for improvement and development of novel clinical indexes.

With the aim to better investigate the prognostic factors and outcome of Indolent Non-Follicular B-Cell Lymphomas (INFL), in 2010 the Fondazione Italiana Linfomi launched the

[3] Manuscript under submission

NF10 Project as a prospective observational study comprising marginal zone lymphomas (NCT02904577).

Here we describe the clinical characteristics, different treatment, prognostic factors and their impact on outcome in a large cohort of MZL patients enrolled in the NF10 trial focusing on patients in need of treatment.

Patients and Methods

Patients met the inclusion criteria if they had a diagnosis of SMZL, ENMZL, NMZL, lymphoplasmacytic lymphoma (LPL), Small lymphocytic lymphoma (SLL), and CD5-negative chronic lymphocytic leukemia. In our analysis, only marginal zone lymphomas classified as ENMZL, SMZL and NMZL were considered.

A tissue biopsy of a nodal or extranodal lesion, or a bone marrow biopsy consistent with histological diagnosis of marginal zone lymphoma, assessed by local immunopathology, were required for the study. Moreover, for all cases peripheral and/or bone marrow flow cytometry analysis was collected. Patients with histologic features of MZL who have a concomitant involvement of the marrow and/or spleen and/or lymph nodes and/or extranodal sites were categorized as disseminated MZL (dissMZL).

Clinical characteristics and radiologic assessment were recorded at the time of diagnosis. Laboratory reports included data on serology for HIV, hepatitis B and C, LDH, beta2microglobuline , albumin, absolute lymphocyte count (ALC) and platelets according to the common used prognostic score for marginal zone lymphomas: MALT-IPI for ENMZL, MZL IPI and HPLL for SMZL, FLIPI for nodal and dissMZL. Patients were followed according local institutional guidelines.

Patients initially assigned to a watch and wait period were included only if they had started systemic therapy due to disease progression in the absence of transformation.

Watch and wait was defined as the close monitoring of the patients and without giving any treatment within the first 3 months from the date of diagnosis. Systemic therapy included the use of chemoimmunotherapy or anti-CD20 monoclonal antibody alone; antibiotics, radiotherapy, or splenectomy were excluded from systemic therapies.

The main endpoint of the study was progression free survival, considered for patients treated at diagnosis as the time from date of diagnosis to date of progression or death for any cause, or last control. For patients who received an active anti-lymphoma treatment after W&w, progression free survival was analyzed from date of interruption observation to date of progression, death for any cause or last contact. For patients not treated among W&w the only failure event was the death for any cause. Secondary endpoints were PFS-2, overall

survival, and incidence of late event as well as second cancer and transformation. PFS-2 was calculated from date of first progression/re-treatment to date of second progression or death for any cases or last contact. OS was defined as the time from date of diagnosis (treated at diagnosis or in W&w), or date start treatment (stop W&w) to date of death, or last contact. Patients without events were censored at the time of last follow-up.

Registration of patients in the study and data collection were performed online through Electronic Case Report Forms (ECRFs) using the *Openclinica* free version software (Ver.4.0, Waltham MA, USA). The study was conducted in accordance with the Declaration of Helsinki Ethical Principles and Good Clinical Practices and was approved at each site by an ethics committee. A signed consent form was mandatory for all enrolled patients.

Statistical considerations

An analysis of prognostic factors was performed on all validated cases. Categorical variables were reported using with median as central value and range for dispersion; continuous variables were reported absolute values and percentage frequencies. Differences in remission rates between groups were analyzed by the Pearson's 2 test for contingency. Progression-free survival and overall survival were estimated by the method of Kaplan-Meier according to the International Working Group response criteria. The Log-rank test was used to compare different groups and effect estimate as HR with 95%CI from Cox PH regression. A p value of 0.05 (two-sided) was considered the limit of significance for each analysis. Cumulative incidence of second cancer was estimated by Fine-Gray regression.

Results

Between July 2010 and May 2019, 1717 cases of INFL have been registered in the NF10 study by 65 centers in Europe and South America (list of centers is shown in the appendix). Of these, 1532 were considered evaluable for the analysis and 1335 cases had complete data on treatment/histology. Overall 785 were MZLs: 332 ENMZL (42%), 260 SMZL (33%), 87 NMZL (11%) and 106 cases (14%) were classified as diss-MZL due to the lack of a clear pattern of organ involvement. Demographic and clinical characteristics are shown in Table 11 (p. 61).

For the aim of the study, we only considered patients who required a systemic treatment corresponding to 414 patients. 71 patients were excluded due to the treatment received soon after diagnosis (surgery, radiotherapy, antiviral or antibiotic, or not specified treatment).

After diagnosis, 300 patients were immediately referred for a “watch and wait” approach, however, 99 among them subsequently received treatment after a median time of active surveillance of 15 months (3-63 months).

A comparison of the clinical characteristics between the group of “initial treated” and “treated after W&w” showed similar results with the exceptions of bone marrow involvement and histological subtype.

Rituximab was combined with bendamustine in 173 patients (38%), with a peak of use during the two years’ period of 2015-2016 and 2017-2019 (61% and 52%, respectively). R-alkylating agents were administered in 114 patients (28%). R-CHOP like regimens were the first-line therapies in 54 patients (13%), R- fludarabine in 3 patients (1%); 43 patients (10%) and 27 patients (6%) received Rituximab or alkylating drugs as single agents, respectively. A distribution of chemotherapy regimens according to the timeline period is detailed in figure 12 (p. 62).

PFS data

After a median follow-up of 56 months (range 1-114 months), 154 events were recorded for treated patients: 123 progression of disease and 31 deaths for any cause. As results, 5-year PFS was 65% (95 CI, 60-70%) without any difference between patients immediately treated at diagnosis or after a “watch and wait” approach respectively (p=0.220).

A difference in term of PFS was observed according to the histology: ENMZL showed a significantly higher PFS of 76% (95% CI, 69-82%) compared to dissMZL, for whom a 5-year PFS of 61% (95% CI, 48-71%, p= 0.006) was registered and compared to SMZL with a PFS at 5-year of 60% (p= 0.012). Finally, NMZL had a lower 5-year PFS of only 49%, (p <0.006) (Figure 13, p. 62). Patients’ characteristics at progression are shown in Table 12 (p. 63). The association between PFS and clinical characteristics was evaluated and described in Table 13 (p. 63). By multivariable analysis, only age >60 years, stage IV and Hb level correlated with PFS.

Role of PFS2

For the second endpoint analysis, we evaluated PFS-2 in 121 patients for whom 58 events were recorded: 38 progressions and 20 deaths.

