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FOLLICULAR LYMPHOMA: CLINICAL AND BIOLOGICAL FACTORS ASSOCIATED WITH RESPONSE TO THERAPY AND OVERALL PROGNOSIS

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Follicular lymphoma: clinical and biological factors associated with response to therapy and overall prognosis

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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“Life can only be understood backwards, but must be lived forwards”

Søren Kierkegaard

To my beloved father who will always be missed

POPULÄRVETENSKAPLIG SAMMANFATTNING

Lymfom är ett samlingsnamn för tumörsjukdomar utgående från lymfsystemet, bland vilka de follikulära lymfomen tillhör de vanligaste. I Sverige insjuknar ca 250 personer per år. Sjukdomsförloppet är i hög grad varierande; ett fåtal patienter tycks tillfriskna spontant och en minoritet botas men det stora flertalet avlider så småningom i eller med sin sjukdom, efter flera återfall och behandlingsomgångar. Överlevnaden har under senare år förbättrats, mycket med hjälp av riktad antikroppsterapi som alternativ eller komplement till strålning och cellgifter. Nya läkemedel har tillkommit, med nya verkningsmekanismer, varibland en del vänder sig till kroppens eget friska immunförsvar och försöker hjälpa detta att identifiera, uppsöka och slutligen döda cancercellerna. Till skillnad från många andra lymfomsorter krävs vid follikulärt lymfom inte alltid behandling om patienten är helt symptomfri. Alltjämt saknas konsensus kring när behandling skall påbörjas, vilken/vilka terapi(er) som är bäst och i vilken ordning dessa bör ges.

Avhandlingen, med flera ingående delprojekt, syftar till att ge ökad kunskap om vilka olika faktorer hos den enskilde patienten och hans/hennes specifika lymfom som kan förutsäga och påverka sjukdomsutvecklingen och vilken behandlingsstrategi som är den lämpligaste. En viktig målsättning är att genom förbättrad individanpassad handläggning uppnå en överlevnad lik den som finns hos övriga befolkningen, med minimerade sjukdoms- och behandlingsrelaterade komplikationer.

I delarbete I undersökte vi långtidseffekterna av behandling med antikroppen rituximab given ensam eller tillsammans med det immunmodulerande läkemedlet interferon-α2a. Efter en medianuppföljningstid på 10 år var 234 av de totalt 321 studerade patienterna i livet och en tredjedel hade klarat sig så pass väl med denna mildare form av lymfombehandling att de aldrig behövt få cellgifter. I delarbete II och delarbete III tillämpade vi två olika prognostiska modeller, m7-FLIPI och PRIMA-PI, framtagna på patienter som fått traditionell cellgiftsbehandling för att se om de var användbara även på den cellgiftsfria gruppen. Det visade sig att endast PRIMA-PI av de två kunde bidra med prognostisk information. Utifrån en kombination av endast två variabler kunde modellen identifiera en grupp patienter vilkas sjukdom skulle komma att visa sig avsevärt mer aggressiv, vilka torde vara betjänt av annan företrädesvis mer intensiv behandling redan från början. Delarbete IV handlade om de varierande nivåerna av olika sorters immunceller i blodet hos patienter före, under och efter behandling. Även här fick patienterna rituximab men istället för kombinationen med interferon gavs det nyare läkemedlet lenalidomid, besläktat med det på 60-talet omskrivna medlet Neurosedyn. Blodproverna visade mer eller mindre varaktiga förändringar i immuncellernas sammansättning, varav somliga tycktes sammanhänga med bättre behandlingseffekt och kanske i förlängningen förbättrad överlevnad.

ABSTRACT

Follicular lymphoma (FL) is a heterogeneous group of malignancies within the adaptive immune system. The clinical course is highly variable. Treatment includes different chemotherapy regimens as well as the anti-CD20 monoclonal antibody rituximab which has significantly improved the prognosis for patients with all types of B cell lymphomas. The majority of patients with FL respond well to therapy but eventually relapse and generalized disease is still considered incurable. On the other hand, a substantial number appear to have such an indolent disease that the benefit from treatment is unclear. In the clinical setting one challenge is to identiy FL patients in need of therapy upfront as opposed to those who can be managed with active expectancy. Seemingly of importance in addition to the clinical status of the host are characteristics of the tumour cells as well as of the immune cells in the surrounding microenvironment. A greater understanding of the complex intra- and inter-cellular signaling provides new potential targets for therapeutical intervention.

The aim of this thesis was to gain increased insight in clinical and immunological factors of prognostic importance, in indolent lymphoma in general and in FL in particular. This was done by investigation of the long-term outcome in patients treated with rituximab-based immunotherapy and of the validity of the prognostic tools developed in patients receiving standard chemotherapy-based treatment. We also made a study on the interaction of rituximab and the immunomodulator lenalidomide with the healthy immune system during therapy.

In paper I we evaluated the late effects of rituximab monotherapy and rituximab with interferon-α2a in 321 previously untreated symptomatic indolent lymphoma patients. After a median follow-up of 10 years more than one third had never required chemotherapy and 73% were still alive.

In papers II and III we investigated the two prognostic models m7-FLIPI and PRIMA-PI, both recently developed on cohorts of FL patients treated with a combination of rituximab and CHOP/CVP. The clinicogenetic m7-FLIPI model was not valid in our cohort treated with rituximab with or without interferon. The PRIMA-PI on the other hand, although based on only two parameters – beta2-microglobulin and bone marrow involvement, was a useful tool in differentiating a small group of patients with very poor prognosis, that should be considered for a new or more intensive and hopefully more effective therapeutic approach.

In paper IV we followed the changing composition of immune cells in blood in FL patients randomized to therapy with rituximab with or without lenalidomide. Cells were sampled at baseline**,** after 2 weeks of lenalidomide (combination arm), 24 hours after first rituximab dose and at follow-up weeks 10 and 23 and analysed by flow cytometry. With lenalidomide alone a transient increase in monocyte and NK cell fractions appeared, the latter decreasing again after first rituximab infusion. Post-treatment effects included an increased fraction of T cells as a group and an increased CD4/CD8 ratio. A high proportion of monocytes at baseline was associated with clinical response at week 10 as were a larger fraction of naïve T cells in the rituximab monotherapy treatment arm. Possibly lenalidomide may help overcome the negative impact of few naïve cells, by a beneficial effect on their activity.

LIST OF SCIENTIFIC PAPERS

Chemotherapy-Free Initial Treatment of Advanced Indolent Lymphoma Has Durable Effect With Low Toxicity: Results From Two Nordic Lymphoma Group Trials With More Than 10 Years of Follow-Up

Lockmer S, Østenstad B, Hagberg H, Holte H, Johansson AS, Wahlin, BE, Wader KF, Steen CB, Meyer P, Maisenhølder M, Smedby KE, Brown P, Kimby E. *J Clin Oncol.* 2018, 36, 3315-3325. doi: 10.1200/jco.18.00262. Epub 2018 Oct 04

M7-FLIPI is not prognostic in follicular lymphoma patients with first-line rituximab chemo-free therapy

Lockmer S, Ren W, Brodtkorb M, Østenstad B, Wahlin BE, Pan-Hammarström Q, Kimby E. *Br J Haematology.* 2020 Jan;188(2):259-267. doi: 10.1111/bjh.16159. Epub 2019 Aug 18.

The Follicular Lymphoma PRIMA-Prognostic Index is Useful in Patients with First-Line Chemo-Free Rituximab-Based Therapy

Kimby E, **Lockmer S**, Holte H, Hagberg H, Wahlin BE, Brown P, Østenstad B. *In submission*

Immunce Cell Dynamics in Follicular Lymphoma Patients Treated Within a Randomized Trial with Rituximab and Lenalidomide

Lockmer S, Wahlin BE, Østenstad B, Jeppson-Ahlberg Åsa, Sander B, Kimby E. *Manuscript*

RELATED PUBLICATIONS

A clinico-molecular predictor identifies follicular lymphoma patients at risk of early transformation after first-line immunotherapy.

