From Department of Laboratory Medicine, Division of Pathology
Karolinska Institutet, Stockholm, Sweden

THE ROLE OF CANNABINOID RECEPTORS, G ALPHA Z, AND B CELL RECEPTOR IN LYMPHOMA PATHOBIOLOGY WITH FOCUS ON CHEMOTAXIS

Magali Merrien

Stockholm 2021

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2021

ISBN 978-91-8016-083-4
Cover picture illustrates the release of a lymphoma cell out from its microenvironment – Painting by Josiane Jannès.

The role of cannabinoid receptors, G alpha z, and B cell receptor in lymphoma pathobiology with focus on chemotaxis
THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Magali Merrien

The thesis will be defended in public at 4U, Entréplan, Alfred Nobels Allé 8, Huddinge, 22nd January 2021, 10:00am.

Principal Supervisor:
Professor Birgitta Sander
Karolinska Institutet
Department of Laboratory Medicine
Division of Pathology

Co-supervisor(s):
Professor emerita Eva Kimby
Karolinska Institutet
Department of Medicine

Professor Edvard Smith
Karolinska Institutet
Department of Laboratory Medicine
Division of Clinical Research Center (KFC)

PhD Agata M. Wasik
Karolinska Institutet
Department of Laboratory Medicine
Division of Pathology

Opponent:
Associate Professor Guillermo Velasco
Complutense University, Madrid
Department of Biochemistry and Molecular Biology

Examination Board:
Docent Daniel Molin
Uppsala University
Department of Immunology, Genetics and Pathology

Professor Christopher Fowler
Umeå Universty
Department of Integrative Medical Biology

Docent Jonas Fuxe
Karolinska Institutet
Department of Laboratory Medicine
Division of Pathology

To my family, and my friends that became family.

“It is imperfection - not perfection - that is the end result of the program written into that formidably complex engine that is the human brain, and of the influences exerted upon us by the environment and whoever takes care of us during the long years of our physical, psychological and intellectual development.”

- Rita Levi-Montalcini -

POPULAR SCIENCE SUMMARY

Lymphoma is a blood cancer of B lymphocytes, the cells that are an important component of our immune system involved in producing antibodies. Malignant B lymphocytes are dividing and proliferating excessively without becoming fully mature, and spreading to other tissues. When lymphoma cells are in tissues, such as lymph nodes and bone marrow, they can disturb the development of other blood cells and lead to complications.

In tissues, lymphoma cells interact with surrounding non-malignant cells, helping the malignant cells to hide and survive from therapies. Some mechanisms used in these interactions have already been found, and various treatments exploit these features to force the malignant cells to leave that pro-survival environment and reach the blood circulation. In the blood, malignant cells become more sensitive to external factors, such as chemotherapy. However, not all patients respond to these treatment strategies and more knowledge is necessary to improve the outcome. This thesis focuses on important mechanisms involved in the cancer pathobiology.

One of my research projects explored the way lymphoma cells from different tissue origin (blood and lymph node) interact with and attach to non-malignant cells, in a co-culture *in vitro* system. This system could be useful to test lymphoma cells from patients before deciding on the treatment, in order to improve the efficacy of therapy from the start. This might decrease the risks of relapse and/or the development of aggressive disease.

During my PhD, we discovered a protein, G alpha z, to be highly expressed in mantle cell lymphoma compared with healthy B lymphocytes. G alpha z conveys signals from cell surface bound receptors to inside the cell and might play a role in the localization of malignant cells in tissues. I also show in this thesis that another molecule, 2-AG, has a similar impact on lymphoma cells. 2-AG is a ligand to the cannabinoid receptors, which are best known for being expressed in neurons where they mediate the effects of marijuana. Cannabinoid receptors and their ligands are dysregulated in many malignancies including lymphoma. Here, we provide evidence that 2-AG attracts the lymphoma cells.

Finally, THC and CBD administration to lymphoma patients modulates levels of blood leukocytes and is probably inducing the migration of lymphoma cells and normal lymphocytes away from the blood into the tissues. These effects are unwanted during lymphoma therapy since the lymphoma cells thrive in lymph nodes and bone marrow where they receive protection from chemotherapy.

In summary, these studies have provided new information on factors that regulate the migration of lymphoma cells from blood to tissues and might pave way for increased understanding of novel mechanisms involved in the interaction between lymphoma cells and cells of the tissue microenvironment.

ABSTRACT

Mantle cell lymphoma (MCL) and chronic lymphocytic leukaemia (CLL) are two incurable B cell malignancies, with an overall survival of 5 to 8 years and 6 to 10 years, respectively. Therapies are available but are often very aggressive, and patients relapse due to minimal residual disease. Minimal residual disease is defined by the presence of few malignant cells that escaped from therapy, mainly due to the survival signals provided by non-malignant cells from the tissue environment, in lymph nodes and in bone marrow. Alternative and targeted therapies are under investigation to increase patient overall survival and to reduce the risks of relapses. However, some patients do not respond to these treatments, as malignant cells develop mechanisms that prevent the drug efficacy. Many factors have already been depicted to contribute to MCL pathogenesis, and in this thesis, a new potential actor in MCL pathobiology is described, the protein G alpha z (Gαz).

The gene encoding for Gαz, *GNAZ* is overexpressed in most MCL cases compared to B lymphocytes from reactive lymph nodes. It was found that *GNAZ* expression correlates with lymphocytosis, and inversely correlates with the cannabinoid receptor type 1 previously described as a receptor potentially involved in the egress and/or retention of MCL cells within the tissue. Although the downregulation of *GNAZ* did not affect cell survival, proliferation or chemotaxis *in vitro*, its potential role in MCL pathobiology is of interest and needs further investigation.

Moreover, we characterize a co-culture *in vitro* system of MCL cell lines with mesenchymal stromal cells that permitted to identify differentially expressed genes between cells from different tissue origin. The JeKo-1 MCL cell line from peripheral blood origin, utilized the BCR signalling pathway to adhere to stromal cells, while the Rec-1 MCL cell line from lymph node origin did not, which conferred resistance to BCR targeted therapies. This system could be useful for testing patient samples to determinate a potential resistance before treatment decision.

Finally, the endocannabinoid system has been previously identified as dysregulated in both MCL and CLL. Here, we provide a new role of the endogenous cannabinoid 2-arachidonoylglycerol in chemotaxis of malignant B cells, regulated by both cannabinoid receptors type 1 and type 2. This endocannabinoid did not only induce chemotaxis by itself but also modulated the chemotaxis towards the chemokine CXCL12. In addition, a single administration of the natural cannabinoids, THC and CBD, in lymphoma patients promoted the redistribution of malignant cells from peripheral blood, and also affected non-malignant immune cells in blood. This potential negative effect of cannabinoids on the immune cells should be taken into consideration, knowing that around 25% of cancer patients use cannabinoids to alleviate symptoms and side effects from therapy.

LIST OF SCIENTIFIC PAPERS

I. Mundt F,* **Merrien M**,* Nygren L, Sutton LA, Christensson B, Wahlin BE, Rosenquist R, Sander B, Wasik AM.
Expression of GNAZ, encoding the Gαz protein, predicts survival in mantle cell lymphoma.
Br J Haematol. 2019 May;185(4):708-712. doi: 10.1111/bjh.15810. Epub 2019 Feb 20. PMID: 30788840.

II. Sadeghi L,* Arvidsson G,* **Merrien M**, Wasik AM, Görgens A, Smith CIE, Sander B, Wright AP.
Differential B-Cell Receptor Signaling Requirement for Adhesion of Mantle Cell Lymphoma Cells to Stromal Cells.
Cancers (Basel). 2020 May 2;12(5):1143. doi: 10.3390/cancers12051143. PMID: 32370190; PMCID: PMC7281289.

III. **Magali Merrien**, Agata M. Wasik, Christopher M. Melén, Kristina Sonnevi, Henna-Riikka Junlén, Birger Christensson, Björn E. Wahlin and Birgitta Sander.
2-arachidonoylglycerol modulates the CXCL12-mediated chemotaxis in mantle cell lymphoma and chronic lymphocytic leukemia.
Manuscript.

IV. Christopher M. Melén*, **Magali Merrien***, Agata M. Wasik, Georgios Panagiotidis, Olof Beck, Kristina Sonnevi, Henna-Riikka Junlén, Birger Christensson, Birgitta Sander**, Björn Engelbrekt Wahlin.**
A single dose of cannabinoids transiently affects malignant B cells and CXCR4 expression in patients with indolent B-cell leukemia.
Manuscript.

* first authors equal contribution
** last authors equal contribution

CONTENTS

LIST OF ABBREVIATIONS

2-AG	2-Arachidonoylglycerol
BCR	B Cell Receptor
BM	Bone Marrow
BTK	Bruton's Tyrosine Kinase
C	Constant
CARD11	Caspase Recruitment Domain family member 11
CB	Cannabinoid receptor
CBD	Cannabidiol
CD	Cluster of Differentiation
CFSE	Carboxyfluorescein Succinimidyl Ester
CLL	Chronic Lymphocytic Leukaemia
cMCL	Classical Mantle Cell Lymphoma
D	Diversity
del	Deletion
DAG	Diacylglycerol
ECM	Extracellular Matrix
ERK1/2	Extracellular signal-Regulated Kinases 1/2
FAAH	Fatty Acid Amide Hydrolase
FAK	Focal Adhesion Kinase
FBS	Foetal Bovine Serum
FISH	Fluorescence In Situ Hybridization
GC	Germinal Centre
GPCR	G Protein Coupled Receptor
GPR	G Protein Receptor
HUVEC	Human Umbilical Vein Endothelial Cells
ICAM-1	Intercellular Adhesion Molecule-1
Ig	Immunoglobulin
IGHV	Immunoglobulin Heavy chain Variable region
IL	Interleukin
IP3	Inositol (1,4,5)-triphosphate
J	Joining
Kv	Voltage-gated Potassium channel
LN	Lymph Node
MAGL	Monoacylglycerol Lipase
MAPK	Mitogen-Activated Protein Kinase
MCL	Mantle Cell Lymphoma
M-CLL	Mutated Chronic Lymphocytic Leukaemia
MHC	Major Histocompatibility Complex
MSC	Mesenchymal Stem/Stromal Cell
NAPE-PLD	N-Acyl-Phosphatidyl-Ethanolamine Phospholipase D
NFκB	Nuclear Factor κB
NHL	Non-Hodgkin Lymphoma
nnMCL	Non-nodal Mantle Cell Lymphoma
PB	Peripheral Blood
PCR	Polymerase Chain Reaction
PD-1	Programmed cell Death protein 1
PD-L-1	Programmed cell Death protein Ligand 1
PI3K	Phosphoinositide 3-Kinase
PIP3	Phosphatidylinositol-3,4,5-triPhosphate
PKC	Protein Kinase C
PLCγ2	Phospholipase Cγ2
PPAR	Peroxisome Proliferator-Activated Receptor
R-CHOP	Rituximab-Cyclophosphamide Doxorubicin Vincristine Prednisone
RFI	Relative Fold Increase
S1PR	Sphingosine-1-Phosphate Receptor
siRNA	Small interfering RNA
STAT3	Signal Transducer and Activator of Transcription 3
THC	Δ9-Tetrahydrocannabinol
TNF	Tumour Necrosis Factor
TRPV1	Transient Receptor Potential Vanilloid type-1
U-CLL	Unmutated Chronic Lymphocytic Leukaemia
V	Variable
VCAM-1	Vascular Cell Adhesion Molecule-1
VLA-4	Very Late Activation antigen 4

1 INTRODUCTION OF THE RESEARCH FIELD

In this thesis, molecular and mechanistic pathways are investigated in two B lymphoid malignancies: mantle cell lymphoma (MCL) and chronic lymphocytic leukaemia (CLL), with the aim to increase knowledge on their pathogenesis and pathobiology. To understand how these cancers arise, it is important to first describe the development of a normal B lymphocyte (Figure 1).

1.1 B CELL DEVELOPMENT

The immune system is divided in two types of responses: the innate immune response and the adaptive immune response. The innate response is the fast first response to foreign bodies, germs or tissue injury but is a non-specific and short-term response. The adaptive response on the other hand, will produce a specific and long-lasting response, with a memory for already encountered pathogens that will facilitate and accentuate the next response (Abbas *et al*, 2014).

The adaptive immune response consists of two main parts: the humoral immunity and the cell-mediated immunity. B lymphocytes are responsible for the humoral immunity, due to their production of proteins called antibodies. Antibodies are formed by a combination of immunoglobulin chains (described below) and specifically target antigens that come from the pathogens. Only fully mature B lymphocytes will be able to produce efficient antibodies after encountering antigens.

During development and maturation, B lymphocytes go through several steps of differentiation. Each step is characterized by changes in: i) cell morphology, ii) cluster of differentiation (CD), which are used for the recognition of cell surface molecules and for immunophenotyping of cells, iii) the immunoglobulin (Ig) heavy and light chains that form the B cell receptor (BCR) and secreted antibodies (Burger *et al*, 2018, DeFranco 2015). It is a dynamic process involving different stimuli and checkpoints (Melchers 2015, Perez-Andres *et al*, 2010).

After birth, B lymphocytes start their development in the bone marrow (BM) from a haematopoietic stem cell, characterized by expression of CD34 and CD45 (pan-leukocyte marker). The CD34+/CD45+ stem cell first differentiates into an early stage called pro-B cell, that expresses the B lymphocyte marker CD19, which will be expressed during the whole development until latest stage of plasma blast when it will be lost. The next step of the development is the pre-B cell, characterized by the Ig surface expression due to the rearrangement by DNA break and repair mechanism to assemble exons from variable (V), diversity (D) and joining (J) genes of the Ig heavy chain (Lieber 2009). This VDJ arrangement forms the antigen recognition and binding part of the Ig. Additionally, the association of the Lambda5 and VpreB surrogate light chains will form the pre-BCR. At that stage, pre-B cells

have lost CD34 but have attained CD20, another B cell marker, in addition to CD45 and CD19. Once the pre-BCR matures into a fully functioned BCR with heavy (VDJ segments and constant region Cμ region) and light chains (VJ combination coupled with Cκ or Cλ), the pre-B cell becomes an immature B cell, expressing IgM at the cell surface (Figure 1). Immature B cells that will come out of the BM, acquire also surface IgD due to alternative splicing that will assemble the VDJ segments with a different constant region C gene (here from Cμ to Cδ) and become fully mature B cells (Abbas, *et al* 2014).

The maturation process of B lymphocytes in BM requires the expression of the chemokine receptor CXCR4 and the cannabinoid receptor (CB) type 2. Indeed, knock-out studies in mice showed that the absence of CXCR4 resulted in impaired lymphopoiesis and reduced haematopoiesis in BM (Ma *et al,* 1998), and that the absence of CB2 led to a defective retention of immature B cells in BM, thus an accumulation in the blood instead (Pereira *et al,* 2009).

The mature but naïve B cells encounter pathogens in follicles of secondary lymphoid organs such as lymph nodes (LN), spleen or Peyer's patches in small intestine. The entry and maintenance of immune cells into the lymphoid organs are driven by the presence of chemokines such as CXCL12, CXCL13, CCL19 and CCL21, secreted by stromal cells. Chemokines are low-molecular weight proteins that have a chemo-attractive effect on cells that express the respective receptor, in this case: CXCR4, CXCR5 and CCR7 (Okada *et al,* 2002). In the secondary lymphoid organs, B cells become activated after antigen binding to their BCR, and the process from naïve mature B cells to fully activated competent plasma or memory cells mostly occurs in the germinal centres (GC) of these organs. It can also take place outside GC, so called extrafollicular B cell activation (Chappell *et al,* 2012).

Germinal centres are important histological structures that also involve the presence of chemokines, and that lymphocytes express the receptors CXCR4, CXCR5 and CCR7. Knock-out of these receptors results in altered LN and spleen GC structures due to the impaired migration of lymphocytes in those areas (Förster *et al,* 1996, Förster *et al,* 1999, Müller *et al,* 2002). The sphingosine-1-phosphate receptors (S1PR) 1 and 2 are also participating in the presence of B lymphocytes in GC. B lymphocytes downregulate S1PR1, which is an egress receptor binding to its ligand S1P in high concentration in peripheral blood (PB), and instead express at the cell surface S1PR2 that inhibits the migration of cells, therefore keeping them in GC (reviewed in (Cyster *et al,* 2012)). Cells that do not encounter antigen, meaning that the BCR is not activated, will upregulate S1PR1 and egress from the LN (Arnon *et al,* 2013).

The B cell in the GC proliferates in the "dark zone" of the GC first and acquire somatic mutations in the variable region of the heavy and light chains (hypermutation), in order to possess a wide variety of BCR to recognize as many antigens as possible. The proliferating and activated B cells are called centroblasts. Once the centroblasts have proliferated, they will arrive at the "light zone" where follicular dendritic cells will display the specific antigen for high affinity selection of the specific B cell clone that recognizes that antigen. In addition, the B lymphocyte will interact with T lymphocytes via different mechanisms, including the binding of CD40 to CD40 ligand on T lymphocyte, and the major histocompatibility complex (MHC)

II to T cell receptor. These stimuli induce the clonal expansion and Ig class switch resulting in the expression of a new heavy chain isotype (Cα, Cγ or Cε). These clones express IgA, IgG and IgE, which provides other functions of the Ig such as Fc-receptor binding, localization to mucosal areas (IgA), and binding to mast cells and eosinophils (IgE), therefore activating a relevant type of immune response (Abbas, *et al* 2014, Cyster *et al*, 2019).

These mature cells expressing different types of Ig are smaller in size and are called centrocytes. The centrocytes will leave the GC to become memory B cell or antibody secreting plasma cells, which are both long lived cells (Reviewed in (Cyster and Allen 2019, LeBien *et al*, 2008)).

During this development process, checkpoints are in place to ensure that the BCR recognizes antigens with sufficient strength, and on the other hand, that the BCR does not recognize self-antigens that would damage the surrounding cells and lead to autoimmune diseases. In such cases, the B cell will go through another round of Ig genes rearrangement or be eliminated by apoptosis (Melchers 2015).

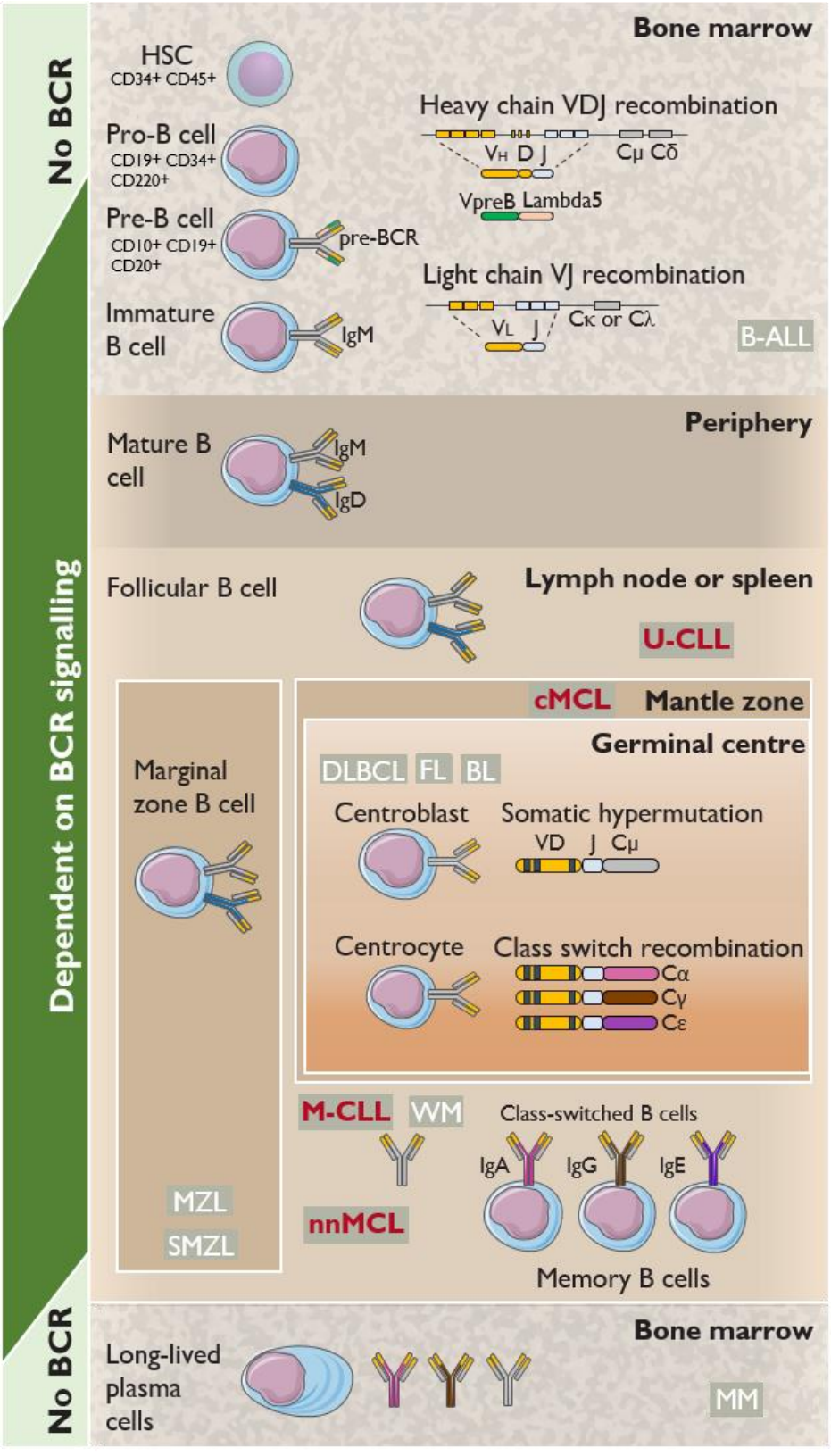

Figure 1. Representation of key steps of the B cell development and different B-cell lymphoma that can arise from cells at the different stage of their differentiation. Abbreviations: BL, Burkitt lymphoma; cMCL, classical mantle cell lymphoma; DLBCL, diffuse large B cell lymphoma; FL, follicular lymphoma; HSC, haematopoietic stem cell; MCL, mantle cell lymphoma; M-CLL, mutated chronic lymphocytic leukaemia; MM, multiple myeloma; MZL, marginal zone lymphoma; nnMCL, non-nodal mantle cell lymphoma; SMZL, splenic marginal zone lymphoma; U-CLL, unmutated chronic lymphocytic leukaemia; WM, Waldenstrom macroglobulinaemia (adapted with permission from Burger, J.A. & Wiestner, A. (2018) Targeting B cell receptor signalling in cancer: preclinical and clinical advances. Nat Rev Cancer, 18, 148-167).