After a median follow-up of 26 months (95% CI 14-54 months), 2-year PFS-2 was of 51% (95% CI, 41-61). As well as for PFS, also the PFS-2 resulted higher in ENMZL group with 2-year PFS-2 of 77% (95% CI 58-89) compared to SMZL with 2-years PFS-2 of only 37% (p=0.001). NMZL counted a PFS-2 of 49% (p=0.094) and diss-MZL of 40% (p=0.004). (Figure 14 p. 64).

Overall, the median survival after a second progression for the 121 evaluable patients was of 64 months (95%CI 41-NA) (Figure 15, p.64).

Overall survival data

After a median follow-up of 56 months, 72 deaths were recorded in the whole group of treated patients, 55 in upfront treated at diagnosis and 17 in treated after W&w.

Overall, 5-year OS was 86% (95% CI, 82-89) for patients treated at diagnosis and 84% after a “watch and wait” approach, respectively (p=0.626). ENMZL showed a significantly higher OS (93%) compared to dissMZL, SMZL (78% and 79%, p-value <0,001, respectively) and NMZL (76%, p-value 0.039) (Figure 16, p.65).

Progressive disease was reported as the cause of death in 31 cases (42%), 24 cases treated at diagnosis and 7 after observation, followed by second cancer in 10 cases (15%), and infections in 6 patients (8%), all treated soon after diagnosis.

Late Toxicities and Transformation

Out of 536 patients treated with ICT, second cancers were reported in 63 patients. At 5 years, the cumulative incidence of second cancer was 10% (95% CI, 7.7-12.8). 35% of second cancers were registered after R-Bendamustine therapy, 31% after R-Alkyl and 14% after R-CHOP regimens. Only 1 patient experienced a second cancer after receiving R-fludarabine.

A high-grade transformation was documented in 7 patients, all treated at diagnosis. Five histological transformations occurred in POD24 group and 2 in patients who were not POD24. The cumulative hazard risk of transformation at 5 years was 1.8% (95% CI, 0.8-3.9).

Discussion

Our analysis confirmed the long natural history of MZLs both for patients treated at diagnosis and after “watch and wait” approach, highlighting that active monitoring is a valid initial strategy for the management of MZL without treatment criteria. However, different behaviors and outcomes were observed for different subtypes. Specifically, we documented a higher PFS, PFS-2 and OS for ENMZL compared to the other subtypes, underlining a more indolent course for this subtype and a worse outcome for NMZL and DissMZL, for whom a more aggressive disease and shorter interval of remission were observed. Besides the histological subtype, other clinical factors seemed to correlate with outcome. In multivariable analysis, age >60 years, stage IV and Hb level correlated with PFS. These factors confirmed the role of some parameters already used and could represent the basis to construct a simplified prognostic model.

While lymphoma progression remains the most frequent cause of death, we observed important rates of non-lymphoma related death correlated to second cancer and infections. So far, little is known about long term treatment related toxicities in INFL and more data are needed to have a better estimate of the risk associated with specific treatment. A risk-to benefit balance of treatment, considering the toxicity, is important given the long natural history of the disease. Moreover, addressing patients to risk-adapted therapy with less toxic therapy is an urgent need to be investigated.

Finally, in our analysis, the risk of transformation was 1.8% at 5 years with a higher risk observed after 6 months from diagnosis and for patients who relapse within two years from diagnosis (POD24 group). This data seems to be lower compared with other series of indolent marginal zone lymphomas (107,108). Given the adverse outcome associated with transformation, efforts that focus on clinical or biological risk are needed.

Among the limitations of our study, we highlight the lack of central histologic review that might have partly affected subgroup analysis on MZL subtypes. However, local pathologic reports with clinical, radiologic, immunophenotypic and molecular findings were centrally collected and reviewed, allowing to exclude a few numbers of cases with insufficient diagnostic criteria for MZL. Finally, we were not able to validate our findings on an independent series of patients with MZL.

NF10 confirms the high value of prospective observational studies to provide relevant new data outside clinical trials or for diseases that are not frequently studied within clinical trials.

Table 11. Clinical Characteristics of patients enrolled in the NF10 FIL trial (785 pts).

Group		**Histology, n(%)**				**Total, n (%)**	**p-value**	**NA**
		ENMZL (n=332)	**SMZL (n=260)**	**NMZL (n=87)**	**DissMZL (n=106)**	**(n=785)**		**n**
Age	≥60	205 (61)	182 (70)	58 (67)	81 (76)	**526 (67)**	0.023	-
Sex	M	158 (48)	138 (53)	37 (43)	59 (56)	**392 (50)**	0.169	-
Stage	IV	142 (44)	249 (98)	60 (71)	105 (99)	**556 (73)**	<0.001	22
BM	+	44 (16)	246 (96)	36 (48)	79 (80)	**405 (57)**	<0.001	76
ECOG-PS		10 (3)	15 (6)	4 (5)	12 (11)	**41 (5)**	0.014	7
LDH	>UNL	33 (10)	116 (47)	24 (30)	33 (32)	**206 (27)**	<0.001	35
B2M	>UNL	92 (34)	160 (77)	31 (48)	54 (62)	**337 (54)**	<0.001	155
Albumin	<3.5	30 (11)	37 (18)	13 (21)	14 (19)	**94 (15)**	0.044	177
ALC	<1000	51 (16)	38 (15)	23 (27)	23 (22)	**135 (18)**	0.040	23
Platelet	<100	8 (2)	75 (29)	4 (5)	7 (7)	**94 (12)**	<0.001	4
HCV	+	24 (7)	13 (5)	10 (12)	13 (12)	**60 (8)**	0.045	15
HBV	+	50 (16)	53 (21)	14 (17)	18 (18)	**135 (18)**	0.412	31
Planned	Treatment	236 (71)	126 (48)	56 (64)	67 (63)	**485 (62)**	<0.001	-
	WW	96 (39)	134 (52)	31 (36)	39 (36)	**300 (38)**		
	WW treated					***99 (33)***		
Treatment	CHT	216	126	51	67	**453**		
(**n=485**)	Only RT/Surg	27	-	5	-	**32**		
Surgery		17 (5)	31 (12)	3 (3)	1 (1)	**51 (6)**	<0.001	-
CHT	Mono ALK	10	10	2	5	**27 (6)**		
(**n=453**)	R-Mono	13	24	2	4	**43 (10)**		
	R-ALK	73	20	3	18	**114 (28)**		
	R-CHOP	15	14	10	15	**54 (13)**		
	R-B	67	54	31	21	**173 (38)**		
	R-Fluda	1	-	-	2	**3 (1)**		
	Total	179	122	48	65	**414**		
	Antiviral	*3*	*1*	-	-	*4 (1)*		

	Other	*25*	*3*	*3*	*2*	*33 (7)*		
	NA	*2*	-	-	-	*2 (0.4)*		
CHT (n=414)		**179**	**122**	**48**	**65**	**414**		