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LIST OF ABBREVIATIONS

ADCC	Antibody-dependent cellular cytotoxicity
ß2m	Beta 2-microglobulin
BTK	Bruton tyrosine kinase
CAR T cell	Chimeric antigen receptor
CD	Cluster of differentiation
CRF	Case report form
DLBCL	Diffuse large B-cell lymphoma
ECOG	Eastern Cooperative Oncology Group
FDG	Fluorodeoxyglucose
FFS	Failure-free survival
FL	Follicular lymphoma
FLIPI	Follicular lymphoma international prognostic index
FOXP3	Forkhead box protein P3
HPF	High-power field
IFN	Interferon-α2a
LDH	Lactate dehydrogenase
LON	Late-onset neutropenia
MIU	Million international units
MRD	Minimal residual disease
NHL	Non-Hodgkin lymphoma
NK cell	Natural killer cell
NLG	Nordic Lymphoma Group
NOS	Not otherwise specified
OS	Overall survival
PD-1	Programmed death-1
PFS	Progression-free survival
POD	Progression of disease
R	Rituximab
RL	Rituximab and lenalidomide
R-CHOP	Rituximab – cyclophosphamide, doxorubicin, vincristine, prednisone
R-CVP	Rituximab – cyclophosphamide, vincristine, prednisone
SAKK	Swiss Group for Clinical Cancer Research
SCT	Stem cell transplant
PET	Positron emission tomography

tFL	Transformed follicular lymphoma
TTF	Time to treatment failure
WHO	World Health Organization

1 INTRODUCTION

1.1 OVERVIEW

After the early description of Hodgkin's disease, R. Virchow was the first to launch the term lymphosarcoma for the non-leukemic tumours later collectively named non-Hodgkin's lymphoma (after dropping of the possessive form simply non-Hodgkin lymphoma, NHL). Many years followed, during which pathologists had only a vague idea of these presumably malignant conditions presenting with lymphadenopathy and splenomegaly and applied different terminology.[1] In 1942, Gall and Mallory[2] tried to bring order introducing a classification based on clinicopathologic criteria. During the years 1966-1976 the heterogenous entity of NHL was to be found in six different classification systems; the Rappaport[3], Kiel[4], Lukes-Collins, Dorfman, British, and World Health Organization systems.[5] Starting in 1982, the Unites States (US) National Cancer Institute recommended the Working Formulation which was adopted in the US while an updated version of the Kiel classification was preferred in Europe. The Working Formulation and Kiel Classification were in 1994 replaced by the Revised European-American Lymphoma (REAL) classification[6], which making use of scientific advances, included morphologic, immunophenotypic, genotypic, and clinical features into the definition of NHL subtypes.[7]

Follicular lymphoma (FL) is characterized by a usually follicular (nodular) pattern of growth and earlier names for the disease reflected its appearance. It was termed nodular lymphoma in the 1956 Rappaport classification[3] and with reference to cytology centroblastic/centrocytic lymphoma in the 1974 Kiel classification.[4] In the REAL classification[6] it was called follicular centre lymphoma because it is composed of centroblasts and centrocytes of the germinal centre in the follicle.[8] The World Health Organization (WHO) classification of 2001[9] was built upon the REAL and has, including its revised editions, ever since been the international gold standard for all hematopoetic neoplasm. FL is hereby grouped among the mature B-cell malignancies of the NHLs.[7] Although a more accurately defined neoplasm within the adaptive immune system, FL is still a heterogeneous entity for which the clinical course is highly variable.[8, 10, 11]

When Gall and Mallory presented their work in 1942, average survival after diagnosis of FL was around five years.[2] Treatment often consisted of radiation over relatively large fields of the body in lower or higher doses depending on the aggressiveness of the disease, and/or surgical excision, later sometimes with the addition of chemotherapy.[12-14] 1946 saw the first publication on nitrogen mustard therapy[15], cyclophosphamide was introduced into clinical practice in the late 1950s[16] and treatment during the following decades came to include different chemotherapy regimens. In the 1990s steroids, the oral alkylators chlorambucil and cyclophosphamide, the purine analogue fludarabine and the combination of fludarabine, mitoxantrone (topoisomerase inhibitor) and dexamethasone (FND) were all common as first-line therapy, whereas in the relapse situation usually combinations of cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) were applied.[17] When the anti-CD20 monoclonal antibody rituximab was introduced in the late 1990s, it significantly improved the prognosis for patients with all types of B cell lymphomas.

The majority of FL patients respond well to therapy, but eventually relapse and generalized disease is still considered incurable. On the other hand, a substantial number of patients appear to have such an indolent disease that the benefit from treatment is unclear.[8, 10] In the clinical setting one challenge is to identify patients in need of therapy upfront as opposed to

those who can be managed with active expectancy. The typical patient will during his or her lifetime receive a number of different therapies with variable intensity toxicity.[10] Factors that seem to be of importance for the course of disease apart from the clinical status of the host, are features of the tumour cells as well as the immune cells in the surrounding microenvironment. An increased understanding of the complex intra- and inter-cellular signaling provides new potential targets for therapeutical intervention.[11]

1.2 EPIDEMIOLOGY

Follicular lymphomas account for about 20% of all lymphomas with the highest incidence in the Unites States (US) and Western Europe where it is the second most frequent subtype among lymphoid malignancies.[1] However, numbers vary between geographical regions and ethnic groups; compared to the age-standardized incidence of 2-4 per 100000 person-years in the US and Europe[18, 19], FL in Japan and South Korea for instance is 2-3 times less common.[8] Within the US, the lowest incidence 2011-2012 was reported in the minority of people with Asian or Pacific Island descent and second lowest in African Americans.[18] In Sweden the estimated number of new patients each year is around 250.[20] According to data from the National Cancer Institute, the FL incidence is slowly decreasing again after a peak in the year 2003.[21] The reason for the decline is unknown but one hypothesis is the decreasing use of cigarettes.[18] In addition to tobacco use and family history[22, 23], Sjögren syndrome[23, 24] (as opposed to other autoimmune diseases) and lifetime use of antibiotics[25] have been associated to an increased risk of FL. The median age at diagnosis is 60-65 years with a male:female ratio of 1:1.2-1.7.[18-20, 26]

1.3 DIAGNOSIS OF FOLLICULAR LYMPHOMA - SUBTYPES

FLs are composed of centroblasts and centrocytes growing in the follicles of lymph nodes, but also in spleen, tonsils and bone marrow. At diagnosis most patients have disseminated disease involving lymphoid tissue at various sites and occasionally, in addition or primarily, extranodal organs such as skin or testis.[1] Yet, the typical patient is feeling well and does not always present with a palpable enlarged lymph node.[8] In the current WHO classification, FLs are subclassified into grades 1, 2, 3A and 3B, according to the number of large immature centroblasts dispersed among the smaller more differentiated centrocytes, counted in 10 random neoplastic follicles and expressed per x40 high-power microscopic field (Table 1).[27] Since histology varies among follicles, an adequate sample is crucial and fine needle aspiration alone insufficient for grading. The grading system and its clinical significance have been a matter of debate and no method has shown high reproducibility between pathologists.[8, 28] Grades 1, 2 and 3A are considered indolent whereas 3B, by definition holding the greatest number of large cells, is more aggressive sharing features with diffuse large B cell lymphoma (DLBCL).[28] In the latest WHO classification distinction between grades 1 and 2, the most frequent grades, is no longer recommended.[8]

Table 1. WHO Grading System for FL

GRADING	DEFINITION
Grade 1-2	0-15 centroblasts per hpf
1	0-5 centroblasts per hpf
2	6-15 centroblasts per hpf
3	>15 centroblasts per hpf
3A	Centrocytes present
3B	Solid sheets of centroblasts
PROPORTION FOLLICULAR	**PATTERN REPORTED**
>75%	Follicular
25-75%	Follicular and diffuse
<25%	Focally follicular
0%	Diffuse