1.2 DOWNSTREAM SIGNALLING OF BCR

Signalling through the BCR is essential for B cell survival and proliferation, and knocking down the BCR results in cell apoptosis (Lam *et al*, 1997). After binding of antigen, the BCR encounters a conformational change that will activate the SRC protein tyrosine kinases family, such as LYN. These kinases will phosphorylate the two signal transduction proteins CD79A and CD79B that are coupled to the BCR intracellular domain. This will recruit SYK protein that will transduce the signal by activating the BCR co-receptor CD19 together with other adaptor proteins, and recruiting phosphoinositide 3-kinase (PI3K) to the plasma membrane. There, PI3K activation will generate the second messenger phosphatidylinositol-3,4,5-triphosphate (PIP3) that is important for the activation of the Bruton's tyrosine kinase BTK and its downstream target phospholipase Cγ2 (PLCγ2). Several signalling cascades are then activated, including calcium mobilization and activation of protein kinase C (PKC) and CARD11 containing complex, which lead to activation of the transcription factor NFκB. NFκB will induce the release of cytokines, such as CCL3 and CCL4, and activate pathways involved in survival (Sasaki *et al*, 2016), cell adhesion (Spaargaren *et al*, 2003) and migration (de Gorter *et al*, 2007). Several other signalling pathways are also activated upon BCR stimulation, such as the serine/threonine kinase AKT and the mitogen-activated protein kinases (MAPK) ERK1/2 and p38, all regulating cell proliferation and survival (Reviewed in (Burger and Wiestner 2018, DeFranco 2015, Efremov *et al*, 2020, Seda *et al*, 2015); Figure 2).

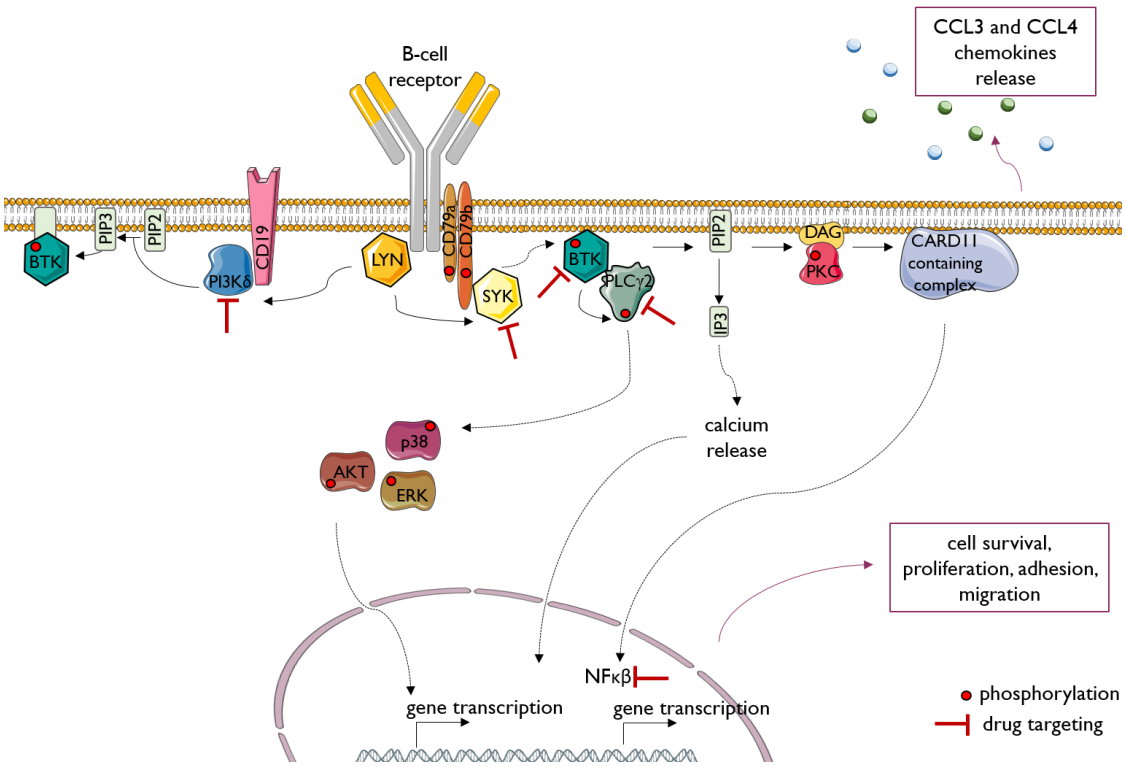

Figure 2. Scheme representing the main signalling molecules involved upon BCR activation (inspired and adapted with permission from Jerkeman, M. *et al.*, (2017) Targeting of B-cell receptor signalling in B-cell malignancies. Journal of internal medicine).

1.3 B CELL MALIGNANCIES

B cell malignancies can arise from any steps of the B cell development, as highlighted in Figure 1. It can be divided in two types: Hodgkin and non-Hodgkin (NHL) lymphoma. Mantle cell lymphoma (MCL) and chronic lymphocytic leukaemia (CLL) are NHLs, both with characteristics of a mature B cell, expressing CD5 and IgM at the cell surface. They are two lymphoma entities with very similar characteristics but also some disparities, summarized in Table 1 below.

Table 1. Characteristics of MCL and CLL.

<table>
<tr><th></th><th colspan="2">MCL</th><th colspan="2">CLL</th></tr>
<tr><td rowspan="2">Immunophenotype</td><td colspan="4">CD5+ CD19+</td></tr>
<tr><td colspan="2">CD23-; CD20+ (bright)</td><td colspan="2">CD23+; CD20+ (dim)</td></tr>
<tr><td>Incidence</td><td colspan="2">100 cases per year in Sweden
(in Europe 1-2 per 100,000 person)</td><td colspan="2">500 cases per year in Sweden
(4 per 100,000 person)</td></tr>
<tr><td>Prevalence</td><td colspan="4">2-10% NHL</td></tr>
<tr><td>Survival</td><td colspan="2">5 to 8 years</td><td colspan="2">6 to >10 years</td></tr>
<tr><td>Median age</td><td colspan="2">65-year-old</td><td colspan="2">72-year-old</td></tr>
<tr><td>Male:Female ratio</td><td colspan="2">3:1</td><td colspan="2">2:1</td></tr>
<tr><td rowspan="2">Therapy</td><td colspan="4">rituximab + chemotherapy combination</td></tr>
<tr><td colspan="2">ibrutinib</td><td colspan="2">idelalisib, ibrutinib, venetoclax</td></tr>
<tr><td rowspan="2">Frequent genetic aberrations</td><td colspan="2">t(11;14)</td><td colspan="2">tri(12), del(13q)</td></tr>
<tr><td colspan="4">somatic mutation on ATM and TP53 genes together with del(11q) and/or del(17p)</td></tr>
<tr><td>BCR</td><td colspan="4">dependent on the BCR signalling</td></tr>
<tr><td>SOX11</td><td>SOX11+</td><td>SOX11-</td><td colspan="2">SOX11-</td></tr>
<tr><td>IGHV mutation</td><td>IGHV unmutated, classical nodal MCL</td><td>IGHV mutated, TP53 mutation, non-nodal leukemic cases</td><td>IGHV unmutated, shorter survival</td><td>IGHV mutated, longer survival</td></tr>
<tr><td>CB1</td><td>CB1 overexpression</td><td>CB1 negative</td><td colspan="2">overexpression in 50% cases</td></tr>
<tr><td>CB2</td><td colspan="2">overexpression in 100% cases</td><td colspan="2">overexpression in 90-95% cases</td></tr>
</table>

1.3.1 Mantle Cell Lymphoma

Mantle cell lymphoma (MCL) is aggressive and incurable lymphoma with a median survival of only 5-8 years (Abrahamsson *et al,* 2014, Jain *et al,* 2019, Nygren *et al,* 2012). MCL cells have the morphological characteristics of mantle zone B cells.

1.3.1.1 Genetic characteristics

The first genetic aberration that defines MCL is the chromosomal translocation t(11;14)(q13;q32), which occurs at the pre-B cell stage, when the V(D)J segments are being joined to form the Ig (Küppers *et al,* 2001, Nadeu *et al,* 2020). This translocation places the gene encoding for cyclin D1 under the control of the *IGH* enhancer, leading to cyclin D1 overexpression, which promotes transition from G1 to S phase in the cell cycle. Since more than 95% of MCL tumours express cyclin D1, while normal lymphocytes and most other lymphomas are cyclin D1 negative, immunohistochemical staining for cyclin D1 (Figure 3A) is part of the diagnostic work-up and the fluorescence in situ hybridization (FISH) method for detecting the translocation (Li *et al,* 1999) (Figure 3B). However, carrying this chromosomal translocation isn't sufficient to develop MCL as cells with the t(11;14) have been detected by sensitive PCR method in 1-7% of healthy individuals without consequences (Hirt *et al,* 2004, Lecluse *et al,* 2009). Other genetic aberrations are therefore necessary and happen later in the B cell development, such as deletion or mutations of *ATM* (Campo *et al,* 2015), which is important for DNA damage recognition. Other additional cellular functions are dysregulated in MCL, including defects in DNA-repair, cell cycle regulation and apoptosis (reviewed in (Jares *et al,* 2012)). SOX11 has also become a diagnostic marker since it is expressed in 90% of MCL cases and is important for the distinction from CLL (described in the next section) in the rare cyclin D1 negative MCL cases (Wasik *et al,* 2015a).

1.3.1.2 Clinical presentation

MCL is usually first detected in LN, but BM involvement is frequent, and the disease can also involve the spleen or gastro-intestinal tract. Two subtypes of MCL can be distinguished: classical/conventional nodal MCL (cMCL) and leukemic non-nodal MCL (nnMCL) (Swerdlow *et al,* 2016).

cMCL is characterized by SOX11 expression and it is believed that the cells have not entered the GC (reviewed in (Ghia *et al,* 2017)). Instead, these cells locate in the mantle zone, and do not experience rearrangement of the variable region of Ig heavy chain (IGHV), and therefore present an unmutated *IGHV* gene (>98% homology with the germline sequence). This subtype can evolve into a more aggressive variant (blastoid MCL), which is characterized by a larger cell size, high proliferation (Ki67+), *TP53* mutations and multiple genetic alterations (Beà *et al,* 1999). *TP53* can also be mutated in the cMCL, which is then associated to worse outcome ((Hernandez *et al,* 1996) and reviewed in (Sander *et al,* 2016)).

nnMCL was first classified as atypical CLL, because of the leukemic presentation rather than LN engagement, and presence of mutated *IGHV* genes (Orchard *et al*, 2003). However, as the malignant cells in the blood carry the t(11,14), which is the hallmark of MCL, the CLL diagnosis was revised. It is now well known that nnMCL has a distinct genomic signature, with fewer chromosomal alterations than cMCL ((Fernàndez *et al*, 2010, Royo *et al*, 2012); Figure 3C). Based on these findings, a molecular assay has recently been developed by Clot *et al*. to identify and discriminate between cMCL and nnMCL in situations where clinical and biological characteristics were not sufficient to determine the subtype of MCL (Clot *et al*, 2018). Among the identified genes, the cannabinoid receptor type 1 (*CNR1* encoding for the protein CB1, described in more details in sections **1.4.4.1** and **1.6**) is described as highly expressed in cMCL and at a low expression level in nnMCL. This molecular assay could be used in the decision of the treatment strategy, as patients with the indolent nnMCL form do not require treatment until symptoms, but most cMCL, also those with leukemic presentation will need therapy.

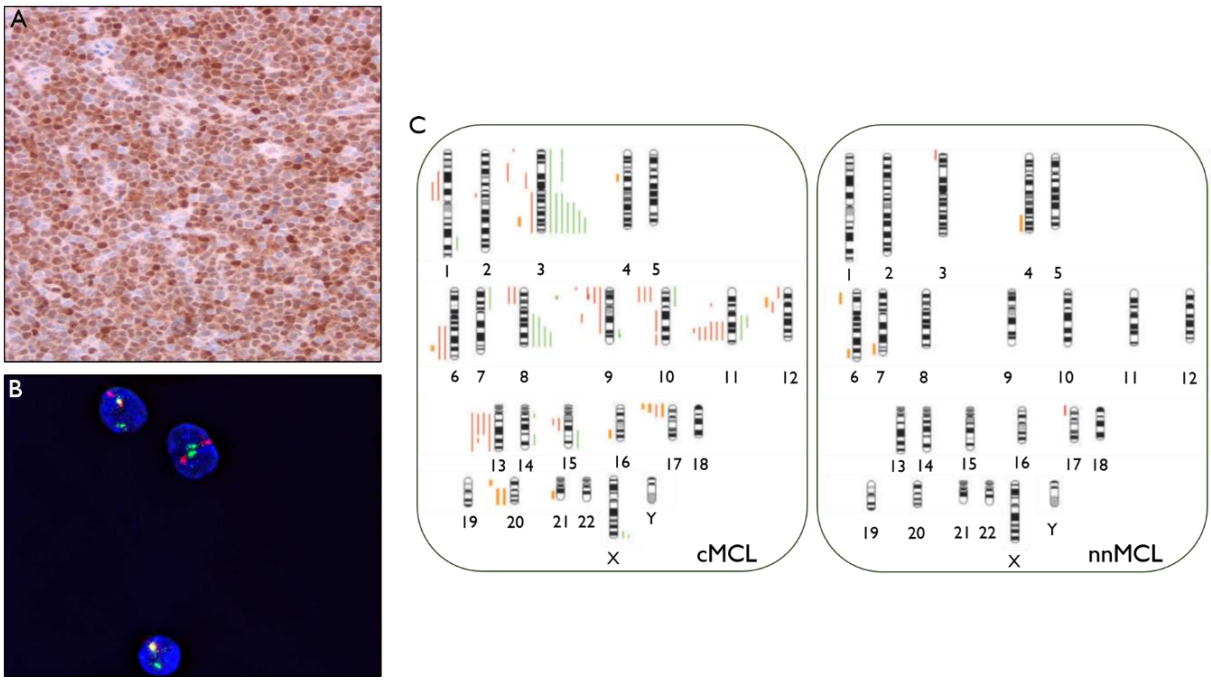

Figure 3. Characteristics of MCL. **A.** Cyclin D1 staining in MCL case visualized by positive staining (brown) detected in the nuclei. The staining is normally variable with both weakly and strongly positive cells (original magnification x10). **B.** FISH staining using probes targeting chromosome 14 (green) and chromosome 11 (red), yellow signal represents gene chromosomal fusion (original magnification x63). **C.** chromosomal alterations differences between cMCL and nnMCL. (A and B were kindly provided by Prof. Birgitta Sander; C was obtained with permission from Fernàndez, V. *et al.*, (2010) Genomic and gene expression profiling defines indolent forms of mantle cell lymphoma. Cancer Res, 70, 1408-1418.).

1.3.1.3 Treatment options

The conventional treatment for MCL is R-CHOP regimen, which consists of rituximab antibody targeting CD20 together with a chemotherapy combination (cyclophosphamide, doxorubicin, vincristine and prednisone). Today, most young patients are treated with an intensified R-CHOP alternating with high dose of cytarabine, followed by stem cell rescue, so called autologous stem cell transplantation. This options has been reported to increase the overall survival for young and fit patients (Dreyling *et al,* 2005, Eskelund *et al,* 2016, Geisler *et al,* 2008, Kolstad *et al,* 2017). Recently, targeting the BCR signalling pathway became a new treatment option (e.g. BTK inhibitors ibrutinib or acalabrutinib). Also inhibitors of Bcl-2 and cyclin-dependent kinases are under investigation (reviewed in (Jerkeman *et al,* 2017)). However, these targeted therapies are still given mostly to relapsed MCL patients (Dreyling *et al,* 2018).

Although nnMCL has a longer survival of 7-10 years, compared with 3-5 years for cMCL, MCL is still an incurable disease.

1.3.2 Chronic Lymphocytic Leukaemia

Chronic lymphocytic leukaemia (CLL) is a common subtype of lymphoma/leukaemia. It is a heterogenous disease which results in various clinical outcome (Hallek 2019).

1.3.2.1 Genetic characteristics

CLL is characterized by four main chromosomal abnormalities: deletion of long arm of chromosome 11 (del(11q)), which deletes *ATM* gene involved in DNA damage detection, like for MCL; trisomy 12 (tri12); del(13q) which deletes the micro-RNA miR15a and miR16-1 that are normally silencing the gene encoding for the anti-apoptotic Bcl-2 protein (Calin *et al,* 2002, Cimmino *et al,* 2005); and deletion of *TP53* on the short arm of chromosome 17 (del(17p), (Döhner *et al,* 2000), Figure 4). All these alterations promote cell survival and proliferation. A worse prognosis is seen when del(11q) and del(17p) are combined with somatic mutations in the remaining *ATM* and *TP53* genes (Zenz *et al,* 2010). Twenty percent of CLL cases, however, do not carry any of these four genetic aberrations (Döhner, *et al* 2000).

The mutation status of *IGHV* gene is also a prognostic factor in CLL, with unmutated *IGHV* (U-CLL) associated to a more aggressive disease course (Hamblin *et al,* 1999, Oscier *et al,* 2002). Gene sequencing analysis of the *IGHV* gene is now considered part of the diagnostic routine for CLL (Davi *et al,* 2020, International CLL-IPI working group. 2016, Langerak *et al,* 2011, Rosenquist *et al,* 2017) and because it has been done already in a large proportion of cases, it is now possible to evaluate and get a risk stratification of CLL disease course and overall survival according to *IGHV* mutation status (International CLL-IPI working group. 2016). The mutation status of *IGHV* and the presence of specific sequences help to predict therapy responses and outcome (Fischer *et al,* 2017, Sutton *et al,* 2017).

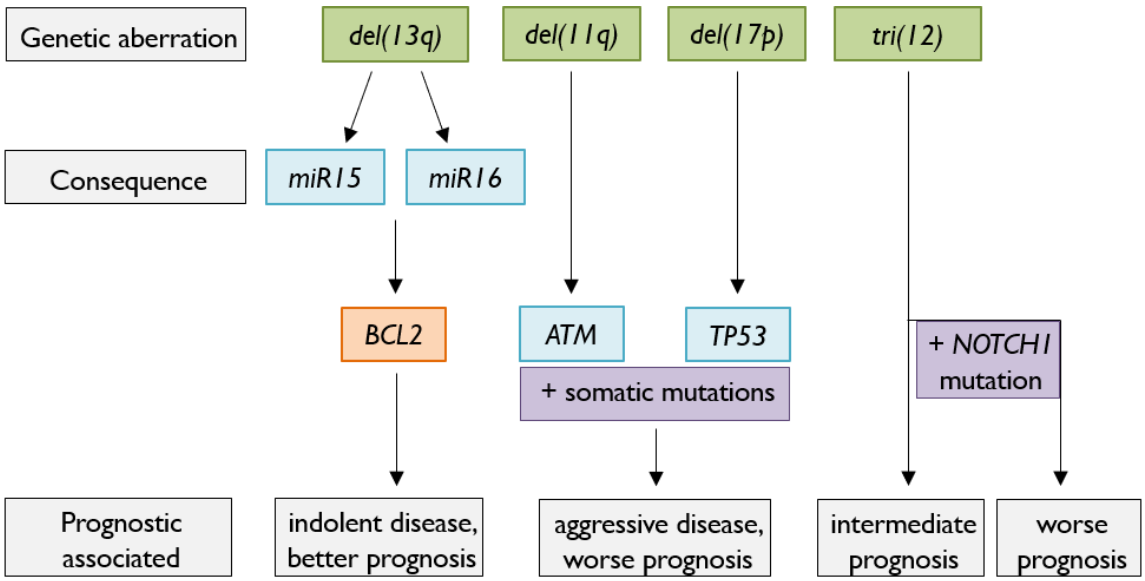

Figure 4. The most common genetic alterations in CLL (blue box represents loss of gene and orange box overexpression of the gene; inspired by Hallek, M., Shanafelt, T.D. & Eichhorst, B. (2018) Chronic lymphocytic leukaemia. Lancet, 391, 1524-1537).