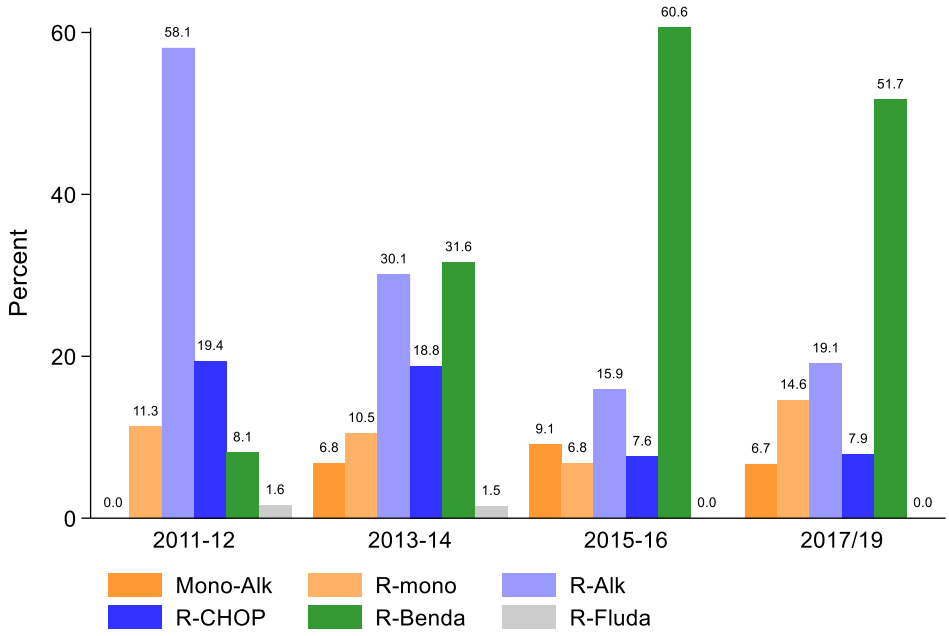


Figure 12. A distribution of chemotherapy regimens according to timeline period.

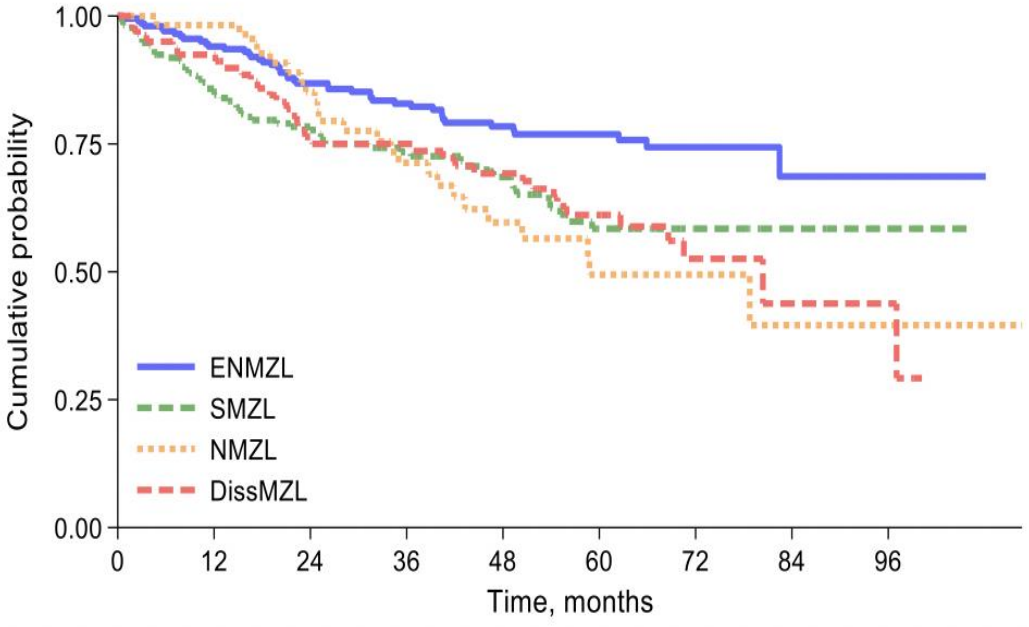


Figure 13. 5-year Progression Free Survival according to subtype of MZL

Table 12. Patients' characteristics at progression (n=125)

Group		Treated n (%)	After WW n(%)	p
		N=105	**N=18**	
Age	≥60	84 (80)	14 (78)	0.760
Stage [n=104]	IV	67 (75)	13 (87)	0.936
BM [n=55]	+	26 (58)	8 (80)	0.287
LDH [n=94]	>UNL	39 (48)	6 (46)	1.000
ALC [n=99]	<1000	36 (41)	2 (17)	0.123
Platelets [n=99]	<100	18 (21)	0	0.118
LodLin [n=92]	>6 cm	15 (19)	1 (7)	0.455
Histology	NMZL	32 (30)	6 (33)	0.780
	SMZL	35 (33)	7 (39)	
	ENMZL	17 (16)	1 (6)	
	Diss	21 (20)	4 (22)	

Table 13 Univariable and multivariable Cox PH regression on PFS (treated at diagnosis and after WW)

		Univariable		Multivariable [n=500]	
Group		**HR (95%CI)**	**p**	**HR (95%CI)**	**p**
Age [n=505]	≥60	1.59 (1.10-2.30)	0.014	1.57 (1.09-2.28)	0.016
Sex [n=505]	F	1.03 (0.75-1.42)	0.841		
Stage [n=500]	IV	2.13 (1.22-3.72)	0.008	2.10 (1.20-3.68)	0.010
ENS [n=504]	>1	0.90 (0.57-1.40)	0.629		
ECOG-PS [n=503]	>1	1.49 (0.84-2.65)	0.176		
LDH[n=496]	>UNL	1.37 (0.96-1.95)	0.082		
B2M [n=420]	>UNL	1.30 (0.86-1.97)	0.215		
Albumin [n=402]	<3.5	1.62 (1.08-2.44)	0.020		
ALC [n=496]	<1000	1.30 (0.89-1.89)	0.172		
Hb[n=505]	<12	1.61 (1.14-2.26)	0.006	1.50 (1.07-2.11)	0.019
Platelets [n=505]	<100	1.57 (0.99-2.46)	0.052		

HCV [n=498]	+	1.28 (0.75-2.19)	0.372		
HBV [n=490]	+	0.93 (0.63-1.38)	0.723		
Treat after WW		1.13 (0.74-1.73)	0.560		