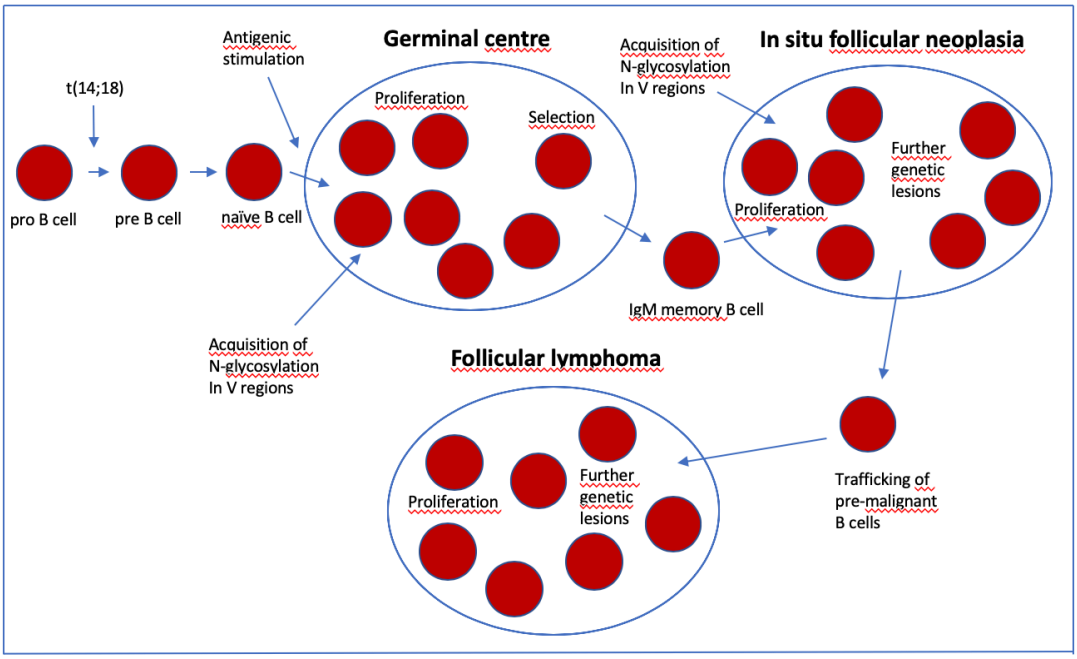
Follicular lymphoma (FL) is graded according to cytological features. CB, centroblast; HPF, high-power microscopy field (×40 objective, 0.159 mm^2). Diffuse areas with >15 centroblasts per hpf are reported as diffuse large B-cell lymphoma.

Rare variants of FL include duodenal-type FL and in situ follicular neoplasia (ISFN) where "in situ" refers to signs of FL restricted to one or more follicles in an elsewhere normal appearing lymph node. Compared to partial nodal involvement of FL, the in situ variant is less likely to progress. In the WHO 2016 revision pediatric FL as well as primary cutaneous follicle center lymphoma are recognized as separate entities not sorting under FL.[27]

1.4 PATHOGENESIS

The characteristic hallmark, present in up to 90% of FL, is the t(14;18) (q32;q21) translocation, an error occurring in the maturing B cell where the immunoglobulin heavy chain gene is juxtaposed to the anti-apoptotic gene B cell lymphoma/leukemia 2 (*BCL2*) leading to an overexpression of the latter. The process is initiated in the bone marrow and followed by migration of affected cells to germinal centers in lymph nodes where secondary events occur.[29] The same t(14;18) translocation can be found in approximately 1 per 10^5 of peripheral blood B cells in healthy individuals with a large inter individual variation but often increasing with age. It is also thought to be present in 2-3% of reactive lymph nodes. For a lymphoma to occur, a number of additional genetic alterations; mutations in chromatin modifiers among others, as well as certain interactions of the B cell with the microenvironment are needed. Due to its complexify the pathogenesis remains poorly understood (Figure 1). Several of the most frequently mutated genes (*CREBBP, EZH2, KMT2D, MEF2B*) may cause a maturation arrest at the proliferative stage of the cell, preventing it from differentiating into a memory or plasma cell. Like for normal B cells signaling downstream of the B cell receptor seems crucial for FL cell survival and retention of immunoglobulin expression is mandatory. The surface immunoglobulin is known to provide both a low-level tonic signal, perhaps from environmental autoantigens with interaction facilitated by mannosylation of the immunoglobulin binding-sites, as well as a true antigen-mediated signal, and their relative importance in FL cell survival is unclear.[30] Several studies have focused on FL with versus without the classical *BCL2* breakpoint.[31-32] Leich et al.[31] found that the BCL2 protein was expressed also in the majority of breakpoint-negative cases, indicating that a mechanism other than t(14;18) leads to BCL2 expression. It is still unknown which events in the FL pathogenesis may compensate for the lack of BCL2 protein in t(14;18) negative cases.

Figure 1. Critical influences on the pathogenesis of FL (Adapted from Küppers et al. 2018)



1.5 TUMOUR MICROENVIRONMENT

Still after all, the FL cell itself much resembles the normal germinal centre B cell, morphologically, immunophenotypically and even genetically, and is surrounded in the tumour by various non-neoplastic cells such as T cells, follicular dendritic cells and macrophages.[33] In 2004, Dave et al.[34] demonstrated a correlation between features of the non-malignant immune cells in the diagnostic biopsy and patient survival and there has since then been increasing evidence that the microenvironment plays a crucial role in the development and progression of lymphoid malignancies. Several studies have been focusing on the complex signaling between tumour cells and non-malignant cells, where dendritic cells, monocytes/macrophages, mesenchymal stromal cells (MSCs) and the plasticity of helper T cells all play a part.[35-38] In summary, a high overall content of tumour infiltrating T-cells, in particular CD8+ T cells, is prognostically favorable in contrast to tumour-associated macrophages (TAMs). Data on T-cell subsets including regulatory T cells (T regs) and T follicular helper (TFH) cells and their relation to prognosis and therapy are however conflicting.[35-38] Not only the number of cell subsets but also their infiltration pattern seem to play a role, as well as their activity and expression of surface molecules such as programmed death-1 (PD-1). PD-1 is involved in T cell function and its expression can reduce the anti-tumour response.[35]
Factors underlying a more aggressive course may be either primary genetic changes in the tumor cells or variability in the host immune response.[29] Clearly chromosomal aberrations and mutations in genes in the neoplastic cell, including the tumour suppressor gene *TP53*, are associated with aggressive disease[26] and are likely to promote a microenvironment further supporting tumor growth as well as suppressing anti-tumoral response.[29, 39]

1.6 PROGNOSTIC MODELS

In general the indolent lymphomas progress slowly and the prognosis for patients with all types of CD20+ lymphoma improved with the introduction of rituximab, the median survival for follicular lymphoma patients across clinical stages, now reaching nearly 20

years from time of diagnosis.[40] However, although FL grades 1-3A are all considered to be indolent lymphoma their clinical course is highly variable. Some patients, also with disseminated disease at diagnosis, remain asymptomatic for many years without any sign of lymphoma progression and even spontaneous remission[41], whereas others early undergo transformation to high-grade disease; 3B or DLBCL. In contrast to FL grade 3B which is treated with intensive chemotherapy with a curative intent, the lower grades are still considered incurable unless strictly localized and thereby possibly eradicated with radiotherapy.[28] Also in the rituximab-era, lymphoma progression has sometimes been shown to be the leading cause of death in all age-groups during the first decade after diagnosis, followed by treatment-related mortality.[42]