1.3.2.2 Clinical presentation

As for MCL, CLL cells proliferate in BM and in secondary lymphoid organs, but also accumulate in PB. For a CLL diagnosis, the threshold of B lymphocyte count is at least $5x10^9$ cells per litre of blood.

CLL is a heterogenous disease, that can present as a pre-leukemic form (small cell lymphocytic lymphoma), an indolent form, or be progressive and resistant to therapy. The severity of the disease is measured according to the Rai and Binet staging system, including the assessment of symptoms, clinical parameters such as anaemia, thrombocytopenia, lymphadenopathy (Hallek *et al*, 2018), presence of genetic aberrations and as mentioned above the *IGHV* mutation status.

Interestingly, CLL cells that are found in the PB are less activated compared with cells in BM and LN as the gene signature was enriched for the NFκB pathway in CLL cells from LN (Burger and Wiestner 2018, Herishanu *et al*, 2011).

1.3.2.3 Treatment options

The standard therapy for CLL includes a chemotherapy cocktail, mostly fludarabine and cyclophosphamide, combined with anti-CD20 antibody rituximab. However, targeted therapy is more used in CLL, especially the BTK irreversible inhibitor ibrutinib (reviewed in (Smith 2017)). Other signalling pathways inhibitors such as the PI3K inhibitor (idelalisib), the Bcl-2 inhibitor (venetoclax) or SYK inhibitors have shown promising results (reviewed in (Jerkeman, *et al* 2017)).

In both MCL and CLL, disease progression and relapse after treatment are very common, due to resistances to treatment (Ondrisova *et al*, 2020), which can be intrinsic or acquired (Zhao *et al*, 2017). Resistances can arise when malignant cells try to escape by mutating the region in the drug-binding sites to avoid recognition from it. For instance, after ibrutinib, a point mutation (BTK C481S) reduces the binding affinity of ibrutinib for BTK. Other types of mutations have been described such as gain-of-function mutations on PLCγ2 gene, which allows a BCR signalling independent of BTK activation (Woyach *et al*, 2014).

Relapses are common also in patients with minimal residual disease. It is hypothesised that minimal residual disease is due to a few lymphoma cells that reside in tissues, supported by non-malignant cells in the microenvironment and receiving survival signals that make them resistant to therapies (Burger et al, 2011, Kurtova et al, 2009, Medina et al, 2012).

1.4 LYMPHOMA MICROENVIRONMENT

Like many cancer types, lymphoma cells need the signals and interactions with surrounding non-malignant cells and other components, called the microenvironment, to survive and proliferate. One striking clue about the importance of the microenvironment came from studies that demonstrated that primary MCL or CLL cells survived up to several months in co-culture with stromal cells, compared with just few days when cultured alone (Medina, *et al* 2012, Panayiotidis *et al*, 1996). Several actors are involved in the communication between lymphoma cells and non-malignant cells in the tissue microenvironment, and the main ones are described below and presented in Figure 5.

1.4.1 The B Cell Receptor

The BCR signalling is a key-player in both MCL and CLL. The initial role of the BCR in a normal B lymphocyte is to recognize the antigen that will stimulate the cells into proliferation and differentiation processes in order to produce antibodies. The malignant B cells are utilizing the same mechanisms and pathways through the BCR for survival and proliferation.

Ibrutinib is a drug that inhibits BCR signalling by targeting the downstream tyrosine kinase BTK. Ibrutinib irreversibly blocks BTK, which causes the egress of tumour cells out from the tissue into the blood stream in both CLL and MCL (Chang *et al*, 2013), confirming the communication between malignant and non-malignant cells via the BCR.

Indeed, in the malignant context, the BCR is continuously active and sometimes over-stimulated as a result of the following mechanisms: i) binding of auto-antigen from the surrounding cells in the microenvironment, ii) acquirement of activating mutations in the BCR downstream signalling molecules, iii) antigen-independent tonic activation of the BCR (reviewed in (Burger *et al*, 2014, Burger and Wiestner 2018)).

1.4.1.1 Auto-antigen stimulation

In CLL, it has been discovered that approximately one third of patients share a similar *IGHV* sequence, specifically the antigen recognition domain (complementarity-determining region 3; (Agathangelidis *et al,* 2012)). These subsets of BCR are called stereotypes. This stereotypy can be explained by the recognition of antigens that are not commonly recognized by normal B lymphocytes (low frequency of these *IGHV* sequences normally) as they are auto-antigens present in the microenvironment, but specifically selected by CLL cells. In MCL the same phenomenon has been reported but with different *IGHV* gene sequence profile compared to CLL (Hadzidimitriou *et al,* 2011, Sutton *et al,* 2013).

1.4.1.2 Activating mutations

Mutations in different proteins involved in the BCR signalling pathway can occur (e.g. mutations in the gene encoding for CD79B) and lead to continuous activation of the pathway together with inhibition of the negative feedback loop that should control the BCR stimulation. However, these mutations are rare in MCL and CLL (reviewed in (Burger and Wiestner 2018)).

1.4.1.3 Tonic antigen-independent BCR signalling

The third way of BCR activation is the tonic signalling, which occurs independently from antigen binding. It is mediated by PI3K pathway, and serves for the normal B lymphocytes as a survival signal when no antigen is binding (reviewed in (Rickert 2013)). Malignant B cells also use this signalling pathway, increasing the signalling by the activation of important protein kinases or the downregulation of PI3K inhibitor protein (Burger *et al,* 2013, Merolle *et al,* 2018).

1.4.1.4 Tissue localization

Gene pathway analysis in both MCL and CLL demonstrated that the BCR and downstream NFκB signalling are enriched, especially within the LN, compared with PB, where there is little interaction with other cells (Herishanu, *et al* 2011, Saba *et al,* 2016). NFκB signalling regulates cell survival and proliferation (reviewed in (Burger and Wiestner 2018, Efremov, *et al* 2020, Ghia, *et al* 2017)). Indeed, non-malignant cells in the microenvironment, especially in LNs, (e.g. lymphoma-associated macrophages) provide signals that activate BCR-signalling (reviewed in (Ten Hacken *et al,* 2016)).

1.4.2 Non-malignant cells

MCL and CLL cells in tissues interact with the surrounding cells, especially other immune cells that are communicating with normal B cells to develop immune responses upon antigen encountering (Cyster and Allen 2019). Malignant cells can reside in BM or other lymphoid tissues such as LN, where different cell types provide support for the tumour cells. The role of the non-malignant cells is very complex and still needs to be investigated further.

1.4.2.1 Bone marrow stromal cells

In BM, the stromal cells (also called mesenchymal stem cells, MSCs) predominantly support CLL and MCL survival by secreting chemokines and expressing essential surface molecules (Honczarenko *et al,* 2006, Li *et al,* 2016). Importantly, the communication between the malignant cell and the non-malignant BM MSCs has to involve direct contact (Lagneaux *et al,* 1998) and is bi-directional (Ding *et al,* 2009). Indeed, malignant cells are attracted to MSCs, bind to them and have the capacity to migrate beneath them, all due to the expression of adhesion molecules and chemokine receptors that recognize chemokines secreted by MSCs (Burger *et al,* 1999, Kurtova, *et al* 2009, Medina, *et al* 2012). In return, signalling pathways such as ERK1/2, AKT and NFκB are activated in MSCs after interaction with CLL cells (Ding, *et al* 2009, Lutzny *et al,* 2013).

In addition, MCL and CLL primary cells co-cultured with BM MSCs induce the secretion of several soluble factors such as interleukin IL-6 (Zhang *et al,* 2012) and chemokines CCL3 and CCL4 (Zucchetto *et al,* 2010), activating different signalling pathways that all promote survival.

Altogether these close interactions activate survival pathways like ERK1/2 (Kurtova, *et al* 2009) and anti-apoptotic proteins, such as Bcl-2 (Lagneaux *et al,* 1999, Lwin *et al,* 2009, Lwin *et al,* 2007), making lymphoma cells protected from therapy by MSCs.

1.4.2.2 Lymph node microenvironment

Compared with BM, the LN microenvironment has been more investigated in haematological malignancies. It is composed of a wide variety of cells, due to the fact that it is the location for presentation of antigen, activation, proliferation and maturation of antigen-specific B lymphocytes. It is also where CLL and MCL proliferate the most, compared to BM and PB (Herishanu, *et al* 2011, Saba, *et al* 2016).

T lymphocytes are an important part of the LN microenvironment. In MCL, the amount of T lymphocytes found in LN biopsies is lower in the aggressive form of disease, and a high ratio of CD4+/CD8+ T cells is correlated to longer overall survival in MCL (Nygren *et al,* 2014). This suggests that the aggressive forms of MCL are less dependent on the microenvironment signals.

In CLL, CD8+ T lymphocytes are enriched in blood (Herrmann *et al,* 1982), and cytotoxic CD4+ T cells are attracted in LN microenvironment by CLL cells that secrete cytokines (Hartmann *et al,* 2016). Interaction between B and T lymphocytes results in proliferation signals in B cells via for instance CD40-CD40L interaction (Abbas, *et al* 2014, Castillo *et al,* 2000).

The presence of CD4+ T lymphocytes in LNs are related to a good patient outcome in MCL (Nygren, *et al* 2014) and in CLL (Nunes *et al,* 2012), due to their anti-tumoral role (Dobrzanski 2013). However, malignant cells develop a way to exhaust T cells. Indeed, once the CD4+ T cells interact with CLL cells, they lose their ability to form immunological synapse (Ramsay

et al, 2012). The immunological synapse consists of changes in cytoskeleton of T cells in contact with the antigen presenting B cell via the MHC-II, or with the tumour cell via self-antigen, which is supposed to activate the T cell for cytotoxic actions (Houghton *et al*, 2004, Nassef Kadry Naguib Roufaiel *et al,* 2015). Nevertheless, this effect is inhibited by the presence of programmed cell death protein 1 (PD) ligand PD-L-1 on MCL/CLL cells (Allahmoradi *et al,* 2017, Wang *et al,* 2013). PD-L-1, which is a negative regulator of T cells in normal condition to avoid auto-immune responses (Jin *et al,* 2011), is then recognized by the receptor PD-1 on T cells, providing signals that abolish their function of tumour cell lysis (Wang, *et al* 2013).

Monocytes, macrophages and nurse like cells attract malignant cells into lymph nodes and other lymphoid organs by secreting chemokines (Burger *et al,* 2000). In addition, these non-malignant cells are attracted by malignant cells via chemokines, such as CCL3 and CCL4 (Zucchetto, *et al* 2010).

1.4.3 Chemokines and adhesion molecules

Many different chemokines, cytokines and other molecules, their respective receptors, as well as adhesion molecules are involved in the communication between the non-malignant cells and CLL/MCL cells, but only the most relevant ones to this thesis are described here.

1.4.3.1 Chemokine and egress receptors

CXCR4, CXCR5 and CCR7 are chemokine receptors expressed in normal B lymphocytes, and they are important for their development, maturation and differentiation. These chemokine receptors are part of the superfamily of G protein coupled receptors (GPCR), and are overexpressed in MCL (Corcione *et al,* 2004, Kurtova, *et al* 2009) and CLL (Burger, *et al* 1999, Till *et al,* 2002).

Malignant cells use essentially the same mechanisms as the non-malignant B cells, to promote their survival and proliferation, while diminishing the effects of the opposite mechanisms such as apoptosis. In a healthy setting, CCR7 ligand (CCL21) is responsible for the entry of lymphocytes into LN, CXCR5 ligand (CXCL13) for the positioning in follicular areas in LN and CXCR4 ligand (CXCL12) for the entry and maintenance of B cells in BM. In lymphoma, the expression of these receptors and ligands is also different according to the tissue compartments, in favour of cell retention (Middle *et al,* 2015). This is explained by the lymphoma tissue microenvironment secreting these ligands, creating a gradient and attracting cells expressing the receptors, therefore playing a major role in homing of lymphoma cells into tissues.

In MCL and CLL, all three chemokine receptors (CXCR4, CXCR5 and CCR7) are upregulated at the cell surface (reviewed in (Burger and Ford 2011, Burger and Gribben 2014)). In CLL, the high levels of CCR7 and CXCR4 could at least partially be explained by the enhanced recycling of the receptors compared with non-malignant B lymphocytes (Patrussi *et al,* 2015).

Additionally, there is a balance, or unbalance in the case of malignancy, of expression between the chemokine receptors that induce the retention of cells in tissues, and S1PR1 which promotes their egress (Cyster and Schwab 2012, Patrussi, *et al* 2015).

S1PR1 is involved in the egress of normal B lymphocytes from LN into PB, where its ligand, the biolipid S1P, is in high concentration. S1PR1 is expressed in most MCL cases (Nishimura *et al,* 2010), with a different expression level observed in different tissue of origin derived MCL cell lines (Sadeghi *et al,* 2020). *S1PR1* gene is also found mutated in 8% of MCL cases, inducing in most cases the reduction of S1PR1 expression, correlating with advanced stages of the disease and promoting the retention of cells within the tissue (Wasik *et al,* 2018). In CLL, the expression of S1PR1 is often impaired, and its surface expression is even more reduced *in vitro* upon several stimuli like BCR stimulation with anti-IgM antibody or CD40-CD40L interaction (Borge *et al,* 2014, Till *et al,* 2015).

Both CXCR4 and CXCR5 were shown to be involved in the migration of malignant cells beneath MSCs, thus protecting them from therapies (Burger, *et al* 1999, Kurtova, *et al* 2009). CXCR4 expression is important for the malignant cell survival as it was demonstrated that culturing MCL cell lines with its ligand CXCL12 increased survival compared with medium alone, and that knock-down of CXCR4 reduced cell proliferation (Chen *et al,* 2016b). In addition, CXCR4 expression is dynamic, allowing the cells to recirculate from LN to PB and vice-versa (Chen *et al,* 2016a). CXCR7 is also a receptor for CXCL12 but is mostly involved in cell adhesion rather than migration (Burns *et al,* 2006) and is not expressed in leukocytes (Berahovich *et al,* 2010).

Because all interactions are dynamic, cross-activation of specific downstream signalling pathways happens, as for instance the phosphorylation and activation of BTK by CXCL12 in CLL (Montresor *et al,* 2018, Nore *et al,* 2000), involving both CXCR4 and BTK signalling pathways in adhesion of cells. In MCL, we found that these signalling pathways are differently activated in different cell lines (Sadeghi, *et al* 2020). Interaction between BCR and CXCR4 signalling is also suggested by the fact that CLL and MCL cells are released in PB after ibrutinib treatment (Chang, *et al* 2013), due to the inhibition of surface CXCR4 expression (Chen, *et al* 2016a).

Also, BCR signalling results in secretion of CCL3 and CCL4 from CLL, which will further attract non-malignant immune cells such as monocytes and T cells as mentioned earlier (Burger *et al,* 2009).

1.4.3.2 Adhesion molecules

Adhesion molecules are participating in cell-to-cell adhesion, as well as cell-to-extracellular matrix (ECM) contacts. They are part of the process of homing and retention of B lymphocytes into lymphoid tissue.

There are several types of adhesion molecules, categorized based on their structures. Integrins are transmembrane receptors composed of two subunits: α and β. Different α and β subunits can be paired together, creating a variety of different integrins. The very late activation antigen (VLA-4, also known as CD49d) is a α4β1 integrin expressed on leukocytes. It can recognize ECM components such as fibronectin and vascular cell adhesion molecule-1 (VCAM-1). CD49d is also expressed at the cell surface of MCL and CLL cells. This integrin is part of the mechanism used by non-malignant and malignant lymphocytes to migrate beneath the MSCs (Burger *et al,* 2001, Kurtova, *et al* 2009, Miyake *et al,* 1992). In malignancies, VLA-4 is therefore associated with nodal and extra-nodal involvement (Strati *et al,* 2017, Terol *et al,* 1999) and worse prognosis due to drug resistance (Kurtova, *et al* 2009, Shanafelt *et al,* 2008).

Another adhesion molecule of importance is ICAM-1, expressed mainly on stromal cells but also on some leukocytes, and in some of MCL and CLL cases (Jacob *et al,* 1999, Molica *et al,* 1995). ICAM-1 is upregulated upon adhesion to stromal cells in co-culture *in vitro* system (Sadeghi, *et al* 2020), validating the fact that its expression is higher in MCL and CLL samples from LN compared with PB (Arvidsson *et al,* 2018).

1.4.4 Cannabinoid receptors and ligands

1.4.4.1 Cannabinoid receptors

Gene expression analysis comparing LN biopsies from MCL patients and non-malignant cells from reactive tonsils and LNs described for the first time the overexpression of cannabinoid receptors (CB) type 1 and type 2 in MCL (described in section **1.6.1**; (Ek *et al,* 2002, Islam *et al,* 2003)). Screening of different NHL subtypes identified CB1 and CB2 overexpressed in most of the B cell lymphoma subtypes included in the study such as CLL, marginal zone, follicular and diffuse large B cell lymphomas (Gustafsson *et al,* 2008, Rayman *et al,* 2007).

The mRNA expression of CBs had already been identified in human immune cells in the 1990s (Bouaboula *et al,* 1993, Galiegue *et al,* 1995) after the publication of earlier reports describing effects of the CB ligands from the marijuana plant (delta-9-tetrahydrocannabinol, THC) in immune cells function, hypothesizing that the receptors should then be expressed on those cells. Both CB1 (encoded by *CNR1* gene) and CB2 (encoded by *CNR2* gene) mRNA expression could be quantified in lymphoid organs and immune cell subtypes. *CNR2* is there expressed at higher levels compared to *CNR1*, up to 100-fold increase, and the highest expression levels for both receptors were found in tonsil, and B lymphocytes (Galiegue, *et al* 1995).

In MCL and CLL, the range of expression measured by quantitative PCR is wide for *CNR1* (relative fold increase (RFI) compared to non-malignant B cells from 0.63 to nearly 5000) and *CNR2* (RFI from 1.06 to nearly 200), with *CNR1* reaching the levels of *CNR2* for some cases, if not higher (Wasik *et al,* 2014). Protein expression was also confirmed in patient samples and in MCL cell lines (Gustafsson, *et al* 2008, Islam, *et al* 2003). *CNR1* has two splice variants in addition to the full-length, which causes impaired binding to endocannabinoids (Ryberg *et al,* 2005). However, only splice variant *CNR1a* and not *CNR1b* seems to be expressed in MCL, and at very low levels since it was not detected on the first round of PCR but instead on the second round of PCR performed on the product from the first round (Gustafsson, *et al* 2008).

The role of the CBs in lymphoma is not yet deciphered. However, analysis of CB expression and clinical data from a study in our group showed that low *CNR1* levels correlated to lymphocytosis (>5x10^9 lymphocytes/litre of blood) (Wasik, *et al* 2014). *CNR1* was also found at lower levels in indolent (low progression of disease) MCL (Fernàndez, *et al* 2010), and confirmed recently from gene expression analysis comparing cMCL and nnMCL profiles, in which *CNR1* is downregulated in leukemic nnMCL compared with cMCL (Clot, *et al* 2018). Therefore, it is hypothesized that CB1 might be involved in homing, retention and/or egress of the malignant B-cells from the tissue to the PB.

In CLL, low *CNR1* cases displayed more frequently unmutated *IHGV* genes (U-CLL), and low *CNR1* expression levels correlated to better overall survival (Freund *et al,* 2016).

1.4.4.2 Endogenous ligands to cannabinoid receptors

In addition, endogenous ligands to cannabinoid receptors (endocannabinoids, described in further detail in chapter **1.6**) are released on demand by MSCs in BM (Kose *et al,* 2018). Endocannabinoids might play a role in the tumour microenvironment as their expression levels are found dysregulated in plasma of cancer patients compared with healthy individuals, as well as at the site of tumour in mice models (Sailler *et al,* 2014, Zhang *et al,* 2016).