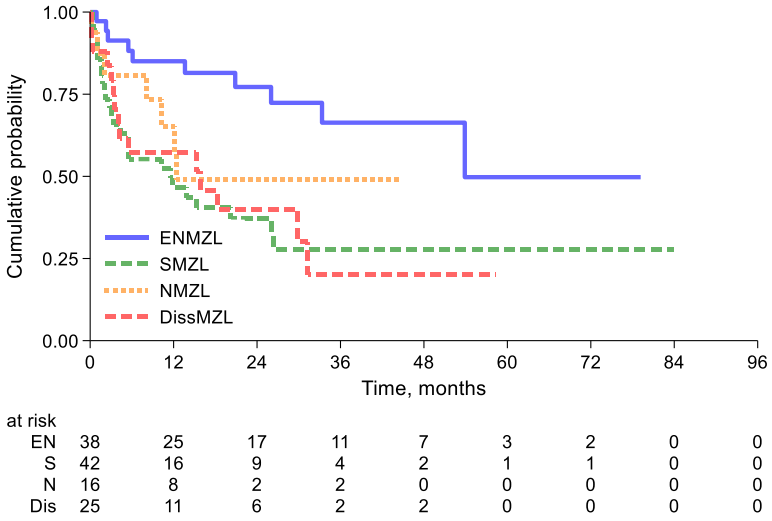
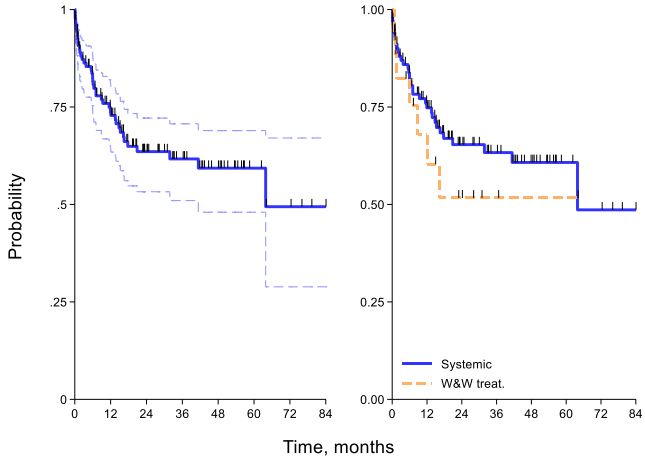


Figure 14. 2-year PFS-2 according to subtype of MZL



Group	N	5-yr SAR % (95CI)	HR (95CI)	P value
Treated	103	61 (49-71)	1.00	
WW treat.	18	52 (24-74)	1.38 (0.61-3.11)	0.442
Total	121	59 (48-69)		

Figure 15. Survival after progression in W&w and treated patients (n=121)

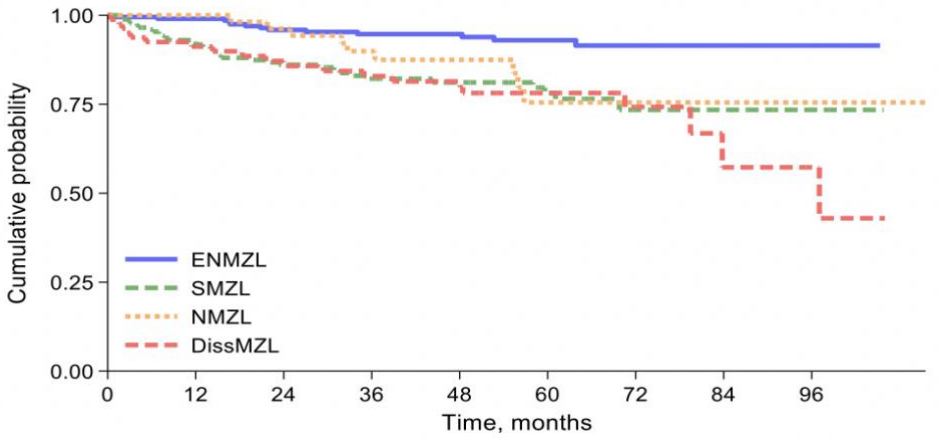


Figure 16. 5-y Overall Survival in NF10 patients according to histological subtypes

Early Progression as a predictor of survival in Marginal Zone Lymphomas: An Analysis from the Prospective International NF10 Study By Fondazione Italiana Linfomi [4]

Introduction

As previously described, the analysis of progression free survival has been used to identify surrogate endpoints in B-cell NHLs, with progression of disease at 24 months identified to stratify the overall survival in Follicular Lymphoma. Association of POD24 with OS has been confirmed also in Mantle Cell Lymphoma, Diffuse Large B-Cell and in Peripheral T-cell Lymphoma but it has not been analyzed in MZL. NF10 trial contributed to evaluate the impact of early progression on survival also in MZL (109). Our contribution on the analysis of POD24 both in FL and MZL patients is summarized in Table 16 (p. 72)

The main aim of this analysis was to validate the prognostic role of POD24 in the subgroup of early progressor patients enrolled in the NF10 study. The secondary objective was to identify additional patients' features that were associated with early progression and with patient outcome and to establish the accuracy of POD24 to predict OS.

Patients and Methods

For the purposes of this study only patients with MZL were included in the analysis and were classified as SMZL, ENMZL, NMZL and dissMZL, according to pathologic diagnosis.

The main endpoint of this study was overall survival, which was calculated from the date of diagnosis to the date of death for any cause. Secondary endpoints were PFS, and cause-specific survival (CSS). PFS was defined for all patients and calculated as the time from the date of initial diagnosis to progression, re-treatment, or death due to any cause; CSS was defined for all patients and calculated as the time from diagnosis to death due to lymphoma progression. Regarding OS, PFS and CSS, patients without events were censored at the time of last follow-up. POD24 was calculated only for patients requiring immediate therapy and was defined as experiencing lymphoma progression within 24 months from diagnosis. OS analysis, according to POD24, was calculated only for patients with events within 24

[4] S. Luminari, M. Merli, S. Rattotti, V. Tarantino, L. Marcheselli, F. Cavallo, M. Varettoni, B. Bianchi, F. Merli, A. Tedeschi, G. Cabras, F. Re, C. Visco, M. Torresan Delamain, E. Cencini, M. Spina, S. Ferrero, A. Ferrari, M. Deodato, D. Mannina, O. Annibali, A. Rago, L. Orsucci, I. Defrancesco, M. Frigeni, M. Cesaretti, and L. Arcaini

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months. For patients without early progression, OS was computed starting at 24 months from diagnosis, to reduce the effect of early progressive disease patients. Patients censored or those who died before 24 months were excluded from the analysis.

The study was approved by local ethic committees at any active center and a signed consent form was mandatory for all enrolled patients.

Results

Clinical characteristics of 706 MZL patients enrolled in NF10 are shown in Table 11 (p. 61). Overall, 321 patients who received immediate systemic therapy and who had an adequate follow-up were identified as the study population.

The median follow-up was 43 months (range 1-92). Five-year PFS was 64% (95% CI, 56% to 71%). Overall, 31 patients died; progressive disease was reported as the cause of death in 19 of 31 cases (61%). As result, 5-year OS was 88% (95% CI, 83% to 92%).