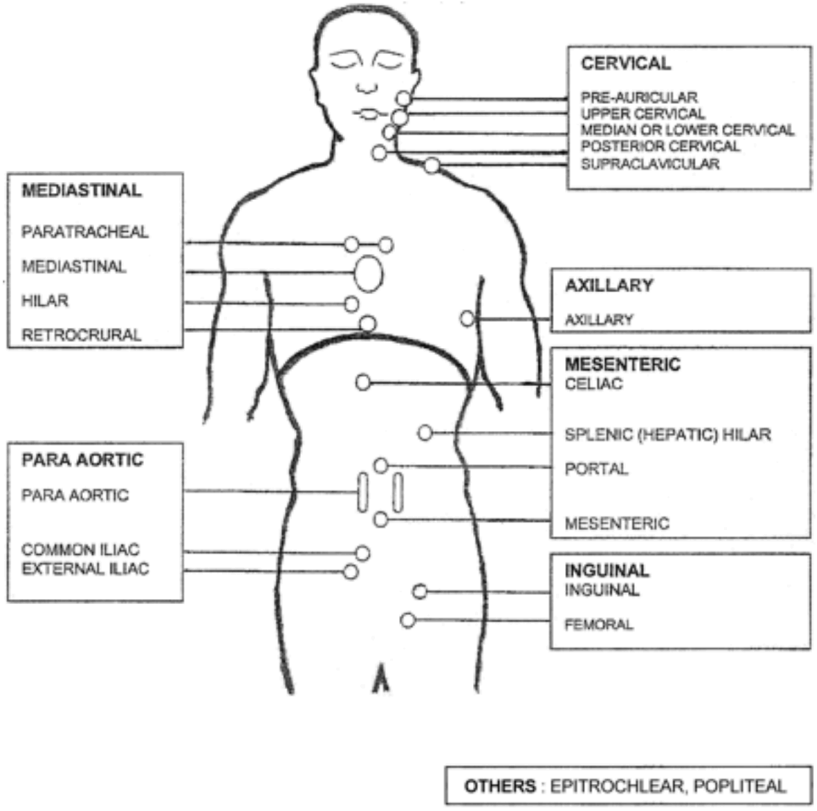
Traditionally, a lower grade was thought to mean better prognosis[26] but more recent data show that in patients given rituximab as first-line therapy, those with grade 3A survive longer than those with grade 2, and grade 2 patients have better survival than do grade 1.[43-44] Proliferation is not included in the grading criteria, but may be associated with grade as well as with prognosis as cases with high proliferation index often behave more aggressively. However, as with grading, interobserver variability cannot not be disregarded nor can the proliferation, as estimated, of the surrounding T cells.[45]

The optimal timepoint, choice and sequence of therapeutic regimens remain matters of debate [46-48] and is one major challenge for the treating physician to decide upon. Several attempts have been made to find a way of predicting the clinical course[49-50] but so far none has proven a useful tool in guiding therapeutic intervention. In 2004 the clinical Follicular Lymphoma International Prognostic Index (FLIPI)[49] was presented and it has since then become widely used. It is based on data from patients before rituximab was introduced and includes age, clinical Ann Arbor stage[51], hemoglobin and lactate dehydrogenase levels and number of affected lymph node stations according to a specified anatomical map, with overall survival (OS) as endpoint. However, among clinicians and radiologist the assessment and calculation of areas of lymphadenopathy has not always been straightforward (Figure 2). In 2009, the FLIPI was revised by Federico et al.[50] who introduced the FLIPI2 including slightly different baseline variables such as serum beta2-microglobulin and bulky disease defined as a largest tumour diameter of > 6 cm. The FLIPI2 was developed in patients treated with chemotherapy, some with the addition of rituximab and its primary endpoint is failure-free survival (FFS) instead of OS. Still the FLIPI2 model is used less than the FLIPI, perhaps because many centres do not include beta2-microglobulin in their routine blood analyses and because of the required measurement of lymphatic mass (Table 2).

Table 2. FLIPI and FLIPI2 variables

	RISK FACTOR	
	FLIPI	**FLIPI2**
Age, years	> 60	> 60
Hb	< 120 g/l	< 120 g/l
Serum marker	LDH > ULN	ß2m > ULN
Lymph node	Nodal sites > 4	Size > 6 cm
Stage	III - IV	Bone marrow+
Low risk, 0 to 1; Intermediate risk, 2; High risk, 3 to 5.		

Figure 2. Nodal stations according to FLIPI



More recently the impact of a number of gene mutations in the tumour was evaluated by Pastore et al. in the m7-FLIPI prognostic model, derived from the FLIPI score combined with Eastern Cooperative Oncology Group (ECOG) and the mutational status of seven specific genes (*EZH2, ARID1A, MEF2B, EP300, FOXO1, CREBBP, CARD11*). Patients with FL were stratified into "low-risk" and "high-risk" with respect to their 5-year FFS after first-line immunochemotherapy (R-CHOP or R-CVP), according to a sum of predictor algorithm. Mutation in *EZH2* gene, coding for the catalytic subunit of a histone methyltransferase, was found to be associated with better prognosis.[52] Perhaps the most simple scoring system is the newly introduced PRIMA-prognostic index (PRIMA-PI), taking into account only bone marrow involvement and level of beta2microglobulin both as dichotomous variables. Based on the prospective PRIMA trial cohort treated with immunochemotherapy[53] it divides patients into three risk categories of subsequent progression-free survival (PFS).[54] As opposed to the FLIPI, FLIPI2 and m7-FLIPI, the PRIMA-PI does not include high age per se as a risk factor. Alig et al. reporting on the impact of age and genetics in FL conclude age itself should not guide treatment decisions.[55]

Apart from patient baseline characteristics their future risk profile can be based on the course of disease, the depth and duration of therapy response. Data from the National LymphoCare Study showed that relapse of FL within 24 months of first-line chemoimmunotherapy is associated with very poor OS, establishing the concept of POD24 as progression of disease status at 24 months.[56] Shi et al.[57] analyzed data from 13 multicenter trials and found the remission status at 30 months after induction therapy (CR30); chemotherapy, immunotherapy and chemoimmunotherapy likewise, serve as a surrogate marker for progression-free survival. This is of interest as early identification and intervention in patients with poor prognosis might help improve both progression-free and OS. Baseline metabolic tumor volume (TMTV) assessed by fluorodeoxyglucose-positron emission tomography (FDG-PET) scan has been shown a strong predictor of PFS and OS[58],

as has the remaining metabolic activity after induction immunochemotherapy.[59, 60] Like the finding on PET scan, bone marrow involvement at baseline and after therapy measured as minimal residual disease (MRD) levels of BCL2/IgH(+) cells by real-time quantitative polymerase chain reaction (RQ-PCR) has been shown associated with prognosis[61] although not as strongly as the in the case of PET.[60]

1.7 TRANSFORMATION

During the course of the disease some patients with FL acquire additional genetic aberrations thereby transforming to a highly malignant lymphoma, most often diffuse large B-cell lymphoma (DLBCL) but sometimes Burkitt lymphoma or lymphoma with features intermediate between these two or lymphoblastic lymphoma. In rare cases blast transformation occurs and the patient develops acute B-cell lymphoblastic leukemia. Histologic transformation from indolent to aggressive lymphoma is reported to occur in 10-30% of FL patients over time at a rate of 2-3% per person-year and is known to confer a much worse prognosis than de novo DLBCL[26]. However, recent studies indicate that the prognosis for transformed FL (tFL) has been improved in the rituximab era[62-65] and that the risk of transformation has slightly decreased.[66]

The process of transformation was first described by Gall and Mallory[2] nearly 80 years ago but similar to FL pathogenesis, it is still poorly understood. One problem when studying tFL is the different definitions used among the published series, including clinical, histological and cytological criteria. The gold standard definition is the most conservative and is based solely on histological criteria. For statement of a completely true transformation, a clonal relationship with the original FL should be demonstrated.[67-68] However, as a biopsy large enough to verify this is not always achieved, a definition of clinical transformation has been proposed. Clinical suspicion of transformation is raised when a patient with known FL presents with a rapid progression of lymphadenopathy, engagement of extranodal sites, B symptoms and/or elevated serum LDH.[69] What also accounts for the wide variation in findings on incidence and outcome for patients with transformed disease, are beside the diagnostic procedures applied, the follow-up time of the studies. Moreover, in survival analyses death as a competing risk is not always taken into consideration although advisable.