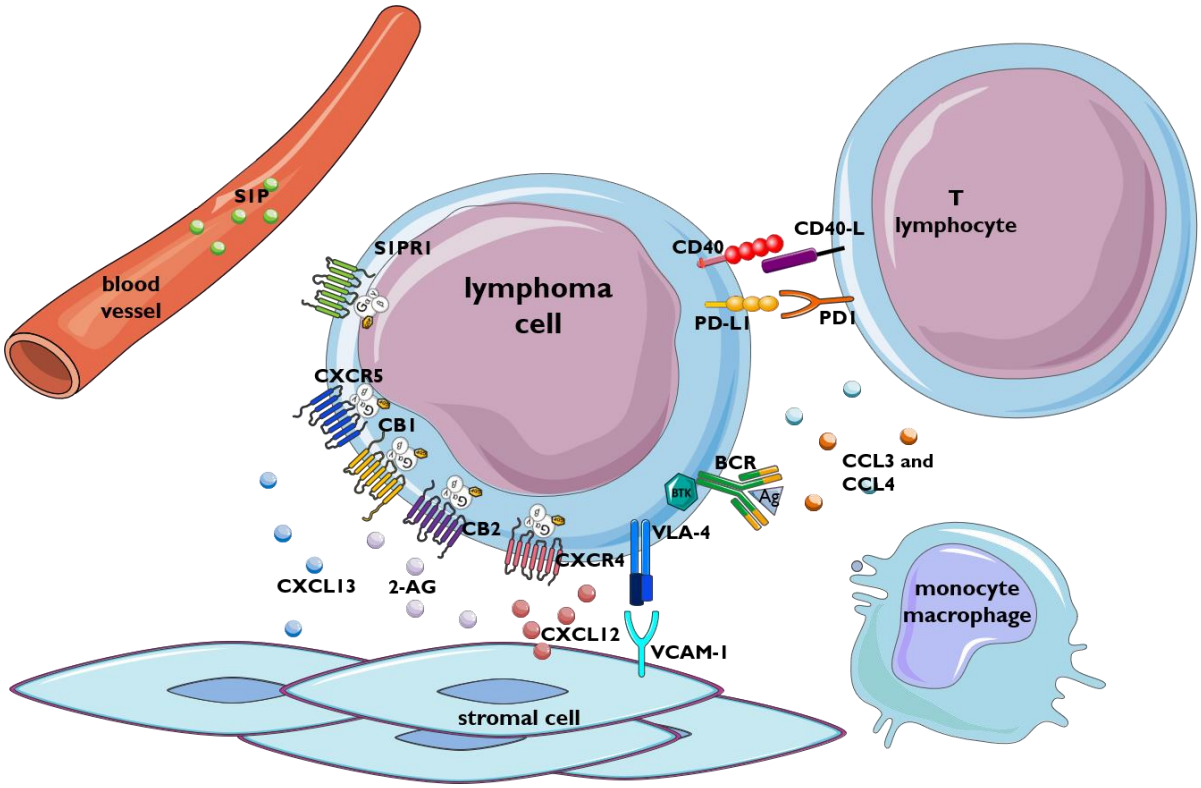

Figure 5. Simplified scheme representing the interactions between the lymphoma cell and the non-malignant microenvironment. *Note that only the factors of relevance to this thesis are represented.*

1.5 G PROTEIN AND G PROTEIN COUPLED RECEPTORS

Chemokine and cannabinoid receptors described in previous chapters are part of the G protein coupled receptors (GPCR) superfamily (Fredriksson *et al,* 2003, Nugent *et al,* 2017). The structure, general mechanism of action and downstream signalling are similar for all GPCRs, including chemokine and cannabinoid receptors.

1.5.1 G protein coupled receptors

The G protein coupled receptor (GPCR) superfamily contains around 800 different proteins divided into five families that share similar phylogeny origins (Fredriksson, *et al* 2003, Katritch *et al,* 2013). Chemokine and cannabinoid receptors are part of the same rhodopsin-like receptor family (also called family A).

The main common feature of GPCRs is the structure: seven transmembrane helical domains, all linked by loops, and with an extracellular N-terminus and a C-terminus inside the cell (Figure 6A). This kind of structure allows transmission of extracellular signals into the cell and the subsequent induction of intracellular signalling pathways. The presence of an eighth helix (H8) has also been described in most of the rhodopsin family receptors, located in the C-terminus, close to the intracellular part of the membrane (Weis *et al,* 2018).

Upon activation, mainly due to ligand binding at the N-terminus, or after homo- or hetero-dimerization (reviewed in (Faron-Górecka *et al,* 2019)), the GPCR encounters conformational change that leads to recruitment of the heterotrimeric G proteins complex composed of subunits α, β and γ. It also induces the exchange of guanosine diphosphate (GDP) to guanosine triphosphate (GTP, abundant in the cell) that triggers the separation of the G protein complex. The subunit Gα is released from the GPCR and from the two other subunits, and activates signalling pathways that are involved in different cell biology processes such as glucose metabolism, synaptic transmission, cell survival and migration (reviewed in (Neves *et al,* 2002, Syrovatkina *et al,* 2016)), through the activation of second messengers and signalling pathways such as PI3K, MAPK and AKT (reviewed in (Gutkind 2000); Figure 6B).

Once the receptor is in active state, it becomes phosphorylated by specific kinases and recruits β-arrestin protein (Figure 6B). This promotes desensitization of the receptor signalling and receptor internalization (reviewed in (Moore *et al,* 2007)). After internalization, the receptor is either recycled back at the cell surface or degraded. Additionally, β-arrestin itself induces downstream signalling upon binding to the activated and phosphorylated receptor, creating a biased signalling (one ligand binding to one receptor, which results in two responses; reviewed in (Rajagopal *et al,* 2010)).

Many different types of ligands can bind to GPCRs, provoking different levels of activation or inactivation depending on their specificity and efficacy to induce receptor conformational changes and recruitment of G protein (reviewed in (Hilger *et al,* 2018)). Depending on the downstream response upon binding, the ligand is considered as agonist or antagonist. If the

binding induces 100% of activation of receptor, the ligand is considered as full agonist. However, it is possible that the receptor becomes only partially activated, which makes the ligand a partial agonist. An antagonist will compete for the binding site with the agonist, without affecting the conformation of the receptor. The last type of ligand is the inverse agonist, which upon binding will induce the inactive state of the receptor and inhibit the basal signalling (Figure 6C).

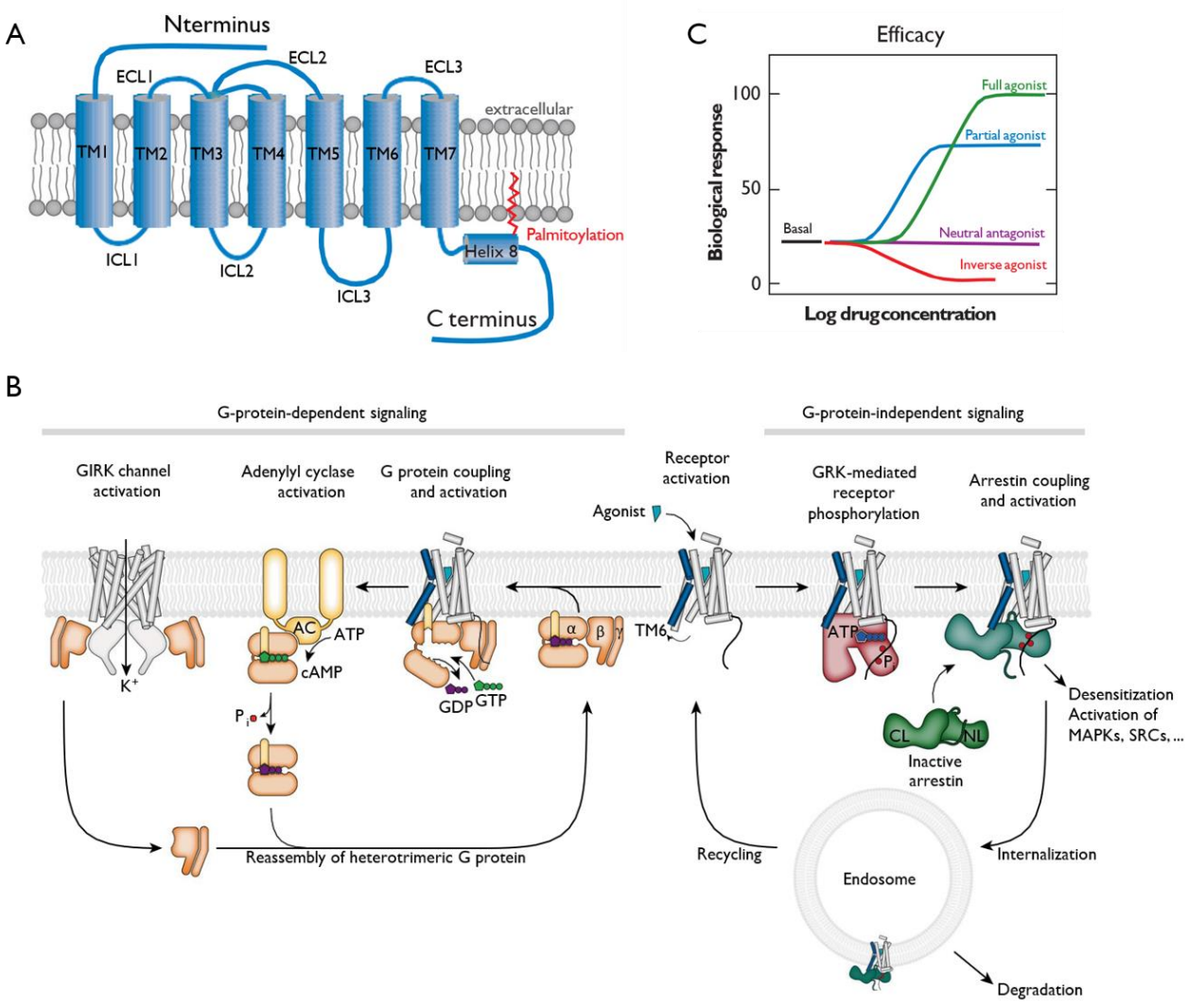

Figure 6. GPCR structure and activation mechanism. **A.** Structure of GPCR. **B.** Steps of a GPCR activation upon ligand binding. **C.** Ligand response. (A and C were obtained with permission from Weis, W.I. & Kobilka, B.K. (2018) The Molecular Basis of G Protein-Coupled Receptor Activation. Annu Rev Biochem, 87, 897-919.; B was obtained with permission from Hilger, D., Masureel, M. & Kobilka, B.K. (2018) Structure and dynamics of GPCR signaling complexes. Nature Structural & Molecular Biology, 25, 4-12.).

1.5.2 G proteins

G proteins are responsible for the signal transduction after GPCR activation. Only 21 Gα proteins have been identified, implicating a conserved G protein binding pocket domain on GPCRs. Six Gβ and eleven Gγ subunits have been identified so far (reviewed in (Syrovatkina, *et al* 2016)).

The subunit Gα is divided into four families, based on their sequence similarity and similar functions: i) Gαi: inhibits the enzyme adenylyl cyclase thus decreasing the levels of the second messenger cAMP in the cells; ii) Gαs: stimulates adenylyl cyclase, which increases cAMP levels; iii) Gαq: activates phospholipase C (PLC); iv) Gα12/13: targets small Rho-GTPases.

The Gαi family is the largest of all four families, containing seven members including Gαo and Gαz. Gαi are expressed in many different cell types, and chemokine receptors usually bind to the Gαi/o family.

1.5.2.1 G protein alpha z

Although it is considered as part of Gαi family, due to the inhibition of adenylyl cyclase, Gαz is the only member of Gαi to be insensitive to pertussis toxin (PTX) (Fong *et al,* 1988, Matsuoka *et al,* 1990). PTX is blocking the binding of Gαi protein to the GPCR by catalysing ADP ribosylation at the C-terminus of the G protein. However, that part is lacking in Gαz. Indeed, Gαz is sharing only 60% of sequence homology with Gαi. Nevertheless, Gαz is binding to similar receptors recognized by Gαi, as indirectly shown by the fact that similar downstream signalling is activated upon GPCR stimulation, but resistant to PTX treatment (Ho *et al,* 2001).

Not much is known regarding the function or expression of Gαz. Gαz has mostly been described in neuronal cells where it couples neurotransmitter receptors to ion channels regulating calcium mobilization (Jeong *et al,* 1998) and in retina cells (Hinton *et al,* 1990). Gαz can also be found in platelets (Gagnon *et al,* 1991) and other non-neural cells where it might control the Golgi structure maintenance (Nagahama *et al,* 2002).

Moreover, we described recently the overexpression of Gαz in a subset of mantle cell lymphoma patients, compared with expression in reactive LN (Mundt *et al,* 2019). Analysis of clinical data showed that high Gαz mRNA expression correlated with poor patient survival, which was also the case for other malignancies such as endometrial, gastric and liver cancers. Its expression also correlated to spread of lymphoma cells to the blood (lymphocytosis) and an inverse correlation was found with *CNR1* expression. Downregulation of Gαz in MCL cell lines did not affect cell survival, and the chemotaxis properties of the cells were intact (Mundt, *et al* 2019). The role of Gαz in mantle cell lymphoma has therefore to be investigated further.

1.5.3 CXCR4 signalling

CXCR4 is a chemokine receptor (described earlier in section *1.4.3.1*) expressed on many different cell types. This section is focused on CXCR4 expression on B lymphocytes (malignant and non-malignant).

As mentioned, CXCR4 binds to Gαi protein family, reducing levels of the second messenger cAMP due to the inhibition of adenylyl cyclase by Gαi subunit. The other two subunits, βγ activate two enzymes responsible for signal transduction: PLCβ and PI3K. These enzymes will allow the activation of the second messengers inositol (1,4,5)-triphosphate (IP3) and diacylglycerol (DAG), that are responsible for calcium release and activation of PKC and MAPK. Additional signalling pathways are activated upon CXCL12 ligation, including ERK1/2, p38, STAT3 and NFκB involved in cell survival and chemotaxis (Ganju *et al,* 1998, Teicher *et al,* 2010).

CXCR4 can also recruit G protein Gα13, that activates the Rho GTPase, complementing Gαi activation of the small GTP binding protein Rac, and inducing chemotaxis and phosphorylation of ERK1/2 (Tan *et al,* 2006). Rho and Rac are indeed regulating cell cytoskeleton reorganization and cell movement upon signalling, that will permit the cells to migrate towards the gradient of chemoattractant (reviewed in (Rikitake *et al,* 2011)).

Apart from G protein signalling, CXCR4 also interacts with β-arrestin, mediating its internalization and activation of ERK1/2 (Cheng *et al,* 2000).

CXCR4 has been described to dimerize with other receptors, such as CB2. This occurred when both receptors were activated by respective agonists, CXCL12 and the synthetic selective CB2 agonist JWH-015 in breast and prostate cancers. This interaction prevented the internalization of CXCR4, thus inhibiting chemotaxis towards CXCL12 (Nasser *et al,* 2011). CXCR4 and cannabinoid receptor signalling are also interacting in T lymphocytes (Ghosh *et al,* 2006), MCL and CLL cells (Merrien et al., manuscript), affecting the chemotaxis. However, it seems that a different mechanism than in breast and prostate cancer is involved, as CXCR4 surface expression was not affected in either studies.

Due to the increased interaction with cells from the microenvironment, signalling pathways through CXCR4 are dysregulated in haematological malignancies. For instance, it was shown that lymphoma cells use the signalling downstream of CXCR4 to promote cell survival, rather than to respond by chemotaxis (O'Hayre *et al,* 2010).

1.5.4 Signalling through the cannabinoid receptors

Cannabinoid receptors are GPCRs that also signal via Gαi/o, inhibiting adenylyl cyclase (Howlett *et al,* 2002). In some circumstances, CB1 can bind to Gαs protein instead of Gαi, inducing an inverse effect on signalling pathways, mainly reported in neuronal cells (Abadji *et al,* 1999, Glass *et al,* 1997). Cannabinoid receptors have also been described to activate MAPK signalling pathway by phosphorylating ERK1/2, which is involved in cell proliferation (reviewed in (Zhang *et al,* 2002)). CB2 has been reported to recruit at the cell membrane β-arrestin, a scaffold protein involved in regulation of signalling proteins such as ERK (McGuinness *et al,* 2009). Signalling pathways activated upon ligand binding to cannabinoid receptors can vary depending on the tissue localization (reviewed in (Howlett *et al,* 2017)).

1.6 THE ENDOCANNABINOID SYSTEM

The endocannabinoid system refers to the cannabinoid receptors, their endogenous ligands (endocannabinoids) and the enzymes synthesizing and metabolizing the endocannabinoids. The role of the endocannabinoid system has been described in many organs, tissues, cell types and biological processes (reviewed in (Bukiya 2019)). In addition, the endocannabinoid system is dysregulated in diseases including solid cancers and haematological malignancies (reviewed in (Laezza *et al,* 2020, Schwarz *et al,* 2018, Wasik *et al,* 2015b, Velasco *et al,* 2015)). This section is describing the endocannabinoid system in the context of the immune system and lymphoma.

1.6.1 Cannabinoid receptors

Cannabinoid receptors were first identified as the receptors binding the main active component of *Cannabis sativa* plant, Δ^9-tetrahydrocannabinol (THC). *CNR1* gene is located in chromosome 6q16, and *CNR2* gene in chromosome 1p36. CB1 and CB2 protein sequences share approximately 44% of identity.

1.6.1.1 Cannabinoid receptor type 1

Cannabinoid receptor type 1 was discovered and described for the first time in rat central nervous system using the radiolabelled synthetic cannabinoid analogue [^{3}H]CP-55,940 (Devane *et al,* 1988). Two years later, CB1 was cloned from rat cerebral cortex (Matsuda *et al,* 1990). It was subsequently described to be expressed (mRNA and protein levels) in hippocampus, cerebral cortex and cerebellum (Herkenham *et al,* 1990, Matsuda *et al,* 1993, Tsou *et al,* 1998), and particularly in presynaptic neuron terminals (reviewed in (Howlett, *et al* 2002)). There, CB1 regulates synaptic signalling by inhibiting the neurotransmitter release upon 2-AG binding (Freund *et al,* 2003), to avoid excessive neuronal activity. The mapping of CB1 in the central nervous system helped to understand the psychoactive effects of THC.

Knock-out studies showed behavioural defects, including reduced activity and locomotion of animals, and defects in the brain development (reviewed in (Howlett, *et al* 2002)). It has also helped to identify non-CB1 and non-CB2 receptors for endocannabinoids (Di Marzo *et al*, 2000).

CB1 is expressed in other tissues like skeletal muscle, fat tissue, liver and pancreas. Its expression was also described in immune cells, although at low levels (Bouaboula, *et al* 1993, Galiegue, *et al* 1995). In T lymphocytes, *CNR1* expression can be upregulated upon stimulation with THC and the pro-inflammatory IL-4 (Borner *et al*, 2007), which indicates a role for CB1 in the immune system.

Importantly, CB1 is involved in cell migration of neuronal cells, via the activation of focal adhesion kinase (FAK) upon treatment with the CB1 synthetic agonist WIN55,212-2 (Dalton *et al*, 2013). FAK is a tyrosine kinase that is implicated in actin cytoskeleton reorganization, in response to chemokines or other stimuli for cell adhesion and migration (reviewed in (Schaller 2010)). Interestingly, FAK is upregulated in BM infiltrates and some of the LN biopsies from MCL cases, promoting cell adhesion and providing survival signals through NFκB, AKT and ERK1/2 activation (Rudelius *et al*, 2018). This suggests, together with the involvement of CB1 in chemotaxis of MCL and CLL primary cells and MCL cell lines (Merrien *et al*., manuscript), that investigating the role of CB1 and FAK in MCL would be of interest.

1.6.1.2 Cannabinoid receptor type 2

Cannabinoid receptor type 2 was discovered and cloned from a promyelocytic leukemic cell line HL60 (Munro *et al*, 1993). Two years later, CB2 was described to be expressed in peripheral and lymphoid tissues such as tonsils, spleen, and BM, and in immune cells with higher expression in B, NK cells and monocytes (Galiegue, *et al* 1995). CB2 is also expressed in neuronal cells such as microglia and astrocytes, however in much lower levels than CB1, and it has mainly been described in condition of inflammation (reviewed in (Atwood *et al*, 2010) and (Howlett and Abood 2017)).

Knock-out of CB2 in mice does not induce any strong morphological phenotype. However, deficiency in CB2 impairs osteoblast and osteoclasts balance in favour of osteoporosis (Ofek *et al*, 2006). Pereira et *al.*, demonstrated that mice deficient for CB2 had an accumulation of immature B lymphocytes in blood and a lack of that cell population in BM. This data showed the importance of CB2 expression in keeping the immature B cells in BM because of high levels of 2-AG there, in association with the integrin VLA-4 (Pereira, *et al* 2009). CB2 was also demonstrated to participate in the B cell repertoire generation and response to antigen, as CB2 lacking mice displayed rare B lymphocytes with λ light chain, and mostly κ light chain instead (Pereira, *et al* 2009). Additionally, B lymphocytes that lacked CB2 failed to produce IgM as a results of impaired homing and localization in marginal zones of the spleen, where blood antigens are carried and scanned by immune cells (Basu *et al*, 2011, Muppidi *et al*, 2011).

In B leukaemia cells, *CNR2* expression was associated with regulation of glucose uptake in response to the transcription factor *PAX5*, which was defined as a tumour-suppressor in acute lymphocytic leukaemia (Chan *et al*, 2017). Chan *et al*., reported that *CNR2* inhibits glucose uptake, which might confer a negative impact on the malignancy development, as glucose is a source of energy for cancer cell transformation (Chan, *et al* 2017).

Both cannabinoid receptors play a role in the immune response, as double knock-out of CB1 and CB2 receptors in mice induced chronic inflammation and increased the response to influenza infection, due to higher capability of antigen presentation by dendritic cells, which lead to increased T cells activity and increased pro-inflammatory IL-17 and interferon gamma cytokines production ((Karmaus *et al*, 2011), reviewed in (Kaplan 2013)). In line with that, the expression of both cannabinoid receptors genes is increased in T lymphocytes upon stimulation with pro-inflammatory cytokines such as the tumour necrosis factor (TNF)-α (Jean-Gilles *et al*, 2015), emphasizing the role of cannabinoid receptors in the balance of the immune response.

1.6.2 Ligands to cannabinoid receptors

There are at least 15 endogenous cannabinoids identified (reviewed in (Pertwee 2015)). 2-arachidonoylglycerol (2-AG) and anandamide are the two most abundant endocannabinoids, therefore the most studied ones. They are active lipids (eicosanoids) synthesized "on demand" by neuronal cells as well as immune cells from precursor lipids (arachidonic acid) present in the cytoplasmic membrane.

Phytocannabinoids are cannabinoids that are contained in the *Cannabis sativa* plant. Almost 500 compounds have been extracted from the *Cannabis sativa* plant, and around 100 are considered as cannabinoids (reviewed in (Andre *et al*, 2016, Flores-Sanchez *et al*, 2008)).