A histological transformation in aggressive lymphoma was documented in 7/85 patients with progressive disease. Two histological transformations occurred in POD24 achieved group and 5 in patients who fail POD24. Analyzing the Hazard risk function of lymphoma progression, the higher risk was observed after 6 months from the time of diagnosis with a decreasing risk in the subsequent follow-up time.

POD24 was reported in 59 patients (18%). The frequency of patients who failed to achieve POD24 was lower in the NMZL (9%) and ENMZL (16%) compared to the group of SMZL (25%) and DissMZL (20%). Three-year OS for patients with POD24 was 53% (95%CI, 37-67%) with a HR of 19.5 (95%CI, 8.4–45.4) when compared to patients without POD24 (88%, 95%CI, 89-98%) (Figure 17 p. 70). Three-year CSS for patients with POD24 was 62% (95%CI, 46-75%) with a HR of 19.5 (95%CI, 8.4–45.4) when compared to patients without POD24 (99%, 95%CI, 92-99%). The effect of POD24 on OS was confirmed in ENMZL, SMZL and Diss-MZL subgroups (Figure 18, p.70).

Logistic regression analysis to predict the risk pf POD24

Univariate logistic regression analysis was conducted to identify associations between clinical and prognostic features at the time of diagnosis, treatment modalities and the risk of POD24 (Table14, p. 70). When the multivariate analysis was conducted, four parameters were independently associated with the risk of POD24, namely hemoglobin, platelet count, lymphocyte count, and treatment (Table 15, p. 70). In addition to laboratory parameters, both the use of Rituximab either alone or in combination resulted in a significant reduction

of the risk of POD24 compared to non-Rituximab based therapies, or regimens other than immunochemotherapy, respectively.

Discussion

Briefly, among the 1325 patients enrolled in the NF10 study, we identified 321 patients for whom immediate therapy was planned right after lymphoma diagnosis and that represent our population target. Overall, POD24 was reported in 18% of cases, which is lower than the POD24 rate of 28% observed for FL and likely corresponds to a more indolent behavior of MZL. A direct relationship between disease aggressiveness and the risk of early progression was also suggested by the observation of different POD24 rates among different MZL subtypes, being higher in SMZL and lower in the less aggressive ENMZL. Similar to FL, a substantial inferior survival was observed in early progressors compared to those with late or without relapse, with a 19.5-fold increase in the risk of death (3-year OS of 53% vs 95%). Association of POD24 with OS was confirmed for the subgroup of splenic and extranodal MZLs. Unfortunately, we were not able to show a correlation of POD24 with OS in NMZL, mainly due to the small size of this subgroup (Figures 17 and 18, p. 69).

Available data raise important questions to identify the major drivers of the lower survival for POD24 patients. Similar to FL, also in MZL 66% of deaths for POD24 patients were referred to lymphoma progression. Recent data on FL suggest that early events could be enriched with a transformation in more aggressive lymphoma. In our study, we were able to identify 7 patients with histologically transformed MZL, 5 of them in the POD24 cohort. Thus also if the rate of transformation in our series was low compared to other reported series (73,110), our report suggests that histological transformation (HT) might play a role in defining the quality of early events. At this regard, there is a need to investigate the underlying molecular mechanisms of both HT and early relapse in indolent lymphoma, as divergent driver genetic lesions or alternative altered pathways may sustain these two kinds of relapse.

Another possible driver of the increased risk of death among MZL cases with POD24 might be represented by an inadequate salvage therapy. Similar to what was initially reported by Casulo for FL (111), very few POD24 patients were treated with aggressive regimens in our series. High dose therapy followed by autologous stem cell transplant (ASCT) was reported in only 3 cases. Early administration of ASCT could be effective to overcome the detrimental effect of POD24 on OS as recently suggested by three independent retrospective studies (111,112). Considered together, these data suggest a potential clinical benefit of ASCT for younger fit patients with MZL experiencing an early relapse.

The finding of early progression as a strong marker of poor prognosis is useful but its clinical utility to support initial treatment choice is limited. Thus, the identification of baseline features independently associated with the risk of POD24 is warranted. We performed a logistic multivariate analysis and confirmed 3 laboratory parameters (anemia, thrombocytopenia, lymphopenia) and initial treatment choice as independent predictors of early progression (Table 14, p. 71). In addition to laboratory parameters, the use of Rituximab either alone or in combination resulted in a significant reduction of the risk of POD24 compared to non-Rituximab-based therapies, or regimens other than immunochemotherapy, respectively.

This multivariate analysis confirmed known prognostic features in MZL as important predictors of early failure. Of note, the independent correlation of the risk of POD24 with initial treatment is in line with recent data on FL that demonstrated that the risk of POD24 can be modified, but not eliminated, by more active induction therapy (113). In line with this observation, in our study the use of immunochemotherapy was found to have an independent role in reducing the risk of POD24 compared with Rituximab monotherapy or chemotherapy alone. Finally, POD24 had a very strong correlation with the risk of death in our study, which was the highest compared with that of other available prognostic indexes.

These results compared with those reported for FL patients (20,22,29,113) allow to generalize POD24 as a strong surrogate of OS for most indolent B- cell Lymphomas.

Moreover, POD24 can be identified as a criterion to identify more aggressive patients to interpret and design clinical trials for relapsed and refractory MZL patients.