It was usually assumed that transformation occurs within a FL cell but more recent research with genomic hybridization and nucleotide polymorphism data suggest instead a common progenitor cell (CPC) to be the origin, giving rise to a related but distinct population of malignant cells.[63, 70-71] Many studies have tried to find genes and/or protein products associated with transformation.[72] None of the classical actors p53, BCL2 nor BCL6 seem to play a crucial role, perhaps more so the transcription factors MYC and NF-kB in the B cell receptor downstream signaling pathway.[63] Steen et al. did propose a clinico-molecular predictor of transformation risk, the BTK-FLIPI, based on a gene expression score at diagnosis capturing deregulation of the NF-κB pathway.[73]

In the evolution of transformation also the tumour microenvironment has been shown important. CD14+ dendritic cells and PD1+ T cells in a particular arrangement are associated with shorter time to transformation, as are increased intrafollicular levels of macrophages and CD4+ T cells, and an extrafollicular or diffuse localization of FOXP3+ regulatory T cells.[74] To conclude, the transformation process is complex, seemingly influenced by genetic alterations and crosstalk with the microenvironment and possibly with the therapeutic regimen applied.[75, 76]

1.8 THERAPY

1.8.1 First-line treatment

In the minority of patients with strictly localized disease, radiation alone renders an estimated 50% chance of cure and long-term follow-up of these patients suggests a 10-year OS of up to 80%.[48, 77] When patients with generalized disease are asymptomatic watchful waiting may well be considered as there is no documentation that early or intensified chemotherapy will prolong life.[78-80] In a large randomized trial by Ardeshna et al.[78] on FL patients with low tumor-burden stages II-IV asymptomatic disease, rituximab alone as induction therapy was shown to improve PFS compared to "wait and watch" but to have no effect on OS. Similar results were later achieved by an observational LymphoCare study.[81] Tools to determine which patients with disseminated disease are likely to benefit from immediate therapy are the Group Étude des Lymphomes Folliculaire (GELF) critera[82] and the British National Lymphoma Investigation (BNLI) criteria.[79] Still, patient and doctor preferences, local traditions as well as economic considerations may influence the timing of therapy initiation and choice of regimen, apart from comorbidities and potential side effects.

When patients with FL first start treatment they usually respond well. Before rituximab was gradually introduced in clinical practice, in Sweden during the years 2003-2007[20], almost all received chemotherapy with little variation.[10, 83] Since then, a number of regimens with different schedules have been tried, often combining an anti-CD20 antibody with one to three well-known cytostatic substances but also with antibody given as single agent or linked to a radioactive isotope.[84-91] So far rituximab maintenance has shown no benefit over monotherapy retreatment at progression in patients with low-tumour burden.[89] On the other hand the PRIMA randomized trial[53], enrolling 1135 high-burden patients, found progression-free survival improved with rituximab maintenance after frontline therapy with rituximab and chemotherapy, and as a consequence two years of maintenance became the standard of care after rituximab with cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP). However, a long-term follow-up demonstrated no effect of maintenance on OS.[92] In patients perceived in need of chemotherapy practice has changed only these last years after the trial by Rummel et al.[93] to rituximab combined with bendamustin (R-Benda) without maintenance, a regimen with milder haematological toxicity, fewer infections and less peripheral neuropathy than the R-CHOP. Interestingly, lenalidomide, a thalidomide derivative, was recently in the large multicenter RELEVANCE trial shown to be as effective in combination with rituximab as the R-chemo combination, in patients with previously untreated high tumour-burden FL.[94]

1.8.2 Relapse treatment and new drugs

Due to the relapsing nature of the disease and the well-known side-effects of chemotherapy more and more research has been focusing on new antibodies as well as on immunomodulatory agents such as lenalidomide, BCL2 inhibitors, PI3 kinase inhibitors, Bruton tyrosine kinase inhibitors and epigenetic modifiers as monotherapy or in combination regimens.[77] The humanized anti-CD20 antibody obinutuzumab is currently recommended in the case of rituximab-refractory disease.[95] As BCL2 seems strongly connected to the pathogenesis of FL there was much hope for its selective inhibition with the drug venetoclax, however clinical results have been modest.[96-97] The Bruton tyrosine kinase inhibitor ibrutinib soon became standard of care for patients with chronic

lymphocytic leukemia (CLL) with deletion 17p but for FL more promising results have been achieved with inhibitors of PI3 kinase isoforms such as idelalisib although there have been problems with toxicity.[98-100] Inhibition of immune checkpoint interaction between PD-1 and PD-L1 is another target mechanism for novel drugs, as it is important for the ability of tumor cells of evading the immune system. Blockade of PD-1 on T-cells in FL has been shown to promote their antitumor function.[101, 102] Both PD-1 blockade and adoptive cell therapy (CAR-T cells) have shown promising results in smaller clinical trials.[103, 104] Despite the rapidly growing arsenal of new drugs, autologous stem cell transplant (auto-SCT) is often recommended in younger patients with aggressively relapsing or transformed FL.[105-107] Allogeneic stem cell transplant (allo-SCT) is the only known potential cure for indolent lymphoma and may be considered, especially in the case of relapse after auto-SCT.[105, 107, 108]

Newer biological agents are likely to affect the significance of prognostic markers and scoring systems developed in the context of chemotherapy. This has been shown e.g. in the case of rituximab.[109] A related field of research is the optimal method of evaluating therapy response, where the use of PET scans, immunohistochemistry and flow cytometry have increased. The well-established Cheson criteria[110] were based on tumor volume not metabolic activity, prompting a revision presented in 2007 incorporating PET and flow cytometry. The revised criteria also included more precise definitions of endpoints such as progression-free survival and response duration which are, however, still being used without consistency in the literature. There is ongoing discussion about the value of molecular detection of MRD in blood and bone marrow but so far it has not been recommended outside of clinical trials.[111, 112]

In summary, in light of the large number of therapeutical options available for patients with indolent lymphoma one may conclude so far none has proven the optimal solution for all. Questions on what to do, at what time and for whom are yet to be answered one at a time.

2 AIMS

The overall aim of this thesis was to gain increased insight in clinical and immunological factors of predictive value and prognostic importance in a given therapeutical setting, in indolent lymphoma in general and in follicular lymphoma in particular. More specifically, we aimed at:

1. Exploring the long-term outcome after immunotherapy given first-line and discover determinants for OS.

2. Investigating the validity and usefulness of the two prognostic models; the clinicogenetic m7-FLIPI and the simplified two-parameter PRIMA-PI in patients treated with a chemo-free first-line regimen.

3. Increasing our understanding of the effects on immune cells during the course of immunotherapy and identifying any relation to therapy response.