In addition, synthetic cannabinoids have been developed for the investigation of cannabinoid receptors structure and function, and for therapeutic use.

1.6.2.1 2-arachidonoylglycerol

The endocannabinoid 2-AG is the most abundant endocannabinoid in the brain and is considered as a full agonist to both CB1 and CB2. 2-AG is also secreted in other tissues, mediating numerous responses upon binding to its receptors (reviewed in (Sugiura *et al*, 2006)). One of the effects of 2-AG is the induction of chemotaxis, and importantly chemotaxis of immune cells *in vitro* and *ex-vivo*, such as NK cells (Kishimoto *et al*, 2005), monocytes and macrophages (Kishimoto *et al*, 2003), and eosinophils (but not neutrophils; (Kishimoto *et al*, 2006, Larose *et al*, 2014, Oka *et al*, 2004)) from peripheral blood, of the B lymphoblastic cell line Raji (Rayman *et al*, 2004), the leukemic T cell line Jurkat (Gasperi *et al*, 2014), as well as CD34+ hematopoietic stem cells (Köse *et al*, 2018) and BM MSCs (Rossi *et al*, 2013).

Most of the chemotactic effects have been reported to be mediated via CB2 (summarized in Table 2), however, in most studies, the cells did not express or expressed low levels of CB1. In our study, we also show that 2-AG induces chemotaxis of primary lymphoma cells (MCL and CLL) and MCL cell lines (Merrien *et al.*, manuscript). In the case of lymphoma cells, both receptors are expressed at higher levels than in normal B lymphocytes, and they both contribute to the 2-AG mediated chemotaxis.

1.6.2.2 Delta-9-tetrahydrocannabinol

Delta-9-tetrahydrocannabinol (THC) is the main active and abundant component of *Cannabis sativa*, responsible for the psychoactive effects of the plant after ingestion, by binding to CB1 present in the neuronal cells.

THC is also a ligand for CB2, in which it acts as a weak agonist. Nevertheless, in some circumstances, THC can also act as an antagonist to CB2 as THC was shown to inhibit the chemotaxis mediated by 2-AG in NK cells (Kishimoto, *et al* 2005).

Furthermore, THC induces cell death in different cancer types *in vitro* and in xenografts models, including gliomas cell lines (Galve-Roperh *et al*, 2000), melanoma (Armstrong *et al*, 2015), leukaemia cells (Herrera *et al*, 2005, Kampa-Schittenhelm *et al*, 2016, McKallip *et al*, 2002, Powles *et al*, 2005). Cell cycle progression of breast cancer cells was also described to be repressed by THC (Caffarel *et al*, 2006). Importantly, these effects were obtained at micromolar concentrations, but not at lower (nanomolar) concentrations. The effects of THC were mediated by CB1, or CB2, or the combination of CB1 and CB2, depending on the cell type (summarized in Table 2).

In vitro, THC has an immunosuppressive role by inhibiting mitogen-stimulated lymphocyte replication, T-cell proliferation and cytokine production (reviewed in (Klein 2005), (Borner, *et al* 2007, Yuan *et al*, 2002)).

1.6.2.3 Cannabidiol

Cannabidiol (CBD) is a non-psychoactive component of *Cannabis sativa* plant. When combined with THC, it suppresses the psychoactive effects of THC by acting as an antagonist to CB1 (Klein *et al*, 2011, Thomas *et al*, 2007). However, the effects of CBD on CB2 are not fully understood, as some reports describe it as a partial agonism, as well as a negative allosteric and/or orthosteric modulator (reviewed in (Pertwee 2008), (Martinez-Pinilla *et al*, 2017, Tham *et al*, 2019)).

CBD has been reported to modulate the inflammatory response by reducing the pro-inflammatory cytokines such as IL-17, IL-1β or IL-6, increasing the anti-inflammatory ones like IL-10 ((Khuja *et al,* 2019, Kozela *et al,* 2013), reviewed in (Peyravian *et al,* 2020)) and inhibiting the chemotaxis of some immune cells like neutrophils (McHugh *et al,* 2008). The combination of CBD to THC increased the cell death induced by THC alone in melanoma cells *in vitro* and enhanced the THC effect on reducing tumour size in xenograft melanoma model (Armstrong, *et al* 2015).

1.6.2.4 Synthetic cannabinoids

Synthetic cannabinoids have been described to induce cell death. For instance, the synthetic cannabinoids WIN55,212-2 and R-methanandamide, both agonists to CB1 and CB2, generate accumulation of ceramide and induction of apoptosis via the activation of p38 pathway in MCL primary cells, xenograft model and cell lines (Flygare *et al,* 2005, Gustafsson *et al,* 2006, Gustafsson, *et al* 2008). It was also depicted that WIN55,212-2 induces cell death via cytoplasmic vacuolation in apoptosis-resistant MCL cell lines (Wasik *et al,* 2011).

Table 2. Effects of 2-AG, THC, CBD and WIN55,212-2 in healthy and malignant cells of the immune system.

	cannabinoid type	effect	concentration	cell type	receptor mediated	reference
2-AG	endocannabinoid	increased chemotaxis	1µM	NK cells	CB2	Kishimoto et al., 2005
			0.1-3µM	HL-60 promyelocytic leukaemia cell line + differentiated into macrophage	CB1 and CB2	Kishimoto et al., 2003
			0.1-10µM	human eosinophils from PB	CB2	Kishimoto et al., 2006
			0.1-3µM	human eosinophils	CB1 and CB2	Larose et al., 2014
			0.01-10µM	human eosinophilic leukemic EoL-1 cell line	CB2	Oka et al., 2004
			1µM	human eosinophils from PB	CB2	
			0.3µM	B lymphoblastic cell line Raji	CB2	Rayman et al., 2004
			0.3µM, 1µ, 50µM	human CD34+ hematopoietic stem cells from BM	CB1 and CB2	Kose et al., 2018
			10µM	human mesenchymal stromal cells from BM	CB2	Rossi et al., 2014
			100nM	MCL cell lines (JeKo-1, Granta519)	CB1 and CB2	Merrien et al., manuscript
		increased chemotaxis towards HUVEC cells pre-treated with 2-AG	1µM	Jurkat T cell line	CB1 and CB2	Gasperi et al., 2014
THC	phytocannabinoid	inhibition of 2-AG (1µM) mediated chemotaxis	1µM	NK cells	-	Kishimoto et al., 2005
		cell death by apoptosis	1.5µM	Jurkat T cell line	CB2	Herrera et al., 2005
		cell death by apoptosis	30-75µM	Jurkat, acute monocytic leukaemia (MOLM13) cell lines	CB1 and CB2	Kampa-Schitternhelm et al., 2016
		reduction of proliferation	10-80µM		-	
		reduction of cell viability	50µM	acute myeloid and acute lymphoblastic leukaemia primary samples	sensitive samples with higher CB1 and CB2 levels	

	cannabinoid type	effect	concentration	cell type	receptor mediated	reference
		cell death by apoptosis	5μM	T-leukaemia cell lines (Jurkat, Molt-4, U251)	CB2	McKallip et al., 2002
				acute lymphoblastic leukaemia primary samples	-	
		reduction of tumour size	5mg/kg	murine lymphoma EL-4 cells in mice	-	
		reduction of cell viability	50μM	leukemic cell lines (CEM, HEL-92, HL60 and MOLT-4)	CB2	Powles et al., 2005
		reduction of cell viability	2.5μM, 12.5μM	Jurkat T cell line	-	Borner et al., 2007
		increased CB1 mRNA expression	0.1-0.5μM		CB2	
		reduction of cell proliferation	2.5μM, 5μM	T cells activated by dendritic cells (from same PB donor)	-	Yuan et al., 2002
		alteration of cytokine release (increase of IL-4 and IL-5, decrease of IL-2 and IFN-gamma)	5μM		CB2	
CBD	phytocannabinoid	reduction of cell proliferation	10μM	CD3 activated mouse splenocytes	-	Khuja et al., 2019
		alteration of cytokine mRNA expression (increase of IL-10, IL-5 and TNFalpha, decrease of IL-17a)	3μM		-	
		reduction of IL-17 cytokine	1-5μM	mouse T cells from LN cocultured with dendritic cells	CB1 and CB2 independent	Kozela et al., 2013
		inhibition of fMLP (potent chemoattractant)-induced chemotaxis	0.01-1μM	human neutrophils from PB	-	McHugh et al., 2007
WIN55,212-2	synthetic	reduction of cell viability	5μM	primary MCL cells	-	Flygare et al., 2005
		cell death by apoptosis	10μM	MCL cell lines (Rec-1, JeKo-1, JVM-2), 3 primary MCL cells (from tonsil, LN and PB)	CB1 and CB2	Gustafsson et al., 2006
		cell death by cytoplasmic vacuolation	10μM	MCL cell line Granta519	CB1 and CB2 independent	Wasik et al., 2011

Abbreviations: BM, bone marrow; HUVEC, human umbilical vein endothelial cells; LN, lymph node; PB, peripheral blood. "-" means data not available.

1.6.3 Enzymes

Endocannabinoid synthesis is in general dependent on Ca^{2+} influx, induced by neuronal cell depolarization signalling and/or via the activation of Gαq protein (reviewed in (Zou *et al*, 2018)). 2-AG and anandamide are synthesised by several types of reactions (hydrolysis, cleavage or dephosphorylation) from arachidonic-acid phospholipids. The enzyme *N*-acyl-phosphatidyl-ethanolamine phospholipase D (NAPE-PLD) is one of the enzymes responsible for the synthesis of anandamide and 1,2-diacylglycerol (DAG) lipases for 2-AG synthesis. Metabolism of anandamide into arachidonic acids and ethanolamine is mainly regulated by fatty acid amide hydrolase (FAAH). 2-AG can also be metabolised by FAAH, but monoacylglycerol lipase (MAGL) is the major enzyme contributing to its hydrolysis into arachidonic acid and glycerol (Figure 7). Both endocannabinoids can be metabolised by cyclooxygenase-2 into anandamide/2-AG-derived prostaglandins, thus playing a role in inflammation (Jhaveri *et al*, 2008).

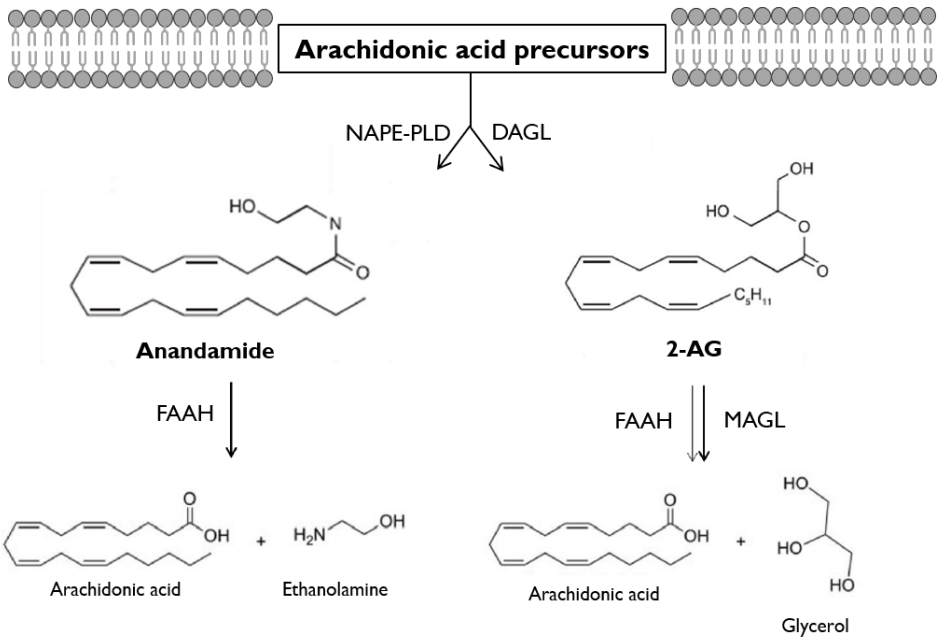

Figure 7. Scheme depicting synthesis and degradation of anandamide and 2-AG.

In MCL, it was shown by our group that NAPE-PLD and FAAH enzymes are dysregulated (Wasik, *et al* 2014). Indeed, NAPE-PLD was found upregulated in all the samples analysed, and the degrading enzyme FAAH was at low levels in almost all samples from the study; all compared to non-malignant B cells. Together with the upregulation of CB1 and CB2 mentioned earlier, this data indicates an accumulation of endocannabinoids, and increased signalling through the receptors, in MCL cells (Figure 8).

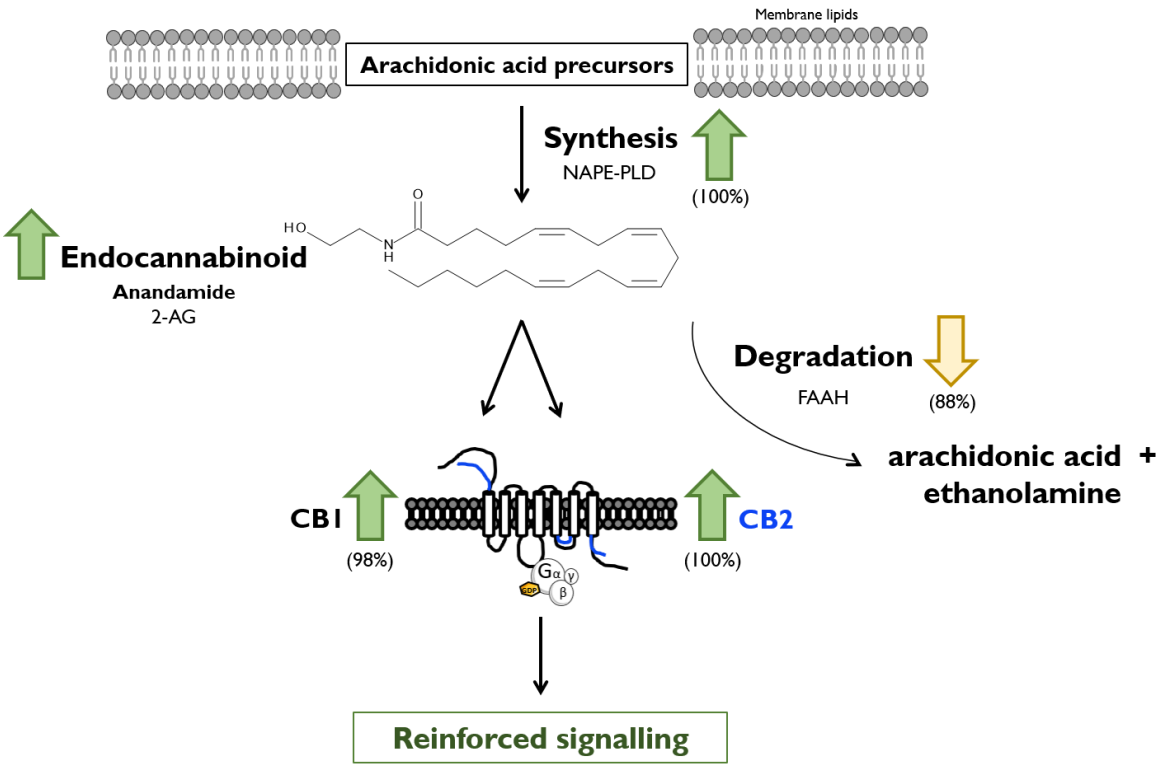

Figure 8. Scheme showing current knowledge on the dysregulated endocannabinoid system in MCL (scheme from Wasik, A.M. & Sander, B. (2015b) Cannabinoid receptors in mantle cell lymphoma. *Cell cycle*, **14,** 291-292.).

1.6.4 Other receptors binding cannabinoids

Apart from CB1 and CB2, cannabinoids can also interact with other receptors, like G protein receptor 55 (GPR55), GPR18, GPR119, and the peroxisome proliferator-activated receptors (PPAR) family (reviewed in (Pertwee 2015)).

GPR55 has been described to promote cancer progression in some malignancies (reviewed in (Morales *et al,* 2016)), to heterodimerize with CB2, and to play a role in the migration of neutrophils (Balenga *et al,* 2014). Expression of GPR55 was reported in immune cells such as NK cells and monocytes (Chiurchiu *et al,* 2015), as well as in Hodgkin lymphoma and the non-Hodgkin lymphoma cell line Karpas 422 (Benz *et al,* 2013), but it has not yet been described in other haematological malignancies. While CBD is an antagonist to GPR55, the effects of THC on the receptor and signalling activation are conflicting (reviewed in (Morales *et al,* 2017, Pertwee *et al,* 2010)).

GPR18 is expressed in lymphoid tissues and lymphocytes (Gantz *et al,* 1997, Kohno *et al,* 2006) and its expression was identified in MCL, without any defined role in the pathobiology (Henson *et al,* 2011, Piccaluga *et al,* 2019).

Cannabidiol and THC can also bind to ion channels, such as voltage-gated sodium and potassium channels, and the transient receptor potential vanilloid type-1 receptor (TRPV1) (Peyravian, *et al* 2020, Sait *et al,* 2020), which are involved in cell proliferation, survival and migration in T and B leukaemia cells (Arcangeli *et al,* 2012, Punzo *et al,* 2018).

Voltage-gated potassium (Kv) channels are especially of interest, since they are important for B and T lymphocytes proliferation and activation, for the immunological synapse during antigen presentation, as well as for the cell migration (reviewed in (Comes *et al,* 2015)). Kv1.3 and Kv1.5 are particularly expressed in lymphocytes, and in B-cell malignancies such as MCL (Vallejo-Gracia *et al,* 2013).

1.7 MEDICAL USE OF CANNABINOIDS

Cannabinoids from *Cannabis sativa* plant have been used in medicine for centuries (Li 1974, Long *et al,* 2017). It is consumed by up to 25 percent of cancer patients to alleviate symptoms and side effects from chemotherapy (Macari *et al,* 2020, Martell *et al,* 2018, Pergam *et al,* 2017), as well as used as a recreational drug.

The use of phytocannabinoids in clinics is controversial due to the psychoactive effects that the *Cannabis sativa* active component THC induces, and the dependence that can be developed (reviewed in (Černe 2020)). The immunosuppressive effects of THC and CBD might also induce progression of certain cancers instead of regression (Bar-Sela *et al,* 2020, McKallip *et al,* 2005). Furthermore, in CLL, a single dose of the combination of THC and CBD appears to induce a redistribution of malignant cells within the tissues without inducing apoptosis, which

might be in favour of cell survival instead of anti-tumoral treatment (Melén, Merrien *et al*., manuscript).

However, cannabinoid treatment has shown positive effects as therapy for multiple sclerosis to reduce neuropathic pain and spasms ((Barnes 2006), reviewed in (Nielsen *et al,* 2018)). CBD alone is approved by the Food and Drug Agency for treatment of certain types of epilepsy (reviewed in (Devinsky *et al,* 2014)). In addition, several studies are conducted in brain tumours, showing the improved effects of alkylating agents by combining with the phytocannabinoids THC and CBD (López-Valero *et al,* 2018).

The endocannabinoid system is in general a very complex system, that acts in a variety of cell types with different responses to stimuli, being context dependent (reviewed in (Joshi *et al,* 2019)). In addition, the endocannabinoids are biolipids released under different conditions such as stress (Dlugos *et al,* 2012), and they can bind to many different receptors, inducing several signalling pathways related to cell migration, inflammation, glucose metabolism or cell survival (Alhouayek *et al,* 2014, Nogueiras *et al,* 2009).

In cancer, the endocannabinoid system is also differently regulated in different cancer types (reviewed in (Das *et al,* 2019)). When using the online data set available for different cancer types (kmplot.com; (Nagy *et al,* 2018)), the expression of cannabinoid receptors or enzymes predicts different outcomes in different cancer types. These features make it difficult to predict whether the use of cannabinoids for cancer therapy will be beneficial or harmful.

2 RESEARCH AIMS

2.1 OVERALL AIM

The overall aim of this thesis was to decipher the role of dysregulated proteins and signalling in the pathobiology of two B cell malignancies, chronic lymphocytic leukaemia (CLL) and mantle cell lymphoma (MCL). The focus was on the possible implication of these dysregulated mechanisms in biological processes of lymphoma including disease presentation, interaction with the tissue microenvironment and chemotaxis.

2.2 SPECIFIC AIMS

Paper I investigated the expression pattern of GPCRs and G proteins in MCL in order to provide information on the global expression pattern as compared to non-malignant lymph nodes. We describe that G protein alpha z is overexpressed in MCL and we further investigated its role in MCL pathobiology.

Paper II aimed at increasing the understanding of factors of importance for interaction of lymphoma cells with stromal cells. We analysed changes in gene expression when MCL cell lines from different tissue origin adhered to stromal cells in a co-culture system.

Paper III focused on the effects of the endocannabinoid 2-arachidonoylglycerol on lymphoma chemotaxis, alone and in combination with the chemokine CXCL12, and therefore its potential role in retention of MCL and CLL cells in tissues.

Paper IV describes the effects of giving patients with indolent B cell lymphoma/leukaemia a combination of THC/CBD. Dosing, vital parameters and patient experienced side effects were investigated as well as effects on lymphoma cells and on non-malignant cells in blood.

3 ASPECTS ON THE METHODOLOGIES USED

The studies included in this thesis were performed using several techniques, for which the material and methods are described in detail in each papers and manuscripts. This section is to highlight some important aspects.