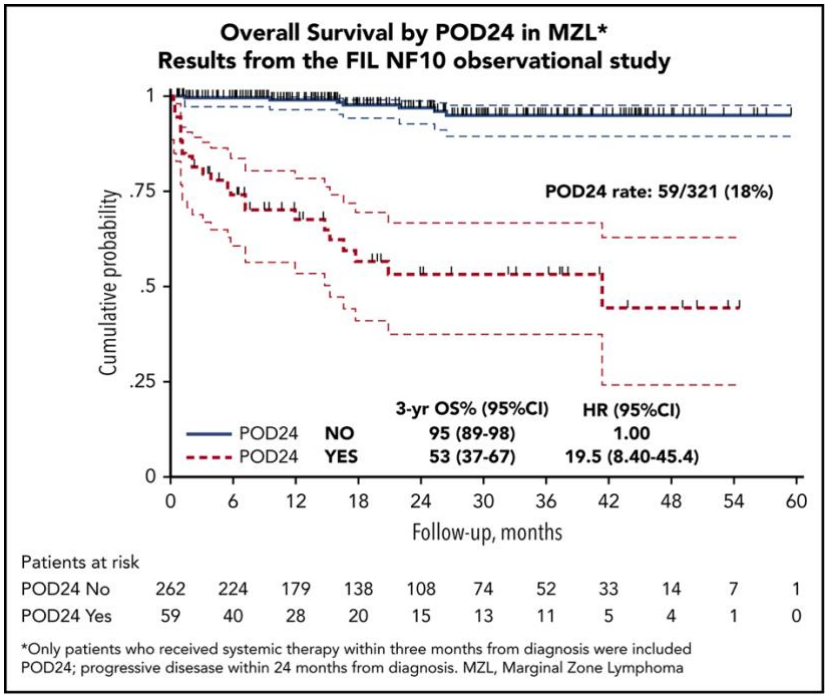

Figure 17. Overall Survival by POD24

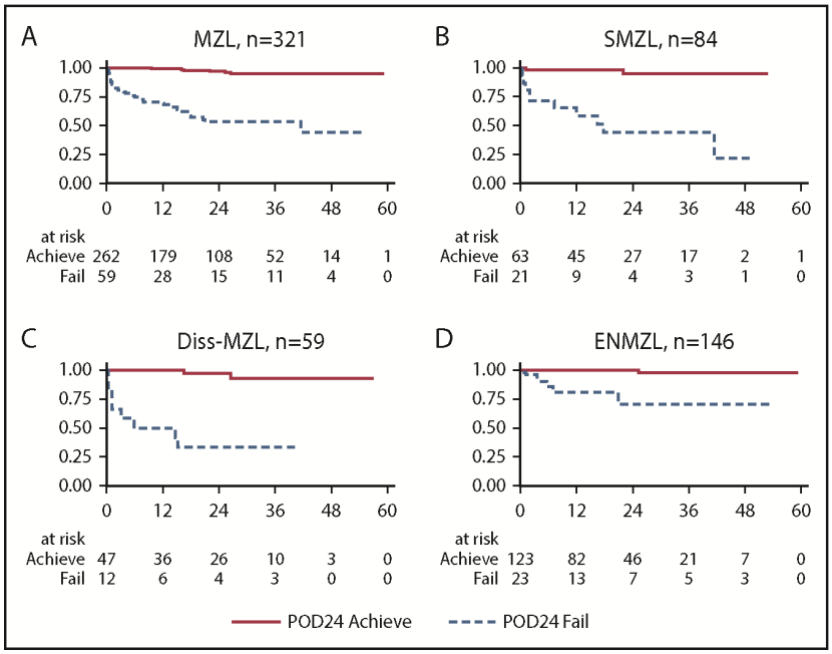

Figure 18. OS by POD24 and by MZL subtypes. (A) Patients with MZL: POD24 rate, 18%; 3-year OS POD24, achieve 95% vs fail 53% (*P* < .001) (HR, 19.5; 95% CI, 8.40-45.4). (B) Patients with SMZL: POD rate, 25%; 3-year OS POD24, achieve 95% vs fail 44% (*P* < .001). (C) Patients with disseminated MZL (Diss-MZL): POD rate, 20%: 3-year OS POD24, achieve 93% vs fail 33% (*P* < .001). (D) Patients with ENMZL: POD rate, 16%; 3-year OS POD24, achieve 98% vs fail 71% (*P* < .001).

Table 14. Univariate logistic regression analysis to predict the risk of POD24

Factor	Missing	Status	POD 24 N (%)	P
Categorical variables				
ECOG PS	-	0-1	51 (17)	0.035
		>1	8 (38)	
Symptoms	-	A	38 (15)	0.004
		B	21 (32)	
BM	20	-	19 (13)	0.049
		+	34 (22)	
Albumin	85	>3.5 g/dL	27 (15)	0.022
		≤3.5 g/dL	15 (29)	
Treated with R-Chemotherapy	-	Yes	34 (14)	0.001
		No	25 (50)	
Treated with Rituximab	-	Yes	40 (14)	<0.001
		No	19 (42)	
Continuous variables			**POD24 OR (95%CI)**	**p-value**
Beta2 microglobuline	17	Log(B2M/B2Mmax)	1.85 (1.15-2.97)	0.010
Hemoglobin,	3	g/dL	0.79(0.70-0.89)	<0.001
Platelet counts	5	Log(Plt)* 10^9/L	0.45(0.27-0.76)	0.003

Table 15. Multivariable logistic regression analysis to predict the risk of POD24

Covariate	**Status**	**POD24**		**p-value**
		OR	**(95%CI)**	
Hemoglobin	g/dl	0.80	0.70-0.91	0.001
Platelet count	Log(Plt), 10^9/L	0.56	0.31-0.98	0.045
Absolute Lymphocyte count	Log(ALC), 10^9/L	0.70	0.51-0.96	0.029
R-Chemotherapy	*yes/no*	*0.26*	*0.12-0.52*	*<0.001*

Table 16. Impact of POD24 on indolent lymphoma patients in our analysis

Impact of POD24 on indolent lymphoma's patients outcome			
Study	**N pts**	**Outcome**	**Factors associated**
FOLL05 (FLpts)	141/502	7-yearsOS 59% vs 91%	FLIPI and FLIPI-2 > 2 (p <0.001) EoT PET+ (p<0.009)
NF10 (MZL pts)	59/329 (18%)	3-yearOS 53% vs 95%	Hemoglobin (p=0.001), plates count (p=0.045), absolute lymphocyte count (p=0.029), R-chemotherapy (p<0.001)
Role of interim PET on outcome			
FOLL12 (FL pts)	211/807	3-year PFS 52% vs 87% 3-year OS 89% vs 99%	Ongoing study

The role of FDG-PET in MZL and our future perspectives: Pimento study

Recently, similar to what was observed in aggressive NHL and in HL, PET has led to a more accurate staging, supporting the identification of cases with transformed histology, and has provided useful prognostic details also in indolent lymphoma, especially it has been extensively studied for follicular lymphoma. For MZL the role of metabolic assessment remains largely undefined.

Most of the controversies derived from the variability of FDG avidity for the different MZL subtypes and from difficulties to interpret metabolic data based on SUV_{max}, that is in median low, ranging from 5 to 7. PET interpretation is further complicated by the frequent bone marrow involvement, which is hard to detected and does not allow to obviate the need for a bone marrow biopsy in the initial patient assessment (114,115).

Moreover, data on the role of PET in MZL were mostly derived from retrospective studies involving a small population. In a small series of 25 MZL patients, PET permitted a more accurate stage in about 20% of patients detecting more involved areas, mainly extra-nodal. Moreover, its prognostic role was demonstrated by the fact that among the 70% of the patients that achieved a negative PET at the end of treatment, none of them relapsed, compared to patients with a fPET+ (116).

More recently, Qi et al retrospectively analyzed a series of 173 cases of MALT lymphomas, correlating PET positivity and intensity of FDG uptake with clinical factors and patient outcome. They found that SUV ≥10 was an independent factor associated with significantly shorter 5-year OS (78% vs. 92%) and a higher rate of subsequent transformation (20% vs 5%). However, only 20 (16%) patients presented with SUV ≥10 and thus this study warrants further validation (117).