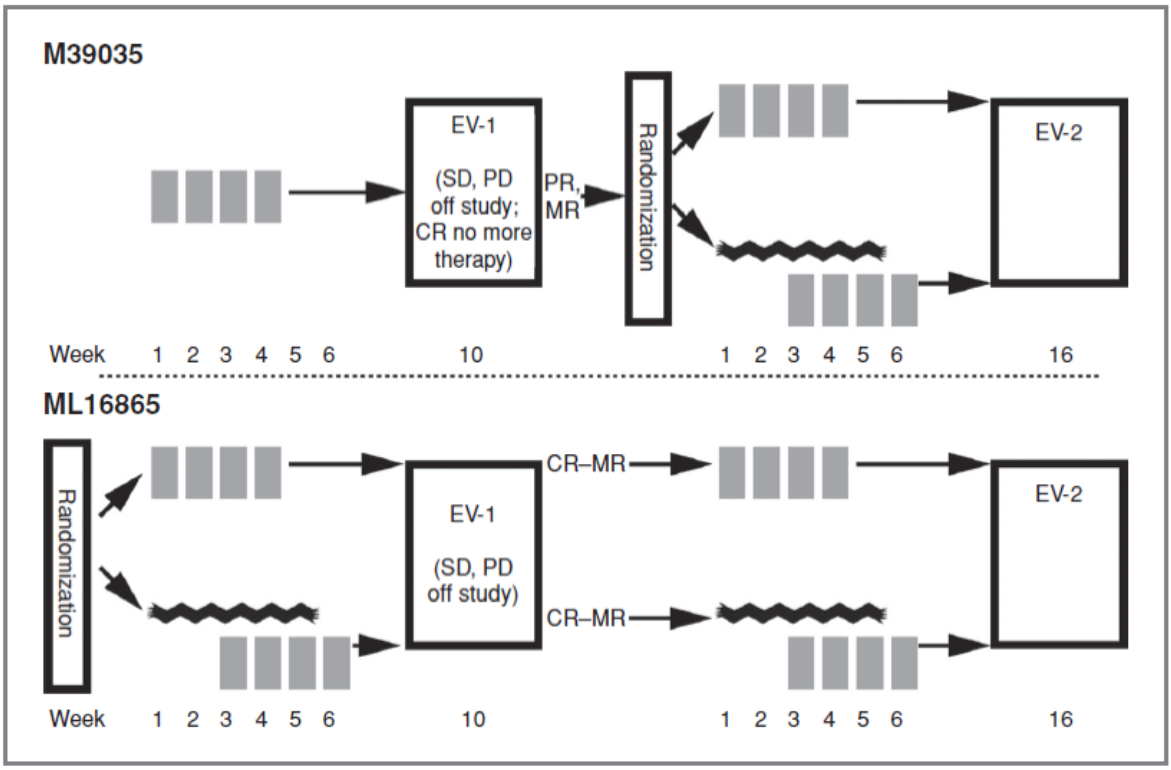
3 PATIENTS AND METHODS

3.1 STUDY POPULATION

3.1.1 Papers I-III

Papers I-III were all based on a larger study population of 439 patients included in either one of two consecutive Nordic Lymphoma Group (NLG) randomized clinical trials; M39035 with accrual 1998-1999 (n=126) and ML16865 with accrual 2002-2008 (n=313).[85, 86] The overall aim of both trials was to deliver effective treatment without the well-known short- and long-term complications of chemotherapy. Patients with the diagnosis of an indolent B-cell lymphoma and generalized symptomatic disease were after biopsy review randomly allocated to treatment with single rituximab or rituximab combined interferon-α2a (IFN). The treatment was first-line for all except a minority who had previously received local radiation and/or a short course of chlorambucil. Therapy consisted of 4 weekly doses of rituximab (375 mg/m^2) and in case of randomization to IFN, IFN- α2a 3 MIU/day subcutaneously (sc) week 1, followed by 4.5 MIU/day sc weeks 2-5 (Figure 3).

Figure 3. Flow charts of trials M39035 and ML16865. Each gray rectangle symbolizes a weekly dose of 375 mg/m2 rituximab and each black zigzagged line represents IFN of 3 MIU/d daily during week 1 and 4.5 MIU/d) during weeks 2 through 5, except on rituximab days. EV-1 and EV-2, signify first and second treatment response evaluation.



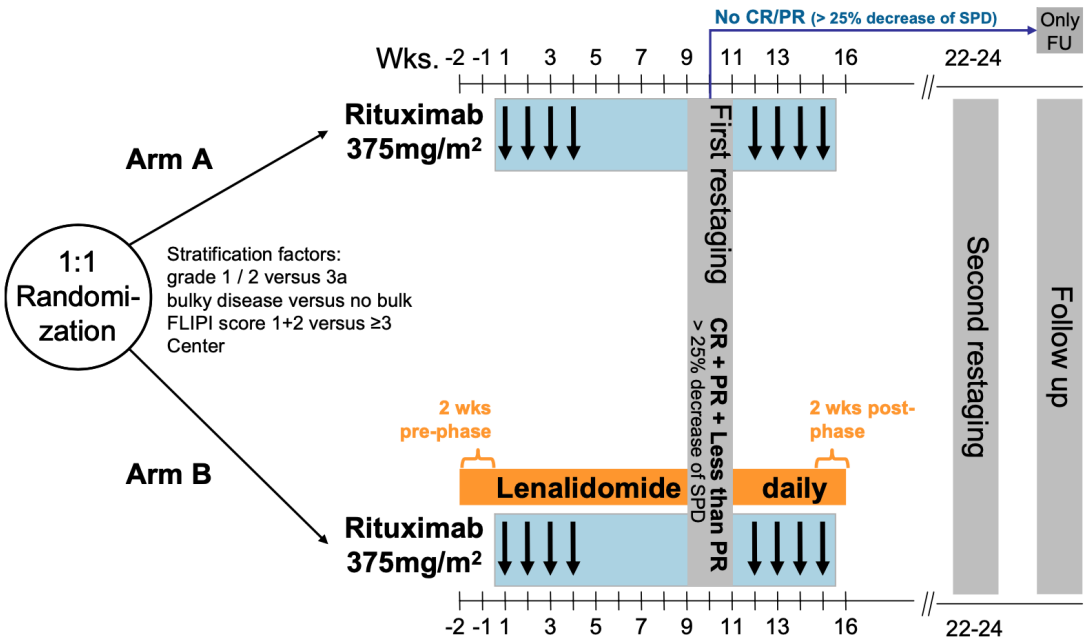
IFN = interferon-α2a. SD = stable disease. PD = progressive disease. CR = complete response. PR = partial response. MR = minimal response.

In **paper I** was included a subgroup of the in total 439 patients treated within the two trials. All Swedish, Norwegian and Danish patients with FL, marginal zone lymphoma (MZL), small lymphocytic lymphoma (SLL) or indolent lymphoma not otherwise specified (NOS) were eligible for the long-term follow-up provided they had previously received neither radiotherapy nor chlorambucil. Patient inclusion in **paper II** was based on the diagnosis of FL or indolent lymphoma NOS (presumably indolent FL without enough material for grading) and most importantly the availability of fresh-frozen biopsies with sufficient material for gene sequencing. As opposed to paper I, also a few patients who had received radiotherapy/chlorambucil were included. **Paper III** was based on all patients in paper I with FL or indolent lymphoma NOS. In conclusion, there was a partial overlap between the study populations of **papers I-III**.

3.1.2 Paper IV

Like papers I-III, paper IV was based upon a randomized clinical trial, this time evaluating, first-line treatment with single rituximab and the combination with lenalidomide (Figure 4). Then multicenter SAKK3510 trial was a collaboration between the Swiss Group for Clinical Cancer Research (SAKK) and the NLG with accrual 2012-2014 and with clinical results published in 2019.[113] Among the 154 patients included in the SAKK3510, 36 patients from Norway and Sweden took part in the translational project and provided consecutive blood samples at up to five times each before therapy, during the course of treatment and after. All had received infusions of rituximab at weeks 1, 2, 3 and 4 followed by 4 additional at weeks 12, 13, 14 and 15, with or without lenalidomide 15 mg p.o. daily starting week -2 and ending at week 17.

Figure 4. Trial design of the multicenter SAKK3510



CR = complete response. PR = partial response.

3.2 DATA COLLECTION

3.2.1 Papers I-III

For **paper I**, a case report form (CRF) for collection of clinical follow-up data was created and distributed to the centres earlier participating in the two clinical trials. Variables assessed included all post-trial therapies with types and dates of initiation, infectious complications, transformation to high-grade disease and occurrence of second primary malignancies. For live patients, dates of last follow-up were noted and for those deceased, the dates and causes of death. Information on diagnosis, sex and age and other clinical variables at the time of inclusion in the randomized trials was retrieved from the original study databases. For **papers II-III**, relevant data were retrieved from the new database created for paper I. In some centres there was no co-investigator in charge at the time of the long-term follow-up, in which case a local temporary permission for external record review was obtained. The variability between investigator access to medical files was reflected in the varying amount of detailed information provided in the new data compilation. The gene sequencing in **paper II** was performed by means of a targeted panel including the seven genes of the m7-FLIPI: *EZH2, ARID1A, MEF2B, EP300, FOXO1, CREBBP* and *CARD11* and the m7-FLIPI score for each patient was calculated according to the published algorithm.