3.1 ETHICAL CONSIDERATIONS

All the studies included in this thesis were performed in compliance with the Declaration of Helsinki.

In **Paper I**, **Paper III** and **Paper IV**, patient samples were used. Patient samples were obtained after informed consent signed from patients. Patients can agree to allow the use of left-over material from diagnosis (paraffin embedded tissue, frozen sample or viable cells). They can also agree to donate an additional sample for research. It can be blood, tonsil or faeces. Patients also agree that clinical parameters of importance for lymphoma disease can be retrieved from hospital records and used for research purpose. In addition, patients are informed that the information obtained from the studies is saved in a protected file at the Pathology department. Patients can also refuse to provide material for research, without giving any explanation and without risking that the refusal affects their treatment or relation with the medical care. The files in which patients' information are stored are made anonymous to us, as researchers. The master data base and the biobank data can be searched by the clinical doctors only.

The written informed consent has been agreed by the ethical committee and comprises the name of the clinical doctor in charge. It includes the agreement of investigating genes, proteins, and functional studies of living tumour cells, tumour tissues and living non-tumour cells. Additionally, research samples have to be given back whenever the clinics needs it for diagnosis, clinical diagnosis always goes first to ensure the best care for the patient.

It is very important that patients are well informed about the possibilities they have regarding their samples. Personuppgiftslagen (PUL) makes it possible for the patients to demand that all information is erased and that samples from biobank and files are destroyed, without having to provide any explanation. Patients obtain the information of whom to contact in such case.

Regarding the clinical trial that we conducted, patients were also very well informed before being included in the study. The patients were followed during the day of the trial, monitored and supervised by the doctor and nurses that reported anything unusual, to ensure the well-being of the patient. Patients could at any time contact the doctors and the nurses responsible for the study.

3.2 CELL LINES AND PATIENTS MATERIAL

3.2.1 MCL cell lines and stromal cells

In three out of four papers, experiments were designed using MCL cell lines. Cell lines are a simplified model for deciphering mechanisms. It has the benefit of being easy to culture and allowing the acquisition of rather reproducible results. We could then, investigate specific roles of proteins (Gαz for **Paper I** and CBs for **Paper III**) and signalling pathways (**Paper II** and **Paper III**) in a "clean" system.

In our lab we have a set of seven MCL cell lines to choose from, which permitted to see variability between them, according to their origin or growth pattern for instance. We chose to work especially with JeKo-1, Granta519, Rec-1 and JVM-2 cell lines. They were all cultured in a similar way (RPMI-GlutaMAX medium, supplemented with 50mg/ml gentamicin and 10% foetal bovine serum (FBS)).

However, the major disadvantage of the monoculture *in vitro* system is that it doesn't represent the *in vivo* settings such as the complex microenvironment that surrounds malignant cells. Moreover, the cells in culture proliferate independently from external signals. The co-culture system in **Paper II** used to investigate the adhesion, is a more complex system and more relevant. We could indeed compare the gene expression data of adherent MCL cells to MSCs to available data from primary MCL cells from LN and PB.

Stromal cells used in **Paper II** were mouse stromal cells. We used it to differentiate between MCL cells and stromal cells regarding the gene expression analysis without having to separate them after adhesion assay. This way, we could distinguish between human (MCL cell lines) and mouse (stromal cells) genome, while reducing the number of sample processing steps which could increase changes in cell genome (as well as epigenome, proteomics, phospho-proteomics).

3.2.2 Patient material

Patients' material was available for us to include in our studies for *ex vivo* experiments (**Paper III** and **Paper IV**), which is the strength of this thesis. *In vivo* data were also collected during the clinical trial (**Paper IV**), which gave immediate insight into the effects of THC/CBD.

This comes with the fact that this work would never have been possible without the close collaboration that we have with clinicians and research nurses to recruit and collect the samples, as well as the routine flow cytometry personal that are providing us with the left-over material from diagnostic samples.

In **Paper III** and **Paper IV**, mostly CLL could be collected and a few MCL samples only, due to the prevalence of both diseases. CLL is a more common disease than MCL. Moreover, at diagnosis, MCL requires more often BM biopsy rather than blood sample. Furthermore, many MCL patients are treated at diagnosis while for CLL, many patients are undergoing watchful waiting. This also makes it more difficult to recruit untreated MCL patients to the studies.

3.3 B CELLS ENRICHMENT FROM PATIENT BLOOD SAMPLES

Patients samples were collected, and only PB was used in the thesis. B cells (mostly malignant cells) were enriched using a cocktail of antibodies (RosetteSep®) targeting platelets and leukocytes except B cells. The antibodies bound to the different cells form rosettes with erythrocytes. Using the density gradient medium Ficoll-Paque and centrifugation for separation of different fractions, only untouched B cells were collected from the interphase layer of cells.

The efficiency of the antibody cocktail was assessed by flow cytometry analysis. We included CD5 and CD19 markers to quantify and control that the fraction was not contaminated by T cells (CD19-). It was important to exclude the presence of T lymphocytes as they express similar chemokine receptors as B lymphocytes (CXCR4, CB2 for instance) and have a higher chemotaxis capacity compared to B lymphocytes, MCL cell lines, and especially compared to CLL cells that lose their CXCR4-mediated chemotaxis in the profit of survival signalling (O'Hayre, *et al* 2010).

In addition, we realized that sometimes a large double negative population was present in the sample, which was due to erythrocytes that might have aggregated and been collected in the interphase layer together with B cells. For this reason, anti-CD45 antibody (CD45 as marker of leukocytes) was later added and used for the first gating.

MCL and CLL primary cells for **Paper III** were frozen and thawed for chemotaxis assay. Importantly, after the cells were thawed, they were kept for one hour in culture media supplemented by 10% FBS, at 37°C in order for the cells to recover from thawing, but not longer time as phosphorylation status could have changed.

3.4 GENE SILENCING USING SIRNA AND ELECTROPORATION METHOD

To knock-down genes, we used siRNA method which consists of small RNA sequence complementary to the gene of interest, which upon recognition will induce degradation of the double-stranded RNA. Subsequently, protein levels should be reduced after RNA degradation. Predesigned siRNA was incorporated using electroporation device AMAXA, using the program X-01. For *CNR2* gene, two different siRNAs were used in order to maximize the downregulation efficiency.

To verify that the genes were downregulated, we performed quantitative PCR (RT-qPCR). However, we noticed that *CNR2* gene was still expressed, and after checking the literature we found out that these signals could be false-positive coming from binding of the qPCR primers to the region that was not degraded upon siRNA incorporation (Herbert *et al,* 2011). We thus designed new primers targeting a different region, which allowed us to see a downregulation.

3.5 PROTEIN EXPRESSION

3.5.1 Western blotting

Fresh protein lysates or maximum one-time thawing were used to investigate phosphorylation of different signalling pathways proteins, because phosphorylation might be affected by the fact of thawing and it would be difficult to detect any signal after more thawing.

In addition, phospho-protein and total protein specific antibodies were added on two membranes, from two gels that were loaded and ran in parallel. This technical detail was implemented because the antibody that recognized phospho-sites blocked the sites for the antibody that should recognize the total-protein. GAPDH was used for loading control and normalization for quantification.

Unfortunately, it was not possible to assess the protein levels for cannabinoid receptors as the currently commercially available antibodies are not specific when used in lymphoma cells, probably because of cross-interaction, as both CB1 and CB2 receptors are at high levels in those cells. In contrast to normal B lymphocyte (low CB1/high CB2) or brain samples (high CB1/low CB2). The antibodies we had in our possession did not give specific bands on Western blot. Other groups have reported the same issue (Freund, *et al* 2016, Grimsey *et al*, 2008, Marchalant *et al*, 2014).

3.5.2 Flow cytometry analysis

Flow cytometry analysis was used to measure cell surface protein expression, to distinguish between different cell populations. We also used it to assess the internalization and recycling of CXCR4 chemokine receptor. Analysis was performed using FlowJo (v10).

3.6 CHEMOTAXIS AND ADHESION ASSAYS

3.6.1 Cells staining

For chemotaxis assays in **Paper I**, **Paper II** and **Paper III**, the cells were stained with calcein-AM. We checked that the dye was not released by the cells in the culture media, which would increase the fluorescence without being an indication of migrated cells (Beem *et al*, 2013).

Carboxyfluorescein succinimidyl ester (CFSE) was used in **Paper II** for adhesion experiments, rather than calcein-AM, as calcein-AM would affect adhesion of the cells.

3.6.2 Boyden chamber assay for chemotaxis assessment

We used a Boyden chamber assay for assessing the chemotaxis of both MCL cell lines and MCL/CLL primary cells. Two pore sizes were used, 8μm for the cell lines, and because primary cells are smaller cells, we had to use a smaller pore size of 5μm.

3.6.3 Co-culture for adhesion assay

Mouse stromal cells were seeded 24 hours prior to the addition of MCL suspension cell line, so they would reach approximately 70% confluence for the experiment.

3.6.4 Read-out

For the chemotaxis assay, two different read-outs were used. For JeKo-1 and primary MCL and CLL cells, images of the migrated cells in the bottom well (in focus) were captured every 30min and fluorescent cells were then counted using NIS-Elements AR software (see Figure 9 below).

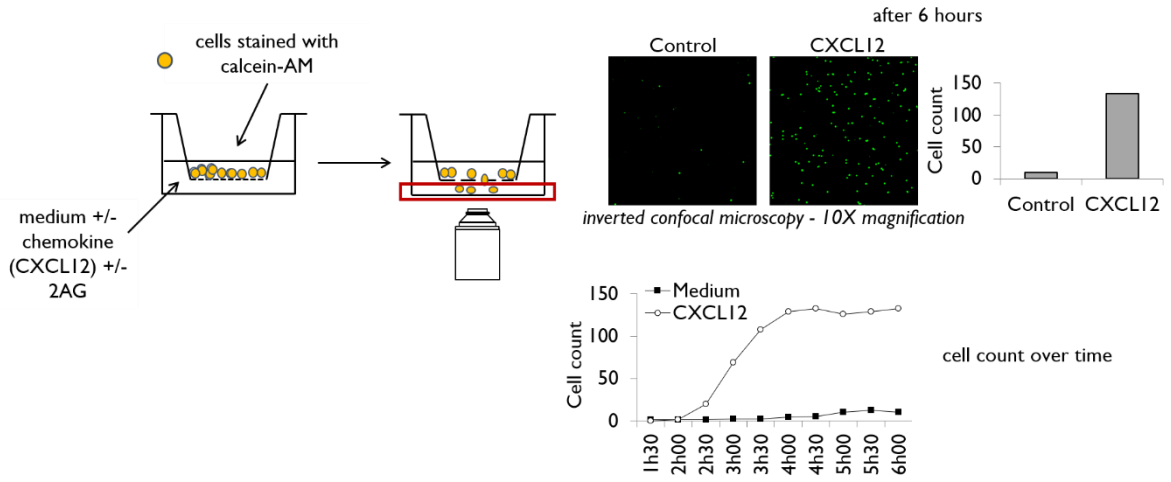

Figure 9. Graphic representation of microscopy read out for chemotaxis assay and an example of chemotaxis performed with JeKo-1 cell line towards CXCL12 (200ng/ml).

Granta519 and JVM-2 are cells that grow in aggregates (cell clumps), which makes evaluation of chemotaxis by microscopy impossible. For these cells, we therefore used the fluorescence intensity measurement at one time point (4 hour), including for each experiment a standard curve to validate that the fluorescence intensity value would represent the number of cells. Fluorescence intensity read-out could also be used for JeKo-1 cell line.

The co-cultured adherent MCL cells were quantified using flow cytometry (CFSE labelled), without the need of separating them from the stromal cells that were not stained.

3.7 BIOINFORMATIC TOOLS

Statistical analysis was mainly achieved with Origin Pro8 for Windows (**Paper I**), GraphPad Prism 8 for Windows (**Paper III**) and Stata 9.2 (**Paper I**) and Stata version 14.2 for **Paper IV**.

Survival was analysed with Kaplan-Meier curves. Principle component analysis in **Paper I** were performed with R and "prcomp" function of its package.

Significance was set with $p<0.05$.

4 RESULTS AND DISCUSSION

4.1 PAPER I

The aim of **Paper I** was to investigate the expression pattern of GPCRs and G proteins in mantle cell lymphoma, as GPCRs are involved in pathways controlling, among others, cell survival, proliferation and migration. Furthermore, GPCRs constitute the most druggable protein family.

4.1.1 Results

Using gene expression data from 17 MCL biopsies and 8 reactive LNs as a comparison, we could identify 29/475 probe sets, representing 26 genes encoding for GPCRs and G proteins, which were differentially expressed in MCL compared to LNs. From the genes encoding for G proteins (18 genes represented by 19 probe sets), *GNAZ* was the most significantly changed in the data set (adjusted p=0.002), being upregulated in 16/17 cases.

We confirmed the over-expression of *GNAZ* by qPCR in a validation cohort of 108 samples and in sorted B cells from 18 MCL cases by qPCR, compared with B cells from reactive LNs.

Furthermore, *GNAZ* mRNA expression predicted overall survival as a continuous variable (Cox regression, p=0.014). Detailed clinical and pathological parameters were available for 77 patients from this cohort allowing for a comprehensive analysis. Among patients that did not undergo autologous stem cell transplantation (55/75), *GNAZ* also predicted worse overall survival (p=0.033). When performing a multivariate analysis of *GNAZ* expression with clinical and pathological parameters such as autologous stem cell transplantation, Eastern Cooperative Oncology Group performance =>2, >4 nodal presentation sites, anaemia, and blastoid morphology, *GNAZ* did not correlate anymore with overall survival.

However, *GNAZ* expression correlated with lymphocytosis (>5x10^9 lymphocytes per litter of blood) (p=0.011), and it moderately inversely correlated with expression of gene encoding cannabinoid receptor 1 *CNR1* (Spearman correlation coefficient = -0.31; p=0.006).

We investigated the potential role of *GNAZ* in MCL pathobiology, first by verifying by Western blot that the gene *GNAZ* translates to the G protein alpha z (Gαz) in MCL cell lines. The protein expression pattern corresponded to the mRNA levels.

We showed that transient downregulation of *GNAZ* by siRNA in MCL cell lines did not affect survival or proliferation. We then assessed whether Gαz participates in chemotaxis of MCL cell lines. However, we could not see any effect of *GNAZ* downregulation on chemotaxis mediated by FBS, the homing chemokines CXCL12 and CXCL13, or the biolipid S1P involved in egress of MCL into the bloodstream.

4.1.2 Discussion

In this article we mapped the gene expression pattern of GPCRs and G-proteins. We identified *GNAZ*, encoding Gαz, as the most significantly overexpressed gene among G-proteins, compared to reactive LNs and showed that high *GNAZ* expression correlates to shorter OS in MCL patients. This was true also for MCL patients who did not undergo autologous stem cell transplantation, suggesting that poor prognosis of high *GNAZ*-expressors is not due to that type of therapy, but probably to MCL pathobiology.

Furthermore, high *GNAZ* expression correlated to lymphocytosis in MCL patients suggesting that Gαz could potentially participate in the localization of MCL cells in tissues or egress of the malignant cells.

We then confirmed that *GNAZ* translates to Gαz protein in MCL cell line Granta519 and our results showed that Gαz participates in neither proliferation nor chemotaxis in this *in vitro* model system. Although Gαz is part of the Gαi family, which mediates signalling for cell migration, also seen in NK cells, it shows that Gαz has a singular role, yet unknown. The previous findings from the group demonstrated that *CNR1* inversely correlates to lymphocytosis. In this study, we showed that *GNAZ* correlates to lymphocytosis and inversely correlates to *CNR1* in the MCL cohort encouraging more studies in this area.

4.2 PAPER II

This study aimed at describing the differences in gene expression of MCL cell lines from different tissue origin upon adhesion to stromal cells, using a co-culture system that reflects some important aspects of the interactions with the microenvironment *in vivo*.

4.2.1 Results

Two MCL cell lines, JeKo-1 and Rec-1 were subjected to adhesion assay to BM-derived mesenchymal stromal cells. While JeKo-1 showed an increased adhesion over time, Rec-1 displayed a plateau very early. The two patterns of adhesion were confirmed by differential gene expression profiling. We identified 590 genes that were differentially regulated upon adhesion to stromal cells between the two cell lines. Several of these genes were adhesion molecules such as ICAM-1, and the chemokine receptor CXCR4. The BCR and NFκB downstream signalling molecules were also differentially expressed.

The BCR and NFκB gene signatures were in fact increased in JeKo-1 upon adhesion, while unchanged in Rec-1. Rec-1 expressed a functional BCR but was not engaged during the adhesion with the stromal cells, seen by a lack of CCL3 and CCL4 secretion, and a lack of response upon downregulation/inhibition of BTK prior to adhesion assay.

We also measured the CXCR4 surface expression in both cell lines. JeKo-1 expressed higher levels of CXCR4 compared to Rec-1, and this receptor was essential for JeKo-1 adhesion and chemotaxis but not for Rec-1. Our results also showed that JeKo-1 uses the same steps for adhesion by CXCR4 and BCR.

Next, we focused on adhesion related genes that were differentially regulated between the cell lines and we investigated the role of ICAM-1 and S1PR1 in the adhesion of JeKo-1 and Rec-1. It appeared that ICAM-1 is upregulated in non-adherent Rec-1 and it is expressed at lower levels in JeKo-1, but upon adhesion, the two cell lines express similar levels. Downregulation of ICAM-1 reduced both JeKo-1 and Rec-1 adhesion. Downregulation of the egress receptor S1PR1 increased the adhesion of Rec-1 to the level of JeKo-1 cells.

4.2.2 Discussion

In MCL, alternative treatments are to target molecules that are participating in the communication between the malignant cells and the non-malignant cells from the microenvironment, in order to disrupt the pro-survival and pro-proliferative signalling that it confers. BTK and CXCR4 inhibitors are for instance used and induce the rapid release of cells from lymphoid tissues. However, some patients are refractory to these therapies.

Here, we show evidence that a co-culture system using mesenchymal stromal cells, could be used to test the resistance to therapy before the treatment. Indeed, while JeKo-1 uses the BCR and CXCR4 downstream signalling for adhesion to and/or chemotaxis towards mesenchymal stromal cells, Rec-1 does not. This represents the variability between the patients and responses to treatments, with some malignant cells being adapted and using non-BCR-mediated survival signalling strategies.

In addition, the egress receptor S1PR1 that is highly expressed in Rec-1 might be the reason for the reduced adhesion as its expression facilitates the mobilization from tissue to the blood circulation.

The main difference between the two cell lines is their origin: JeKo-1 is from peripheral blood origin and Rec-1 from LN origin. Cells that are found in blood are expressing higher CXCR4 than the ones in LN, and CXCR4 is usually downregulated when S1PR1 is expressed. Additionally, gene expression associated with cell adhesion from the MCL cell lines clustered with gene expression from MCL primary cells from different origin, which increases the relevance of this study.

4.3 PAPER III

The aim of **Paper III** was to investigate the effects of the endocannabinoid 2-arachidonoylglycerol (2-AG) on lymphoma chemotaxis, alone and in combination with the chemokine CXCL12, and therefore its potential role in retention of MCL and CLL cells in tissues. We hypothesised that 2-AG is involved in the chemotaxis of lymphoma cells because i) most cases of MCL and CLL overexpress cannabinoid receptors, ii) 2-AG is produced by stromal cells that are present in the microenvironment of lymphoma cells *in vivo* settings.

4.3.1 Results

Five MCL and nineteen CLL samples were collected. All samples expressed *CNR2*, and 22/24 expressed *CNR1*. Comparison between MCL and CLL showed that MCL express higher *CNR1*, and CLL had higher levels of *CNR2* than MCL.

We subjected the primary cells to chemotaxis assay towards 2-AG. Three out of five MCL samples and 17/19 CLL samples had an increased chemotaxis towards 2-AG compared with medium alone. We subjected the primary cells to chemotaxis towards the chemokine CXCL12 which is in high levels in LNs, and the combination of CXCL12 and 2-AG. In CLL, the combination enhanced the chemotaxis compared with CXCL12 alone (median of 1.3-fold increase, $p<0.001$). They were too few MCL samples for statistical analysis, however three out of five MCL had a reduced chemotaxis compared with CXCL12 alone (up to 50% reduction).

We used three MCL cell lines, Granta519, JeKo-1 and JVM-2 to investigate the mechanism of the observed phenomenon. Granta519 and JeKo-1 migrated towards 2-AG, but not JVM-2. In JeKo-1, the 2-AG mediated chemotaxis was not significantly different from the chemotaxis towards medium alone after specific CB1 inverse agonist treatment. 2-AG chemotaxis was completely inhibited by CB2 inverse agonist. All three cell lines had increased chemotaxis towards CXCL12 and behaved differently towards the combination of CXCL12 and 2-AG. CXCL12 and 2-AG combination reduced the CXCL12-mediated chemotaxis in JeKo-1 and JVM-2, while it did not affect chemotaxis towards CXCL12 in Granta519 cells.

We measured the CXCR4 membrane expression and could conclude that 2-AG did not disturb the receptor internalization or recycling. Next, we assessed the phosphorylation status of some key signalling pathways and the present data could only confirm that 2-AG affects the ERK1/2 phosphorylation induced by CXCL12.