Albano et al attempted to evaluate the prognostic impact of qualitative and semi-quantitative baseline PET variables such SUVmax, metabolic tumor volume, and total lesion glycolysis (TLG) in 161 EMZL patients. From the analysis, FDG-avidity seemed to correlate with Ki67 expression and tumor size, however, no correlation between survival and PET parameters was observed (118).

Some efforts have been made to enhance the role of functional imaging in detecting transformation in aggressive lymphoma by correlating abnormally elevated FDG uptake at sites of transformation. In a single center prospective study by Bodet-Milin et al, FDG-uptake driven biopsy was evaluated in 38 indolent lymphoma patients of whom only 3 with MZL. A SUV_{max} of 11 was suggested as cut-off to achieve the best balance between sensitivity and specificity to confirm low-grade histology. SUV_{max} values higher than 17 have been reported

in histologically confirmed transformation suggesting that the probability of aggressive disease rise as the SUV increases (119).

Further investigations in more robust prospective studies are needed to better define PET as an accurate surrogate of transformation in indolent lymphomas.

Taking into account the lack of data on the prognostic and predictive role of FDG-PET we decided to conduct a retrospective study to investigate the role of functional imaging in marginal zone lymphoma patients: the PIMENTO study (NCT04333524) sponsored by International Extranodal Lymphoma Study Group (IELSG). The main aim of the project was to provide robust data on the role of PET for the staging and response assessment of MZL. These results will be used to update the current available recommendation about the use of PET in MZL.

Study Design

The study is designed as a retrospective collection of patients with MZLs enrolled in the prospective IELSG36 and IELSG38 trials and in the observational NF10 study by FIL, with the possibility to add additional cases from participating institutions. Availability of details on clinical presentation, treatment and results, and on follow-up are required for accrual. Moreover, only patients for whom PET scan are available as Digital Imaging and Communications in Medicine (DICOM) format for central review will be considered for the analysis.

The study is being divided into two sections with different aims.

Part A is conducted to understand the role of PET for the staging of MZL. PET scans are analyzed and compared with data retrieved from CT scan and from other staging procedures, also including bone marrow biopsy, ultrasound, and laboratory exams. This part of the study describes the ability of PET to identify pathologic lesions and to contribute to staging definition or to stage migration.

Part B is conducted to validate standardized criteria for response assessment in MZL including FDG-PET among procedures and to define the prognostic role of metabolic response in MZL. The primary endpoint for this part of the study is Progression Free Survival. Secondary endpoints are Overall Survival, Response Rate and rate of histologic transformation.

Primary Endpoints

- To correlate CT and PET results for stage definition

- To evaluate the Progression Free Survival
- To evaluate the Duration of Response

Secondary Endpoints

- To evaluate the prognostic value of PET at baseline with visual, semi-quantitative (MTV and TLG, and radiomics analysis
- To compare the CT scan based staging system to the staging evaluated with PET
- To compare Matutes and Lugano criteria for response assessment vs PET based response (43,120)
- To assess ability of PET to detect histological transformation
- To evaluate the ability of radiomics feature to predict MZL subtypes
- To evaluate the Overall Survival
- To evaluate the Response Rate

Procedures

As a significant proportion of patients considered for this study will be retrieved from previous observational prospective clinical studies, data on clinical presentation, treatment and follow-up will be obtained from the existing dataset of the previous protocols. The additional cases, identified from clinical practice registry, will be collected from patient chart. A unique study e-CRF will be prepared to collect all the required details. All PET will be centralized to perform a blinded independent review of staging and response by a panel of two nuclear medicine physicians. In particular, each case will be evaluated by two reviewers. In case of discordant results, a third reviewer will adjudicate the case.

Due to the retrospective nature and the exploratory aims of the study, we did not plan a sample size, however, we estimate to collect data on 200 patients for part A of the study and 150 patients for part B. These numbers should correspond to all patients enrolled in the prospective IELSG36, IELSG38 trials and in the observational NF10 study.

In order to grant for inclusion of all patients with available PET the researchers will actively screen all patients enrolled in the clinical trials.

PET and CT results will be correlated with treatment response and patients' outcome.

Revised PET results will be correlated with baseline characteristics of patients with Chi square or Fisher exact test as appropriate and with time dependent measures using the log rank test. For the purposes of this study, progression free survival and duration of response will be used as study endpoints. PFS is calculated for all patients as the time between

enrollment to last follow-up or disease progression or death for any cause. Duration of response is calculated only for patients achieving complete response or partial response, from time of response assessment until last follow-up, relapse or death for any cause. All statistical tests will be two sided.

This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, the ICH-GCP as well as all national legal and regulatory requirements.

The study has been recently approved by Reggio Emilia Ethics Committee to start collecting data in Italy. The end date of PET collection is scheduled for February 2023; the duration of the study is from September 2020 to June 2023.

Chapter 6. Conclusions

In the last decades, efforts have increasingly been made to better understand the biological basis and clinical behavior of indolent lymphomas, with significant improvements in patients' outcomes.

Most of the studies conducted so far have consistently confirmed a high heterogeneity in the clinical behavior of indolent lymphomas, with about 20% of patients showing a high-risk profile and a clinical course marked by early relapse or refractoriness to therapy. These vulnerable patients should be identified by the recognition of adverse prognostic factors that can be used to guide a personalized treatment approach to overcome adverse outcome.

Several studies focused on identifying markers of poor prognosis in iNHL, mostly borrowed from FL. An overview of the currently available factors associated with high-risk in FL reveals a multitude of variables including clinical biological and metabolic models applied at the diagnosis and at the end of induction. Among studied factors, those describing the quality of the response either using FDG-PET, applying molecular biology techniques, and describing duration of response, received enough validation to explore their role in risk adapted strategies.

Unfortunately, most of the prognostic information currently available often yielded conflicting results, were not validated in the era of new agents or have not been considered as tools to guide risk-adapted therapeutic recommendations, which remains a significant limitation to daily clinical practice.

Within the above presented framework, our efforts were devoted to refining the description of the outcomes of indolent lymphomas, to improving the definition of the risk through newer and more accurate tools and finally constructing a risk-adapted platform of therapy.

Such endeavor aimed to improve the capacity to define the different risk profiles, to integrate prognostic developments into clinical implementation, and to design a personalized approach to patient care during these years.

Our findings confirmed the excellent control of the disease achieved through the use of standard front-line chemoimmunotherapy both for FL (FOLL05, FOLL12) and for MZLs (NF10 study) in the first and subsequent lines (PETRA study). However, our data also confirmed the heterogeneity of the disease and the impact of early progression on life expectancy, thus validating the prognostic role of POD24 both for marginal zona lymphoma and follicular lymphoma.