3.2.2 Paper IV

FL patients were sampled at baseline before therapy initiation**,** after 2 weeks' of lenalidomide (in patients with combination treatment), 24 hours after first rituximab dose and at follow-up weeks 10 and 23. Fresh whole blood in EDTA tubes was shipped from participating centres to Stockholm immediately after sampling and analysed by flow cytometry, gating upon mononuclear cells and lymphocytes respectively, after the exclusion of doublets and debris. Response data were provided from CRFs and CT scans, reevaluated by the chairs of the SAKK3510 trial.

3.3 DEFINITIONS OF ENDPOINTS AND GROUPS

3.3.1 Papers I-III

OS, the main endpoint in **paper I**, was estimated from the date of randomization in the original trials to death, or end of follow-up in which case patients were censored. The secondary endpoint Time to New Anti-Lymphoma Therapy (TTNT) was considered equivalent to and more robust than Time to Progression (POD). Outcome measures included also Time to New Anti-Lymphoma Therapy restricted to chemotherapy-containing regimens (TTNChemo), Time to Transformation (TTT) and Lymphoma-Specific Survival (LSS). Time to treatment failure (TTF), the primary endpoint of **papers II-III**, was defined as the interval between start of trial therapy and either initiation of new lymphoma therapy due to relapse or intolerance, or death from any cause. The Pastore cut-off score of ≥ 0.8 for discrimination between the m7-FLIPI high- vs low-risk group was applied in **paper II**. In **paper III** patients were classified into the three FLIPI risk groups (low: 0-1, intermediate: 2 and high: 3-5 risk factors)[49] and into the three PRIMA-PI categories: high-risk: ß2-

microglobulin (ß2m) > 3mg/L, intermediate-risk: ß2m ≤ 3 mg/L with bone marrow involvement and low-risk: ß2m ≤ 3 mg/L without bone marrow involvement.[54]

3.3.2 Paper IV

Complete response/unconfirmed (CR/CRu), partial response (PR), stable disease (SD) and progressive disease (PD) were assessed according to the Cheson 1999 criteria.[110] The week 10 evaluation made use of an additional response category called minor response (MR), defined as a >25% but <50% decrease in sum of product of diameters (SPD) of affected lymph nodes.

3.4 STATISTICAL ANALYSES

In all papers the significance level was set to 0.05 and all tests were two-sided.

3.4.1 Papers I-III

Survival across subgroups was analyzed with log rank test and graphically presented in Kaplan-Meier curves. A Cox regression model was used for estimates of hazard ratios both crude and adjusted for independent predictors as described. Patients were censored at last follow-up without event.

3.4.2 Paper IV

The Wilcoxon signed-rank test was applied for comparing changing fractions of cell subsets between any time point and baseline, and the rank-sum test for levels between treatment arms. Kruskal-Wallis and Fischer´s exact tests were used for evaluation of cell subsets as continuous variables as well as predictors dichotomized by the median, in relation to the binary outcome of response vs non-response.

3.5 ETHICAL ASPECTS

The three original randomized trials M39035, ML16865 and SAKK3510 upon which this thesis is largely based, were performed in accordance with good clinical practice (GCP) and approved by the ethical committees in each participating country. All patients had given their informed written consent to the intervention as well as the clinical and translational investigations associated. In preparation for **papers I-III** renewed ethical permissions were obtained from the regional ethical committee of Stockholm and patients who were still alive were asked for renewed consent regarding extensive medical file review but no family of deceased patients were contacted. In **paper II** tissue samples already extracted and saved were further analyzed with respect to genetic aberrations but only genes thought to be of importance to course of lymphoid disease were assessed and contacting all these patients once again was considered neither appropriate nor necessary. As the flow cytometry analyses of **paper IV** were included in the approved protocol and patient information of the SAKK3510 clinical trial, no more permits were asked for.

Although all data are presented only at the group level, the risk that some patients would disapprove of the refined laboratory analyses or thorough review of medical files cannot be completely ruled out.

4 RESULTS AND DISCUSSION

4.1 PAPER I

Our study of first-line rituximab without chemotherapy in indolent lymphoma illustrates that after a median follow-up of 9.8 years, 3/4 of patients can be expected to be alive. This is at least as good as the OS observed in several modern immunochemotherapy trials such as the recently reported long-term results of the PRIMA trial and other follow-up studies.[92, 114-117] The duration of those immunochemotherapies, some also with rituximab maintenance, was longer and toxicities were higher than in our chemotherapy-free therapies. We here find that a substantial proportion of patients (36%) will perhaps never require any chemotherapy with its known early and late side effects. In our study 30% of FL patients required no second-line therapy at all. Surviving patients had a median follow-up time of 10.6 years.

As OS of patients with indolent lymphoma improves, the burden of late side effects becomes of greater concern. In other rituximab trials, most of them including also chemotherapy, late complications have been reported including heart failure, LON, intestinal perforation, interstitial pneumonitis, reactivation of viral infections e.g. herpes and hepatitis and the uncommon but severe progressive multifocal leukoencephalopathy.[118-120] Several studies suggest an increased overall risk of a second primary cancer after treatment for lymphoma[121-126] but it is unclear whether this is due to the treatment (chemotherapy, immune suppression) or inherent to the patient group with shared genetic and/or environmental etiology. A meta-analysis by Fluery et al. showed no such increased risk from rituximab alone after a median follow-up of six years.[127] As many patients in our study had received additional lines of therapy, the potential benefit of first-line rituximab/IFN alone is difficult to evaluate, but the chemotherapy-free approach is suggestive of a slightly reduced risk of LON, viral reactivation and second primary malignancies. Moreover, omitting the cardiotoxicity of anthracycline and the neurotoxicity of vincristine do reduce the risk of heart failure and peripheral neuropathy.[128-130]As very few studies report on follow-up beyond 10 years, comparisons of late secondary outcomes remain hypothetical.

The 10-year cumulative incidence of transformation of 20% at a rate of 2.4% per person and year observed in our study is broadly similar to what has been previously described for FL.[62-65, 116, 126, 131] Management strategies including watch and wait in asymptomatic indolent disease, genotoxic chemotherapy and rituximab maintenance, may influence not only the risk but also outcome of transformation.[78, 62-64, 132] Our results are in line with recent data, suggesting that the risk of transformation in the immunotherapy era has been well reduced from a 5-year cumulative incidence of nearly 20% pre rituximab, and survival following such an event improved from a median of 0.6-2.7 years.[62, 69,126] Of note, our data suggest a relatively constant transformation rate over time with no plateau within 15 years from initial lymphoma diagnosis.

The prognostic value of FL grading[27] has been debated.[133-234] In our cohort with biological therapy the survival of patients with FL grade 1 was inferior to that of higher grades, in line with some previous reports[109, 135, 136] but not with all.[137] B cell lymphoma sub diagnostics being sometimes complex, the higher transformation risk in the non-follicular lymphoma group may in part be due to a number of indolent lymphomas NOS cases in fact representing FLs with more diffuse growth patterns.

As in the LymphoCare Study by Castulo et al, FL patients with progress within 24 months of first-line therapy had a poor OS[27] but the OS for our patients with early POD was better. This is likely due to the several salvage treatments, including chemotherapy, remaining available after a first-line therapy with little toxicity.