4.3.2 Discussion

The results from this study show that 2-AG is a chemoattractant for lymphoma cells and it participates in the signalling for migration of CLL and MCL. We used a low concentration (100nM) of 2-AG to be able to compare with physiological conditions. This concentration of 2-AG did not affect cell survival and proliferation, but induced the chemotaxis of majority of MCL and CLL samples, as well as MCL cell lines. We could also show that both CB1 and CB2 contributed to the chemotaxis.

Importantly, the combination of the chemokine CXCL12 and the endocannabinoid 2-AG affected the chemotaxis of most cells, without impairing CXCR4 expression, internalization or recycling. We also demonstrate that ERK1/2 is affected by incubation of MCL cell lines with the combination of 2-AG and CXCL12 but the direct participation of ERK1/2 in MCL cells chemotaxis remains to be confirmed.

2-AG is agonist to both CB1 and CB2, however in this study we cannot exclude that 2-AG binds to other receptors which could be involved in modulating the CXCR4 response to CXCL12.

4.4 PAPER IV

Paper IV investigated the effects of a single administration of an oro-mucosal spray containing THC and CBD combination in patients with indolent B lymphoid malignancies, specifically the effects on malignant and non-malignant cells. Based on correlative studies and *in vitro* and xenograft *in vivo* studies targeting cannabinoid receptors in MCL, we hypothesised that THC and CBD could be beneficial for lymphoma patients. This study also participated in the understanding of the effects of cannabinoids on immune cells and on lymphoma cells, since THC and CBD are used in the clinics for other indications, and that there is a growing interest of the impact of the use of THC and CBD for palliative treatment.

4.4.1 Results

Twenty-three patients were included in this study, comprising fifteen men and eight women, and the median age was 72 years. Twenty patients were diagnosed with CLL, one with MCL, one with follicular lymphoma and one with marginal zone lymphoma. All patients expressed CB2, and 17/23 expressed CB1.

Patients received THC/CBD at 9AM. Blood was collected just before administration, and at 1, 2, 4, 6 and 24 hours post-THC/CBD. Samples were analysed for cell subsets, chemistry, drug concentration (in plasma), and for functional studies (proliferation, cell death, and chemotaxis).

The maximum tolerated dose was seven actuations, which corresponds to 18.9 mg THC and 17.5 mg CBD. It was given to fifteen patients and it resulted in a median peak plasma concentration of 8.8 ng/mL for THC and 4.9 ng/mL for CBD. All the patients manifested side effects of maximum grade 2. Dry mouth, vertigo, somnolence, hallucination, confusion and euphoria were the most common side effects reported. The severity of side effects was dose dependent.

For thirteen patients, we could measure the different cell count in blood on a day prior to the ingestion of THC and CBD combination. We describe a decrease of malignant B cells in blood, of approximately 10% reduction at 1PM compared with the 9AM sample. This variation was not seen in any other leukocyte subsets during the sampling time (9AM-3PM).

During the day of treatment with THC/CBD, malignant B cells decreased rapidly, from one hour after administration of the drug, which was not due to apoptosis or reduced proliferation. Interestingly, CB1-negative cases displayed a quicker and deeper decrease (up to 10% decrease for CB1-positive cases and 15% decrease for CB1-positive cases at 1PM, compared with 9AM). A quick decrease was also seen in non-malignant B lymphocytes and CD3+ cells. However, there was no change in CD4+/CD8+ ratio. Neutrophils and serum cortisol were however increased four hours after THC/CBD administration. CXCR4 expression was increased in malignant B cells and in T lymphocytes, four and six hours after THC/CBD administration, respectively. The effects of THC and CBD combination on the measured parameters were gone twenty-four hours later.

4.4.2 Discussion

The combination of THC and CBD was safe for an elderly population despite adverse events reported, and it did not affect the course of the disease. The psychotropic side effects were however the dose limiting factor.

The biological effects of THC and CBD combination were seen by a quick decrease in lymphocytes counts in the blood circulation without evidence of apoptosis. Apoptosis upon THC/CBD did not occur probably because the plasma concentrations of THC and CBD were in the nanomolar range and not in micromolar, as reported by studies showing induction of cell death *in vitro* and *ex vivo* settings upon cannabinoids treatment. At a later time-point, an increase of the chemokine CXCR4 at the cell surface of T and B lymphocytes was observed, which could be associated with redistribution of the cells into secondary lymphoid organs.

During this study, we could describe a diurnal fluctuation of malignant B cells, which was not seen in non-malignant B cells or another leukocyte subset. The circadian rhythm of malignant cells from the patients could be perturbed due to aberrant expression of important circadian clock genes, reported in CLL.

Interestingly, a quicker decrease of malignant B lymphocytes occurred in the samples that did not express CB1, which could be due to the increased signalling through CB2. Indeed, CB2 is involved in the circulation of immature B cells, which could then explain the faster redistribution of those cells. However, we cannot exclude that THC and CBD effects could be due to their binding to and signalling activation through other receptors.

5 CONCLUSIONS

This thesis focuses on dysregulated signalling in B cell malignancies and on the interaction between the malignant cells and the microenvironment. Here, I describe my contribution to the identification of new players important for lymphoma B cell localization in the tissue.

We identified, for the first time, the gene *GNAZ*, encoding for the protein G alpha z, as being upregulated in MCL, which was correlated to high lymphocyte count in blood and poor prognosis as a continuous variable in Cox regression analysis. Additionally, its mRNA expression was inversely correlated to the cannabinoid receptor type 1. The impact of G alpha z in MCL pathobiology needs to be investigated further, with emphasis on its potential role in the positioning of the cells in tissue.

We also described differential gene signatures upon adhesion to stromal cells from two MCL cell lines (Rec-1 and JeKo-1) of different tissue origin. We found different signalling signature involved in the cell adhesion of those two cell lines, and identified potential mechanism behind different response of MCL to Btk inhibitor therapy. The co-culture system used could help to identify resistance/sensitivity of lymphoma cells to treatment before therapy.

In addition, we identified that the endocannabinoid 2-arachidonoylglycerol induced chemotaxis of MCL and CLL primary cells and MCL cell lines. We reported that the 2-AG-induced chemotaxis cross-talks with CXCR4 signalling. The current data on 2-AG-mediated chemotaxis of these cells, as well as the apparent redistribution of malignant and non-malignant cells upon the single administration of THC and CBD combination, support the hypothesis that the endocannabinoid system is involved in regulating circulation of lymphoma cells.

Finally, we reported that the use of combined THC and CBD (ratio 1:1) in lymphoma patients has to be considered with caution. Indeed, we observed that THC/CBD affects immune cells, and that it might lead to the malignant cell survival instead of an anti-tumoral treatment due to the possible redistribution of cells in lymphoid tissues.

6 POINTS OF PERSPECTIVE

Although several research questions were answered during my PhD studies, many other aspects of this research topic would need further investigation.

For instance, we identified the protein G alpha z upregulated in MCL, but it would be interesting to see whether it is also dysregulated in other lymphoma subtypes. Moreover, the receptor to which G alpha z binds it is still unknown, as well as the type of signalling that it triggers.

Additionally, we tested the effects of gene downregulation on chemotaxis towards a few chemokines, but it would be interesting to check if *GNAZ* downregulation affects the chemotaxis mediated by the endocannabinoid 2-AG.

As there are currently no reliable antibody for cannabinoid receptors to use in lymphoma cells, it would be of importance to develop new strategies to visualize the protein expression in cells. Also, we could set up an *in situ* hybridization method on tissue sections to see where/which cell expresses the mRNA.

Additionally, an investigation on the expression of other cannabinoid-binding receptors in MCL cell lines, and MCL and CLL primary cells would be important to decipher the mechanism behind the change of chemotaxis when CXCL12 and 2-AG are combined.

Regarding the effects of the combination CBD and THC in lymphoma patients, measurements of cytokines and endocannabinoids (2-AG and anandamide) levels in plasma would provide a more complete view on the impact of cannabinoids *in vivo*. It would also be interesting to understand the decrease of lymphocytes counts in blood by setting up a chemotaxis assay with primary cells treated with THC and CBD *ex vivo*, as well as by doing phospho-proteomic analysis, which would indicate which signalling pathways are affected.

7 ACKNOWLEDGEMENTS

My PhD journey lasted quite a few years, during which I met wonderful people that I will never forget. Each person that I have interacted with has contributed in some way to this thesis, and I would like to express my sincerest gratitude to the people that participated in the completion of my PhD.

First of all, I would like to sincerely thank **Birgitta Sander** for allowing me to join the group, and to start and pursue my PhD studies with you. I remember one day sitting at one of our group meetings during my Master project and wishing being able to continue developing myself as a scientist in your group (or at least in a similar working environment), eventually it happened and I am so grateful for that. Thank you, Birgitta for all the scientific discussions, for letting me become an independent researcher while always being there if I had any question. I am particularly grateful for the important scientific knowledge and the professional network that I could develop thanks to you. I also want to thank you for being so calm and patient in situations where I would (and did) panic! And for all the times that I came out from group meeting with new energy and motivation thanks to your support.

I would also like to thank **Agata M. Wasik**, first for accepting me to be your Master student, then for becoming my PhD co-supervisor. Agata, thank you for teaching me everything in the lab, for sharing with me your broad scientific knowledge and most importantly for being supportive, encouraging and reassuring during this entire process. Thank you for always being kind, always giving good advices in the lab, in the scientific world, and also in life experiences. It started with helping me settling in Stockholm – I remember the first week you showed me shops where to find a winter jacket as it was about to be -20°C and I had my "every weather" coat from Bretagne and I thought I would make it (this is also when I had my first *saffransbulle*). Also thank you for keeping a good environment in our research group (introducing French Fridays with croissants is one example) and in the division – you always made a new person feel welcome, including myself. I would need much more than a paragraph to express how grateful I am to have you as a supervisor, but most importantly, thank you Agata for being like a big sister to me.

Martin Lord, you always cared for me, made sure I wasn't feeling too bad after each adhesion assay that didn't work (came out a nice T-shirt from that), thank you for helping me with some statistics, useful links, and for giving inputs on my projects during group meetings. You were my big brother in the office for many years, you tried to teach me Swedish and I apologize for being so stubborn! Thank you for coping with Agata and me in the office regarding opened window, and thank you for your crazy and creative humour – I won't thank you for trying to train us with cookies – thank you for all the laughter in the office and during the nice dinners, the three of us make a good team! To summarize, thank you for being my friend.

Thank you, **Birger Christensson,** for all your advices regarding research projects, useful tips for experiments and critical views on my data. Thank you for sharing your broad knowledge,

and for pointing out my French mistakes such as *cannabinoir*. You and Birgitta make such a pleasant research group to work in, I am glad to be part of it.

I would also like to thank former group member **Lina Nygren**, we haven't spent much time together but I always enjoyed meeting you and I really appreciated your support. Thank you to recently joined group member **Mohammad Morsy**, we also haven't had the time yet to interact a lot, but I appreciate the scientific discussions we had, thank you for your advices and for being patient with me in the lab during that stressful time finishing my PhD.

Thank you, **Åsa-Lena Dackland**, for teaching me about flow cytometry, for all your help with the ordering and for always being so kind and patient with us. **Lena Hernberg**, thank you for taking over after Åsa-Lena, you also provided an immense help, managing to figure out what went wrong with orders/deliveries, I bet that now when the companies see an order coming from you they are making extra effort on doing a good job! And thank you for being so kind.

Eva Limby and **Edvard Smith**, I was privileged having both of you as my co-supervisors. Thank you for your advices, comments and input in my PhD studies and thesis. Thank you for being responsive when I needed your support.

To all the collaborators that contributed to the projects included in this thesis, without whom none of it could have been possible, especially thank you **Björn Wahlin**, **Christopher Melén**, **Anthony Wright**, **Filip Mundt**, **Gustav Arvidsson** and **Laia Sadeghi**, it is a great pleasure to collaborate with you, our meeting are always fruitful.

I would also like to give a big thank to the routine flow cytometry lab, **Åsa**, **Linh**, **Mariann**, **Elin**, **Branka**, **Shila**, **Bernadette** and **Aleksandra**, for calling us when there is patient material leftover for research. Thank you for always being so kind and cheerful, for inviting me and the rest of the group for mounting the Christmas tree (and eat fika) each year. Thank you for making a lovely atmosphere at F46 corridor!

I am very grateful to **Göran Andersson** for allowing me to join Pathology Division. Thank you for the interesting scientific discussions during lunch, Departmental and Divisional retreats and seminars. Thank you for making the Pathology Division a pleasant working environment by keeping traditions (Lucia Glögg, korv during pubs), joining us for lunch and sharing non-scientific conversations with us PhDs and postdocs (I am still amazed that you had the best combination of meals when you went to France: couscous and Paris-Brest).

Mia Bjerke, thank you so much for all your help. I know that I can always come to you, with any question and you will always be able to answer, if not directly you will do your best to figure this out. You have become the pillar of Pathology Division and I don't know how any of us would manage without you. Thank you also for always listening, and thank you for the fun conversations on the rare lunch that we managed to have together (11:00am is *really* too early!) and during pubs and numerous dissertations we have had the past year (hope you'll like the champagne I'll bring!).

Thank you **Arja Kramsu** and **Ann Mellquist** for being of great help with PhD studies administrative issues. I would also like to thank **Matti Sällberg**, **Anthony Wright**, **Andrej Weintraub**, **Lars Frelin**, **Marjan Amiri**, **Rasmus Larsson** and **Mikael Hammarstedt** for making ANAFutura and LabMed a pleasant working environment.

I would like to thank everyone at Pathology Division, previous and current members, for making this PhD journey enjoyable on a daily basis: **Laia**, thank you for sharing the good and bad times during these past years, I miss you! **ChrisP** and **Anja** thank you for always giving me great advices based on your experiences, and for introducing me to the trilogy. Thank you, **Roberto** for your Italian touch in the Division. **Zurab**, thank you for always being up for a chat, a walk or a beer. Thank you, **Dhanu** for the numerous interesting/crazy scientific/non-scientific discussions, always fun (don't forget my shares). Thank you to my ANAFutura office mates (current and previous): **Suchita**, **Joman**, **Haifeng**, **Ashish**, **Anh**, **Tahira**, **Janne**, and **Mohammad**, I think we have the best office in ANAFutura 😊! **Raghu**, thank you for always being happy and smiling, it was always a pleasure to talk with you in between your visits to the animal house or between 2 PCRs! Thank you **Gosia**, for your cheerful mood, for always being helpful and for being a great cell lab-mate; also, thank you for your Polish treats!! Thank you **Rim** for all the laughter and for sharing chocolate with me. Thank you **Massoud** for the discussions we had and for your encouragement. Thank you **Maria H** for the fun time we always have together when you visit us for fika with little **Gabriel**, when we went to India (the snake dance) and thank you for bearing with me photobombing, especially when your pictures are not blurry..! Thank you, **Sara**, **Carina**, **Helena**, **Camilla**, **Emilie**, **Jiangrong**, **Roxana** and **Sadaf** for always being in a good mood when I see you in the lunchroom. **Karin**, thank you for always asking interesting questions during seminar and for your support (especially by means of coffee, it made our day). Thank you **Mihaela** for always being kind and helpful, and for the good times we had during the few afterwork we had together. **Francesco**, thank you for always caring. Also thank you **Pernilla** for always being very helpful with instruments and methods protocol in the lab, and thank you **Maria N** for being very nice and friendly and for keeping our division in order. **Christina H** thank you for being kind, helpful, for reminding us about keeping the lab clean, and for always being up for dancing during retreats and pubs. **TingTing**, thank you for the nice chats when it was only the two of us in the corridors of old Division. Thank you **Diba** for the fun times at KICancer retreats! **Hanna**, thank you for sharing these 5 past years of PhD with you. Thank you, **Azadeh** for being so cheerful each time I saw you, even though it wasn't many times, you brought brightness in our corridors. A big thank to **Lisa**, **Antje**, **Raul**, **Filip**, **Loffe**, **Ghazal**, **Tunde**, **Ali R**, **Arun**, **Rita**, **Marita**, **Sougat, Nikolina**, **Dina**, **Emmanuelle**, **Gizem**, **Ewa**, **Kristina K**, **Mikhail**, **Katja**, and **Ville** for the scientific discussions during seminars, and for the good times during lunch, retreats, dissertation parties and pubs.

I would like to thank the teachers at Pathology Division, **Gareth**, **Barbro**, **Ulla**, **Anders R**, **Annica**, **Rosita**, **Jolly** and **Sara W**, for all their input into the education and for always trying to improve each year for the benefit of the student.

Thank you to all the PI from Pathology Division, for contributing to my scientific development, I learned a lot from you: **Göran Andersson**, **Mikael Björnstedt**, **Joakim Dillner**, **Katalin Dobra**, **Jonas Fuxe**, **Roberto Gramignoli, Anders Hjerpe**, **Tuomas Näreoja**, **Jaakko Patrakka**, **Birgitta Sander and Stephen Strom.**

To the 8th floor people, **Alen**, **Maria**, **Mirko**, **Ali**, **Lorenzo**, **Amo**, **Seher** and **Johan**, thank you for always passing by our office to say hi, for always asking how I am doing and for being kind, fun, nice, and cheerful people.

To my friends from Pathology course, **Gonzalo**, **Arian**, **Oksana** and **Parisa**. Through the years we managed to meet regularly and I thank you so much for all your advices (regarding lab and life troubles), for all the fun dinners we had, and importantly thank you for always being there for me when I needed. You became very close friends for me, I am so glad that we have each other and I thank you for that.

To the Ethics girls, **Sandra**, **Christine** and **Angelina**, I am so glad that we were together during that course and thank you for the fun dinners we had together afterwards (when we managed to find a suitable time for all of us), I miss this.

I would like to thank **Fu**, for being a very good friend since the microscopy course, we went through this PhD time together, and now we end together by having our PhD defense on the same day.

Thank you, **Teresa** for being my friend since we both started as Master student in the lab in Stockholm. So fare we managed to stay in touch and I hope it will continue because you are a great friend with whom I can talk about anything, which helped me many times.

Thank you **Sylvie** for being an amazing mentor, for checking on me every six months (that is usually when I realize half a year has passed without noticing), and for giving very good advices, often from a different and interesting perspective! Et merci pour les cafés et muffins 😊

Angelina, a special thanks for you, we haven't been very close this year, but you stay one of my very good friends here in Stockholm. Thank you for your support and the very much needed coffee breaks/fika we had.

Sandra, thank you for being a good friend and an amazing scientist! Thank you for including me in the Moving Across project, it was super interesting and great fun. I miss having spontaneous diners with you!

I want to thank my "new friends" (I still call you that although it has been a year now that we spend time together). This year was tough, for all of us, and I really don't know how I would have gone through it without you all, so thank you: **Lucas** for always being positive and a supportive friend, **Robert** for always listening and comforting, **Marco** for always making us laugh, **Giorgio** for always knowing what to say in the right moment, **Esther** for choosing the best songs during afterwork, **Thomas** for all your advices and endless discussions in

French/English/sign languages, **Joman** for always being kind, calm and reassuring, **Suchita** for always being understanding and cheerful. But mostly, thank you all for all the laughter we had, it made me forget the bad things that had happened.

To my friends outside the lab, that helped me to enjoy time outside work. Thank you, **Theresa** and **Karen** for the (often very much needed) long brunches, walks, talks. It is always a pleasure to meet you and I know that I can always count on you. Thank you also **Theresa** and Tom for inviting me to your lovely house for social events (that often includes amazing food).

Thank you **Shahul** for encouraging me, giving me support, always offering your help and for being a good friend for so many years now.

Shuvojit, thank you for all the trips we made together, for all the good (hours long) talks, and for inspiring me with your kindness, open-mindedness and your non-governmental organization.

Joman and **Suchita**, my girls. I had to have a special thank for you two. I am so grateful to have you by my side. You both are always there for me, always supportive, listening, being a shoulder to cry or arms to hug when needed. Thank you for all the laughter, the talks and other moments we shared. You helped me going through this last bit of my PhD (inside and outside work), and I am so thankful for that.

Thank you, **ChrisP** and **Nacho**, for all the support during all these years, you helped me in so many ways to go through everything. And thank you to the rest of the gang: **Ken**, **Barry**, **Steve**, and **Aurélien**, you were all my first non-KI friends in Stockholm! Thank you for making Stockholm feel like home!

A special thanks to my French friends! **Flo**, **Nico**, **Valé**, **Antho**, **Math**, **Franck**, et **Maïwenn** ; **Cindy** et **Jade** ; **Bettina**, **Seb** et **Livio** ; **Manon H** ; **Marion**, **Corentine**, **Manon F**, **Julien** et **Steven** merci de me soutenir et de m'avoir encouragée pendant ces années, le fait de savoir que vous êtes là quand je rentre en Bretagne me rassure.

Finally, and most importantly, I am very grateful to my family, although I am far away from them, they have always been there for me. Merci **Maman**, merci **Papa**, de m'avoir toujours soutenue et encouragée. Si je suis arrivée ici c'est grâce à vous deux. Merci **Sandra** de toujours être à l'écoute, de m'encourager et de me réconforter quand il faut. Merci **Laëtitia**, **Kellyssa** et **Tylia** d'être présentes et de me faire me sentir paisible à vos côtés quand je viens en Bretagne. Merci **Quentin** d'être le plus adorable des frères qu'on puisse avoir. Merci **Mémé** de ta bonne humeur (et tes crêpes) qui m'aide à avancer. J'ai vraiment de la chance de vous avoir tous auprès de moi, malgré la distance, vous m'avez donné beaucoup de courage, je vous aime très fort et vous remercie pour tout.