Our research further triggered other important considerations, in particular from the FOLL12 trial. Despite the strategy of a response-adapted therapy, based on metabolic and molecular

response at the end of induction, applied in this trial, was not mature enough to modify the current therapeutic paradigm, the study opened the horizon to future investigations beyond just a recommendation of a maintenance strategy.

Indeed, there are different aspects that are the key to FOLL12 analysis and that should be considered in the further development of response-adapted strategies:

- The use of anti-CD20 monoclonal antibody maintenance is an important part of the initial treatment that cannot be omitted without affecting the risk of progression;
- Ninety percent of patients are expected to respond to the currently available first-line immunochemotherapy programs, thus challenging the ability to investigate the end of the induction response as an actionable endpoint;
- A different strategy to evaluate response-adapted therapy in FL would be to anticipate response assessment from the end of induction to an earlier treatment phase.

These results supported the development of a novel generation of response-adapted trials. The recently launched FOLL19 platform, anticipating the prognostic assessment, could provide the expected answers about how to early identify patients who could benefit from de-escalation or intensification therapy including novel agent therapies.

The above noted lack of a risk-adapted therapeutic strategy in front-line therapy, also manifests itself in relapse settings.

There is a general consensus that, for patients who experience an early progression of disease, autologous stem cells transplantation should be used. This approach achieved better results on outcome compared to standard chemoimmunotherapy, especially if it is performed within one year from treatment failure (111,112). Nonetheless, the use of ASCT in relapsed/refractory patients is supported by a wide number of retrospective studies with discordant results (121,122) and ASCT has never been compared directly to novel treatment.

In patients who are not eligible for ASCT, several alternative conventional therapies are available. However, a few related recommendations can be made, including suggesting the enrollment into a clinical trial as a first option, if available, and resorting to intensive (but within the limits of the patient’s tolerance) treatment, as alternative option.

Promising data can be drawn from the use of new drugs recently approved by the national health authorities for the treatment of R/R FL, based on their documented activity with phase II or phase III data. These include the pI3K inhibitor Idelalisib, the immunomodulator Lenalidomide and the new anti CD20 monoclonal antibody Obinutuzumab (123–125). Other promising new drugs have recently started their clinical development including the EZH2

inhibitor Tazemetostat (126), antibody-drug conjugates (ADC) and bispecific T cell engagers (Bite) (127–129).

Given the significant improvement in terms of efficacy of the therapy, and the favorable toxicity profile, their application in risk- adapted platform may be an attractive strategy.

Finally, as well as for aggressive lymphoma, also for FL the availability of chimeric antigen receptor-T cell (CAR-T cell) therapy will expand the ability to use cellular therapy also in high-risk indolent lymphoma patients showing promising results (130,131).

Going forward, our next priority must be to tackle the subsets of patients that are early progressors by defining optimum strategies to improve their survival. Successfully achieving this will require improved prognostication, understanding and integration of the disease biology, and delineating molecular determinants of response and resistance to existing and emergent therapies.

In conclusion, for iNHL, the future therapeutic approach should start from the recognition of different risk groups. This approach will facilitate accurate, personalized treatment for most vulnerable patients and will help reducing the risk of progression and death, including by intensifying the treatment and introducing novel strategies other than chemoimmunotherapy. For low-risk patients, who will have excellent survival, this approach would contribute to limiting their exposure to toxicity and to reducing the overall cost of the therapy.

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Appendix

List of centers participating in the studies

FOLL05: Alessandria, Asti, Aviano Oncologia, IRCCS Bari, Ematologia Biella, Ematologia Brescia, Ematologia Catania Ferrarotto, Catania Nesima, Catanzaro, Civitanova Marche, Cosenza, Ematologia Careggi Firenze, Cuneo Ematologia S. Croce e Carle, Genova Galliera, Genova San Martino, Lecce, Lecco, Matera, Ematologia Messina Papardo, Ematologia Messina Policlinico, Milano Fatebenefratelli, Milano Humanitas, Milano Policlinico, Milano Niguarda, Milano San Paolo, Milano San Carlo Borromeo, Milano San Raffaele, Mirano , Modena, Napoli Caldarelli, Nocera Pagani, Novara, , Pavia Policlinico San Matteo, Perugia, Pescara Ematologia, Pescara Oncologia, Piacenza, Pisa, Reggio Calabria, Reggio Emilia, Rionero, Roma Campus Biomedico, Roma Università Cattolica, Rom IFO Regina Elena, Roma Università La Sapienza, Roma Ospedale San Andrea, Roma Università Tor Vergata, San Giovanni Rotondo, Sassuolo, Terni, Torino Candiolo, Torno Molinette, Tricase, Varese, Venezia Mestre

FOLL12: Alessandria, Ematologia Umberto I Ancona, Aviano Oncologia, IRCCS Bari, Bergamo, Ematologia Biella, Ematologia Ospedale S. Orsola Bologna, Ematologia Brescia, Ematologia Brindisi, Ematologia Bolzano, Cagliari, Ematologia Catania Ospedale Vittorio Emanuele, Civitanova Marche, Ematologia Careggi Firenze, Cuneo Ematologia S. Croce e Carle, Genova Galliera, Genova San Martino, Lecce, Matera, IRST Meldola, Ematologia Messina Papardo, Ematologia Messina Policlinico, Milano INT, Milano Ospedale Maggiore, Milano Humanitas, Modena, Monza, Napoli Caldarelli, Nocera Pagani, Novara, Nuoro, Palermo Policlinico, Palermo Ospedale Cervello, Pavia Policlinico San Matteo, Perugia, Pescara Ematologia, Pescara Oncologia, Piacenza, Pisa, Reggio Calabria, Reggio Emilia, Rionero, Roma Campus Biomedico, Roma Università Cattolica, Rom IFO Regina Elena, Roma Università La Sapienza, Roma Ospedale San Andrea, Roma Università Tor Vergata, San Giovanni Rotondo, Sassuolo, Terni, Torino Candiolo, Torno Molinette, Tricase, Varese, Venezia Mestre

NF10: Aviano Oncologia, IRCCS Bari, Bergamo, Brasile Campinas, Brasile San Paolo, Brasile UFRJ, Brescia Ematologia, Cagliari, Catania Nesima, Como, Cosenza, Kiev, Latina Università, Lecce, Lisbona, Matera, Messina Papardo, Milano Ospedale Maggiore, Milano Niguarda, Milano San Carlo Borromeo, Milano San Paolo, Milano San Raffaele, Milano Humanitas, Modena, Monza, Nocera Pagani, Novara, Padova Oncologia, Parigi, Parma, Pavia Policlinico San Matteo, Pescara Ematologia, Pisa, Reggio Calabria, Reggio Emilia, Rionero in Vulture, Roma Campus Biomedico, Roma La Sapienza, Roma S. Eugenio, Sassuolo, Siena, Taranto Ematologia, Terni S. Maria, Torino Molinette, Torino Università, Varese, Vicenza, Vienna.