4.2 PAPERS II-III

In the report by Pastore et al. patients with low-risk m7-FLIPI score clearly outnumbered those with high-risk m7-FLIPI, an imbalance that was even more pronounced in our study cohort where only 17 out of 95 patients had a high m7-FLIPI score. Unlike what was reported by Pastore et al on FL patients´ 5-year FFS after first-line immunochemotherapy (R-CHOP or R-CVP), the m7-FLIPI prognostic model was not able to discriminate between "low-risk" and "high-risk" patients. No difference in TTF nor OS was found between our two m7-FLIPI risk groups. Still, our earlier finding of an existing but weaker association between POD24 and OS after first-line immunotherapy[14] compared to what was shown after R-CHOP[27], suggests that the m7-FLIPI model likewise might have a somewhat predictive value also in the non-chemotherapy setting.

Mutation in the *EZH2* gene, coding for the catalytic subunit of a histone methyltransferase, was here associated with longer TTF only in univariate analysis. This is different from the independent association shown in the Pastore report and in the report by Stevens et al. on end of spectrum FL patients treated with immunochemotherapy.[138] Most surprising and remarkable was the finding of *EP300* as a very clear indicator of inferior outcome in our cohort, stronger than previously reported to our knowledge. These findings are suggestive of other mechanisms involved in the antitumoral response to antibodies and/or immunomodulators than to agents of chemotherapy.

The simplified PRIMA-PI, based only on serum beta2-microglobulin and bone-marrow lymphoma infiltration, had a prognostic value for TTF as well as for OS in our FL cohort with first-line chemo-free therapy, The OS rate, both at 5-and 10-years, was lower in the PRIMA-PI high-risk group than in the FLIPI high-risk group. Thus, the PRIMA-PI better than FLIPI identified the small group of FL patients (19% of all) with a true poor prognosis (Figure 5), although not as poor as that of the 32% high-risk patients in the study of Bachy et al.[54] The model might serve as a tool for stratification of patients upfront and for selection of those likely to benefit from more intensive management, including all patients with high ß2m who by definition are high-risk irrespective of bone marrow results. For patients with low and intermediate PRIMA-PI chemo-free therapy remains an option as the 10-years OS rate was 82% and 78%, respectively, and for patients responding to the first cycle the OS even higher (94 and 80%, respectively).

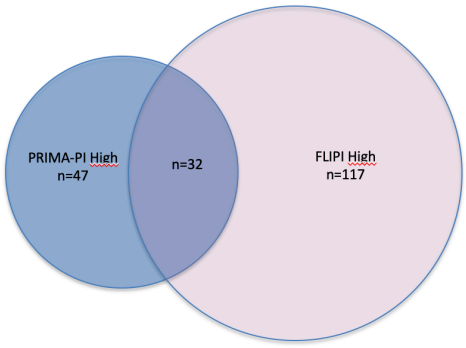


Figure 5. PRIMA-PI and FLIPI high risk groups

Both in the NLG rituximab-interferon trials and in the SAKK3510 trial response was independent of FLIPI. Recently results have been published from the RELEVANCE and the SAKK3510 trial,[94, 113] indicating that a non-toxic regimen with rituximab and lenalidomide even with short duration and without maintenance often can induce durable responses as the drug effects seem synergistic.[139] Perhaps the PRIMA-PI prognostic tool will turn out more valuable than the FLIPI in the chemotherapy-free setting.

4.3 PAPER IV

With lenalidomide alone a transient increase in monocyte and NK cell fractions was found, the latter decreasing again after first rituximab infusion, which might be due to either consumption in ADCC and/or homing to tumour sites, an idea suggested by others.[140, 141] A few patients had very high baseline levels of CD19+CD20+ suggestive of a leukemic component. Overall, the B cell fraction started to decrease already after 14 days with lenalidomide treatment ($p=0.0029$) and was fully depleted in both treatment arms 24 hours after first rituximab infusion. T cells on the other hand first decreased with lenalidomide but increased over time in both treatment arms and remained elevated. The relative increase in total T cell fraction and other immune cell populations could be only partly due the decline in B cells which takes place very soon after therapy initiation. Numerically, B cells are too few in blood to allow such a great increase to be explained only by their replacement. Moreover, the changing NK to T cell ratios, as well as monocyte to T cell ratios, are evidence of processes ongoing irrespective of concomitantly diminishing B cells.

Treatment effects sustained at week 23 included an increased CD4/CD8 ratio. This was due to a large rise in CD4 counts and a less pronounced rise in CD8 counts. A high percentage of monocytes and naïve CD8+ above the median at baseline were suggestive of week 10 response, which is in line with other reports where a larger fraction of naïve T cells at baseline were positively associated with response to therapy.[142-144] We found that the difference in response between higher and lower fractions were often more pronounced, although not always significant, in the rituximab monotherapy treatment arm. This could possibly be due lenalidomide helping overcome the negative impact of, e.g. few naïve T cells, by a beneficial effect on their activity, in analogy with the results presented by Wahlin et al. demonstrating an abrogation by IFN-α2 of the negative impact of few CD8+ cells.[145]

5 CONCLUSIONS AND FUTURE PERSPECTIVES

5.1 CONCLUSIONS

5.1.1 FL grade 1

FL grade 1 is a distinct subentity of FL just like FL grade 3b. In the rituximab era FL grade 1 seems associated with slightly worse prognosis than do grade 2 and 3a, in patients treated with immunotherapy and possibly in those receiving combined immunochemotherapy.

5.1.2 Risk assessment

The therapeutic regimens available in follicular lymphoma is a growing field and PFS as well as OS after diagnosis are both improving. Due to the often indolent course of disease long-term effects including OS hard to assess. Many studies have been conducted in the search of earlier accessible surrogate markers and endpoints such as event-free survival 12 and 24 months from diagnosis, or 30 month complete response (CR30)[57, 146] **Papers I, II and III** all support the hypothesis of markers and scores having different prognostic value in different therapeutic settings.

5.1.3 The chemo-free approach

Also among FL patients in symptomatic advanced-stage disease, a substantial number will achieve long remission with immunotherapy only, a therapeutic approach with usually milder side-effects. It is therefore worth considering and can in many cases be given a try under close monitoring. Any non-responding patient would soon be shifted to salvage therapy. The decision to start therapy in general and chemotherapy in particular, should be based on the presence of symptoms, comorbidity and disease progression or transformation. Quality of life issues, possible infectious complications and the risk of secondary malignancies are all to be taken into consideration when making treatment decisions.

5.1.4 Lenalidomide

The immunomodulation exerted by lenalidomide and the antitumoral effects of rituximab and lenalidomide in combination are reflected in the immune cell dynamics not only in lymphnodes but also in blood. This is evident already after 14 days of lenalidomide monotherapy. The distribution of immune cells before therapy initiation, and the following change in constitution, seem to be associated with anti-tumoural response.

5.2 FUTURE PERSPECTIVES

FL is most common in the western world, which should be due to a combination of genetic and environmental factors. Of the two, genetics is the more fixed whereas environment can change rapidly. Although FL incidence seems to have reached a plateau in the US and western Europe, it is likely to continue growing in other parts of the world as countries

adopt a more western lifestyle exposing their people to new/other carcinogens. As people move around the world and their nutritional, occupational and leisure habits tend to blend with the aid of internet and new social platforms, regional differences in disease panoramas start to fade.

Future studies will increase our understanding of FL pathogenesis at the molecular level. This will not only give us new targets for therapeutical intervention but also provide frameworks for diagnosis and classification of subtypes and variants. Perhaps in a future WHO classification we will see follicular lymphoma not to be one entity but several, some of them with new names.

Advances in the field of genetics will facilitate a more personalized approach to risk assessment and treatment. Germline polymorphisms as well as the mutational profile of the malignant cells will to a larger extent guide our choice of drugs and their dosage for the individual patient.

Many new drugs effectively target the interaction of the microenvironment and the immune system with the tumour. In addition, current regimens such as autologous and allogeneic stem cell transplants will be performed, in selected patients and perhaps in a modified way, with improved results. Overall prognosis will continue to improve but new drugs will expose us to yet unknown side effects. Economic issues will become of increasing importance and as a consequence ethical issues need to be discussed.

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