8 REFERENCES

Abadji, V., Lucas-Lenard, J.M., Chin, C. & Kendall, D.A. (1999) Involvement of the carboxyl terminus of the third intracellular loop of the cannabinoid CB1 receptor in constitutive activation of Gs. *J Neurochem,* **72,** 2032-2038.

Abbas, A.K., Lichtman, A.H.H. & Pillai, S. (2014) *Basic immunology: functions and disorders of the immune system.* Elsevier.

Abrahamsson, A., Albertsson-Lindblad, A., Brown, P.N., Baumgartner-Wennerholm, S., Pedersen, L.M., D'Amore, F., Nilsson-Ehle, H., Jensen, P., Pedersen, M., Geisler, C.H. & Jerkeman, M. (2014) Real world data on primary treatment for mantle cell lymphoma: a Nordic Lymphoma Group observational study. *Blood,* **124,** 1288-1295.

Agathangelidis, A., Darzentas, N., Hadzidimitriou, A., Brochet, X., Murray, F., Yan, X.-J., Davis, Z., van Gastel-Mol, E.J., Tresoldi, C., Chu, C.C., Cahill, N., Giudicelli, V., Tichy, B., Pedersen, L.B., Foroni, L., Bonello, L., Janus, A., Smedby, K., Anagnostopoulos, A., Merle-Beral, H., Laoutaris, N., Juliusson, G., di Celle, P.F., Pospisilova, S., Jurlander, J., Geisler, C., Tsaftaris, A., Lefranc, M.-P., Langerak, A.W., Oscier, D.G., Chiorazzi, N., Belessi, C., Davi, F., Rosenquist, R., Ghia, P. & Stamatopoulos, K. (2012) Stereotyped B-cell receptors in one-third of chronic lymphocytic leukemia: a molecular classification with implications for targeted therapies. *Blood,* **119,** 4467-4475.

Alhouayek, M., Masquelier, J. & Muccioli, G.G. (2014) Controlling 2-arachidonoylglycerol metabolism as an anti-inflammatory strategy. *Drug Discov Today,* **19,** 295-304.

Allahmoradi, E., Taghiloo, S., Tehrani, M., Hossein-Nattaj, H., Janbabaei, G., Shekarriz, R. & Asgarian-Omran, H. (2017) CD4+ T Cells are Exhausted and Show Functional Defects in Chronic Lymphocytic Leukemia. *Iran J Immunol,* **14,** 257-269.

Andre, C.M., Hausman, J.F. & Guerriero, G. (2016) Cannabis sativa: The Plant of the Thousand and One Molecules. *Front Plant Sci,* **7,** 19.

Arcangeli, A., Pillozzi, S. & Becchetti, A. (2012) Targeting ion channels in leukemias: a new challenge for treatment. *Curr Med Chem,* **19,** 683-696.

Armstrong, J.L., Hill, D.S., McKee, C.S., Hernandez-Tiedra, S., Lorente, M., Lopez-Valero, I., Eleni Anagnostou, M., Babatunde, F., Corazzari, M., Redfern, C.P.F., Velasco, G. & Lovat, P.E. (2015) Exploiting cannabinoid-induced cytotoxic autophagy to drive melanoma cell death. *J Invest Dermatol,* **135,** 1629-1637.

Arnon, T.I., Horton, R.M., Grigorova, I.L. & Cyster, J.G. (2013) Visualization of splenic marginal zone B-cell shuttling and follicular B-cell egress. *Nature,* **493,** 684-688.

Arvidsson, G., Henriksson, J., Sander, B. & Wright, A.P. (2018) Mixed-species RNAseq analysis of human lymphoma cells adhering to mouse stromal cells identifies a core gene set that is also differentially expressed in the lymph node microenvironment of mantle cell lymphoma and chronic lymphocytic leukemia patients. *Haematologica,* **103,** 666-678.

Atwood, B.K. & Mackie, K. (2010) CB2: a cannabinoid receptor with an identity crisis. *Br J Pharmacol,* **160,** 467-479.

Balenga, N.A., Martinez-Pinilla, E., Kargl, J., Schroder, R., Peinhaupt, M., Platzer, W., Balint, Z., Zamarbide, M., Dopeso-Reyes, I.G., Ricobaraza, A., Perez-Ortiz, J.M., Kostenis, E., Waldhoer, M., Heinemann, A. & Franco, R. (2014) Heteromerization of GPR55 and cannabinoid CB2 receptors modulates signalling. *Br J Pharmacol,* **171,** 5387-5406.

Bar-Sela, G., Cohen, I., Campisi-Pinto, S., Lewitus, G.M., Oz-Ari, L., Jehassi, A., Peer, A., Turgeman, I., Vernicova, O., Berman, P., Wollner, M., Moskovitz, M. & Meiri, D. (2020) Cannabis Consumption Used by Cancer Patients during Immunotherapy Correlates with Poor Clinical Outcome. *Cancers (Basel),* **12**.

Barnes, M.P. (2006) Sativex: clinical efficacy and tolerability in the treatment of symptoms of multiple sclerosis and neuropathic pain. *Expert Opin Pharmacother,* **7,** 607-615.

Basu, S., Ray, A. & Dittel, B.N. (2011) Cannabinoid receptor 2 is critical for the homing and retention of marginal zone B lineage cells and for efficient T-independent immune responses. *Journal of immunology,* **187,** 5720-5732.

Beà, S., Ribas, M., Hernández, J.M., Bosch, F., Pinyol, M., Hernández, L., García, J.L., Flores, T., González, M., López-Guillermo, A., Piris, M.A., Cardesa, A., Montserrat, E., Miró, R. & Campo, E. (1999) Increased number of chromosomal imbalances and high-level DNA amplifications in mantle cell lymphoma are associated with blastoid variants. *Blood,* **93,** 4365-4374.

Beem, E. & Segal, M.S. (2013) Evaluation of stability and sensitivity of cell fluorescent labels when used for cell migration. *J Fluoresc,* **23,** 975-987.

Benz, A.H., Renné, C., Maronde, E., Koch, M., Grabiec, U., Kallendrusch, S., Rengstl, B., Newrzela, S., Hartmann, S., Hansmann, M.L. & Dehghani, F. (2013) Expression and functional relevance of cannabinoid receptor 1 in Hodgkin lymphoma. *PloS one,* **8,** e81675.

Berahovich, R.D., Zabel, B.A., Penfold, M.E., Lewen, S., Wang, Y., Miao, Z., Gan, L., Pereda, J., Dias, J., Slukvin, II, McGrath, K.E., Jaen, J.C. & Schall, T.J. (2010) CXCR7 protein is not expressed on human or mouse leukocytes. *Journal of immunology,* **185,** 5130-5139.

Borge, M., Remes Lenicov, F., Nannini, P.R., de los Ríos Alicandú, M.M., Podaza, E., Ceballos, A., Fernández Grecco, H., Cabrejo, M., Bezares, R.F., Morande, P.E., Oppezzo, P., Giordano, M. & Gamberale, R. (2014) The Expression of Sphingosine-1 Phosphate Receptor-1 in Chronic Lymphocytic Leukemia Cells Is Impaired by Tumor Microenvironmental Signals and Enhanced by Piceatannol and R406. **193,** 3165-3174.

Borner, C., Hollt, V., Sebald, W. & Kraus, J. (2007) Transcriptional regulation of the cannabinoid receptor type 1 gene in T cells by cannabinoids. *Journal of leukocyte biology,* **81,** 336-343.

Bouaboula, M., Rinaldi, M., Carayon, P., Carillon, C., Delpech, B., Shire, D., Le Fur, G. & Casellas, P. (1993) Cannabinoid-receptor expression in human leukocytes. *European journal of biochemistry,* **214,** 173-180.

Bukiya, A.N. (2019) *Recent Advances in Cannabinoid Physiology and Pathology.* Springer, Cham, Springer Nature Switzerland AG 2019.

Burger, J.A., Burger, M. & Kipps, T.J. (1999) Chronic lymphocytic leukemia B cells express functional CXCR4 chemokine receptors that mediate spontaneous migration beneath bone marrow stromal cells. *Blood,* **94,** 3658-3667.

Burger, J.A. & Chiorazzi, N. (2013) B cell receptor signaling in chronic lymphocytic leukemia. *Trends in immunology,* **34,** 592-601.

Burger, J.A. & Ford, R.J. (2011) The microenvironment in mantle cell lymphoma: cellular and molecular pathways and emerging targeted therapies. *Seminars in cancer biology,* **21,** 308-312.

Burger, J.A. & Gribben, J.G. (2014) The microenvironment in chronic lymphocytic leukemia (CLL) and other B cell malignancies: insight into disease biology and new targeted therapies. *Seminars in cancer biology,* **24,** 71-81.

Burger, J.A., Quiroga, M.P., Hartmann, E., Bürkle, A., Wierda, W.G., Keating, M.J. & Rosenwald, A. (2009) High-level expression of the T-cell chemokines CCL3 and CCL4 by chronic lymphocytic leukemia B cells in nurselike cell cocultures and after BCR stimulation. *Blood,* **113,** 3050-3058.

Burger, J.A., Tsukada, N., Burger, M., Zvaifler, N.J., Dell'Aquila, M. & Kipps, T.J. (2000) Blood-derived nurse-like cells protect chronic lymphocytic leukemia B cells from spontaneous apoptosis through stromal cell-derived factor-1. *Blood,* **96,** 2655-2663.

Burger, J.A. & Wiestner, A. (2018) Targeting B cell receptor signalling in cancer: preclinical and clinical advances. *Nat Rev Cancer,* **18,** 148-167.

Burger, J.A., Zvaifler, N.J., Tsukada, N., Firestein, G.S. & Kipps, T.J. (2001) Fibroblast-like synoviocytes support B-cell pseudoemperipolesis via a stromal cell-derived factor-1- and CD106 (VCAM-1)-dependent mechanism. *J Clin Invest,* **107,** 305-315.

Burns, J.M., Summers, B.C., Wang, Y., Melikian, A., Berahovich, R., Miao, Z., Penfold, M.E., Sunshine, M.J., Littman, D.R., Kuo, C.J., Wei, K., McMaster, B.E., Wright, K., Howard, M.C. & Schall, T.J. (2006) A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumor development. *The Journal of experimental medicine,* **203,** 2201-2213.

Caffarel, M.M., Sarrió, D., Palacios, J., Guzmán, M. & Sánchez, C. (2006) Delta9-tetrahydrocannabinol inhibits cell cycle progression in human breast cancer cells through Cdc2 regulation. *Cancer Res,* **66,** 6615-6621.

Calin, G.A., Dumitru, C.D., Shimizu, M., Bichi, R., Zupo, S., Noch, E., Aldler, H., Rattan, S., Keating, M., Rai, K., Rassenti, L., Kipps, T., Negrini, M., Bullrich, F. & Croce, C.M. (2002) Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proceedings of the National Academy of Sciences of the United States of America,* **99,** 15524-15529.

Campo, E. & Rule, S. (2015) Mantle cell lymphoma: evolving management strategies. *Blood,* **125,** 48-55.

Castillo, R., Mascarenhas, J., Telford, W., Chadburn, A., Friedman, S.M. & Schattner, E.J. (2000) Proliferative response of mantle cell lymphoma cells stimulated by CD40 ligation and IL-4. *Leukemia,* **14,** 292-298.

Černe, K. (2020) Toxicological properties of Δ9-tetrahydrocannabinol and cannabidiol. *Arh Hig Rada Toksikol,* **71,** 1-11.

Chan, L.N., Chen, Z., Braas, D., Lee, J.W., Xiao, G., Geng, H., Cosgun, K.N., Hurtz, C., Shojaee, S., Cazzaniga, V., Schjerven, H., Ernst, T., Hochhaus, A., Kornblau, S.M., Konopleva, M., Pufall, M.A., Cazzaniga, G., Liu, G.J., Milne, T.A., Koeffler, H.P., Ross, T.S., Sanchez-Garcia, I., Borkhardt, A., Yamamoto, K.R., Dickins, R.A., Graeber, T.G. & Muschen, M. (2017) Metabolic gatekeeper function of B-lymphoid transcription factors. *Nature,* **542,** 479-483.

Chang, B.Y., Francesco, M., De Rooij, M.F., Magadala, P., Steggerda, S.M., Huang, M.M., Kuil, A., Herman, S.E., Chang, S., Pals, S.T., Wilson, W., Wiestner, A., Spaargaren, M., Buggy, J.J. & Elias, L. (2013) Egress of CD19(+)CD5(+) cells into peripheral blood following treatment with the Bruton tyrosine kinase inhibitor ibrutinib in mantle cell lymphoma patients. *Blood,* **122,** 2412-2424.

Chappell, C.P., Draves, K.E., Giltiay, N.V. & Clark, E.A. (2012) Extrafollicular B cell activation by marginal zone dendritic cells drives T cell-dependent antibody responses. *The Journal of experimental medicine,* **209,** 1825-1840.

Chen, S.S., Chang, B.Y., Chang, S., Tong, T., Ham, S., Sherry, B., Burger, J.A., Rai, K.R. & Chiorazzi, N. (2016a) BTK inhibition results in impaired CXCR4 chemokine receptor surface expression, signaling and function in chronic lymphocytic leukemia. *Leukemia,* **30,** 833-843.

Chen, Z., Teo, A.E. & McCarty, N. (2016b) ROS-Induced CXCR4 Signaling Regulates Mantle Cell Lymphoma (MCL) Cell Survival and Drug Resistance in the Bone Marrow Microenvironment via Autophagy. *Clinical cancer research : an official journal of the American Association for Cancer Research,* **22,** 187-199.

Cheng, Z.J., Zhao, J., Sun, Y., Hu, W., Wu, Y.L., Cen, B., Wu, G.X. & Pei, G. (2000) beta-arrestin differentially regulates the chemokine receptor CXCR4-mediated signaling and receptor internalization, and this implicates multiple interaction sites between beta-arrestin and CXCR4. *The Journal of biological chemistry,* **275,** 2479-2485.

Chiurchiu, V., Lanuti, M., De Bardi, M., Battistini, L. & Maccarrone, M. (2015) The differential characterization of GPR55 receptor in human peripheral blood reveals a distinctive expression in monocytes and NK cells and a proinflammatory role in these innate cells. *Int Immunol,* **27,** 153-160.

Cimmino, A., Calin, G.A., Fabbri, M., Iorio, M.V., Ferracin, M., Shimizu, M., Wojcik, S.E., Aqeilan, R.I., Zupo, S., Dono, M., Rassenti, L., Alder, H., Volinia, S., Liu, C.G., Kipps, T.J., Negrini, M. & Croce, C.M. (2005) miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proceedings of the National Academy of Sciences of the United States of America,* **102,** 13944-13949.

Clot, G., Jares, P., Giné, E., Navarro, A., Royo, C., Pinyol, M., Martín-Garcia, D., Demajo, S., Espinet, B., Salar, A., Ferrer, A., Muntañola, A., Aymerich, M., Rauert-Wunderlich, H., Jaffe, E.S., Connors, J.M., Gascoyne, R.D., Delabie, J., López-Guillermo, A., Ott, G., Wright, G.W., Staudt, L.M., Rosenwald, A., Scott, D.W., Rimsza, L.M., Beà, S. & Campo, E. (2018) A gene signature that distinguishes conventional and leukemic nonnodal mantle cell lymphoma helps predict outcome. *Blood,* **132,** 413-422.

Comes, N., Serrano-Albarrás, A., Capera, J., Serrano-Novillo, C., Condom, E., Ramón y Cajal, S., Ferreres, J.C. & Felipe, A. (2015) Involvement of potassium channels in the progression of cancer to a more malignant phenotype. *Biochimica et Biophysica Acta (BBA) - Biomembranes,* **1848,** 2477-2492.

Corcione, A., Arduino, N., Ferretti, E., Raffaghello, L., Roncella, S., Rossi, D., Fedeli, F., Ottonello, L., Trentin, L., Dallegri, F., Semenzato, G. & Pistoia, V. (2004) CCL19 and CXCL12 trigger in vitro chemotaxis of

human mantle cell lymphoma B cells. *Clinical cancer research : an official journal of the American Association for Cancer Research,* **10,** 964-971.

Cyster, J.G. & Allen, C.D.C. (2019) B Cell Responses: Cell Interaction Dynamics and Decisions. *Cell,* **177,** 524-540.

Cyster, J.G. & Schwab, S.R. (2012) Sphingosine-1-phosphate and lymphocyte egress from lymphoid organs. *Annu Rev Immunol,* **30,** 69-94.

Dalton, G.D., Peterson, L.J. & Howlett, A.C. (2013) CB_1 cannabinoid receptors promote maximal FAK catalytic activity by stimulating cooperative signaling between receptor tyrosine kinases and integrins in neuronal cells. *Cell Signal,* **25,** 1665-1677.

Das, S., Kaul, K., Mishra, S., Charan, M. & Ganju, R.K. (2019) Cannabinoid Signaling in Cancer. *Adv Exp Med Biol,* **1162,** 51-61.

Davi, F., Langerak, A.W., de Septenville, A.L., Kolijn, P.M., Hengeveld, P.J., Chatzidimitriou, A., Bonfiglio, S., Sutton, L.A., Rosenquist, R., Ghia, P. & Stamatopoulos, K. (2020) Immunoglobulin gene analysis in chronic lymphocytic leukemia in the era of next generation sequencing. *Leukemia,* **34,** 2545-2551.

de Gorter, D.J., Beuling, E.A., Kersseboom, R., Middendorp, S., van Gils, J.M., Hendriks, R.W., Pals, S.T. & Spaargaren, M. (2007) Bruton's tyrosine kinase and phospholipase Cgamma2 mediate chemokine-controlled B cell migration and homing. *Immunity,* **26,** 93-104.

DeFranco, A.L. (2015) B Lymphocytes: Receptors. In: *eLS*, pp. 1-15.

Devane, W.A., Dysarz, F.A., 3rd, Johnson, M.R., Melvin, L.S. & Howlett, A.C. (1988) Determination and characterization of a cannabinoid receptor in rat brain. *Molecular pharmacology,* **34,** 605-613.

Devinsky, O., Cilio, M.R., Cross, H., Fernandez-Ruiz, J., French, J., Hill, C., Katz, R., Di Marzo, V., Jutras-Aswad, D., Notcutt, W.G., Martinez-Orgado, J., Robson, P.J., Rohrback, B.G., Thiele, E., Whalley, B. & Friedman, D. (2014) Cannabidiol: pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. *Epilepsia,* **55,** 791-802.

Di Marzo, V., Breivogel, C.S., Tao, Q., Bridgen, D.T., Razdan, R.K., Zimmer, A.M., Zimmer, A. & Martin, B.R. (2000) Levels, metabolism, and pharmacological activity of anandamide in CB(1) cannabinoid receptor knockout mice: evidence for non-CB(1), non-CB(2) receptor-mediated actions of anandamide in mouse brain. *J Neurochem,* **75,** 2434-2444.

Ding, W., Nowakowski, G.S., Knox, T.R., Boysen, J.C., Maas, M.L., Schwager, S.M., Wu, W., Wellik, L.E., Dietz, A.B., Ghosh, A.K., Secreto, C.R., Medina, K.L., Shanafelt, T.D., Zent, C.S., Call, T.G. & Kay, N.E. (2009) Bi-directional activation between mesenchymal stem cells and CLL B-cells: implication for CLL disease progression. *British journal of haematology,* **147,** 471-483.

Dlugos, A., Childs, E., Stuhr, K.L., Hillard, C.J. & de Wit, H. (2012) Acute stress increases circulating anandamide and other N-acylethanolamines in healthy humans. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology,* **37,** 2416-2427.

Dobrzanski, M.J. (2013) Expanding roles for CD4 T cells and their subpopulations in tumor immunity and therapy. *Front Oncol,* **3,** 63.

Dreyling, M., Aurer, I., Cortelazzo, S., Hermine, O., Hess, G., Jerkeman, M., Le Gouill, S., Ribrag, V., Trněný, M., Visco, C., Walewski, J., Zaja, F. & Zinzani, P.L. (2018) Treatment for patients with relapsed/refractory mantle cell lymphoma: European-based recommendations. *Leukemia & lymphoma,* **59,** 1814-1828.

Dreyling, M., Lenz, G., Hoster, E., Van Hoof, A., Gisselbrecht, C., Schmits, R., Metzner, B., Truemper, L., Reiser, M., Steinhauer, H., Boiron, J.M., Boogaerts, M.A., Aldaoud, A., Silingardi, V., Kluin-Nelemans, H.C., Hasford, J., Parwaresch, R., Unterhalt, M. & Hiddemann, W. (2005) Early consolidation by myeloablative radiochemotherapy followed by autologous stem cell transplantation in first remission significantly prolongs progression-free survival in mantle-cell lymphoma: results of a prospective randomized trial of the European MCL Network. *Blood,* **105,** 2677-2684.

Döhner, H., Stilgenbauer, S., Benner, A., Leupolt, E., Kröber, A., Bullinger, L., Döhner, K., Bentz, M. & Lichter, P. (2000) Genomic aberrations and survival in chronic lymphocytic leukemia. *The New England journal of medicine,* **343,** 1910-1916.

Efremov, D.G., Turkalj, S. & Laurenti, L. (2020) Mechanisms of B Cell Receptor Activation and Responses to B Cell Receptor Inhibitors in B Cell Malignancies. *Cancers (Basel),* **12**.

Ek, S., Hogerkorp, C.M., Dictor, M., Ehinger, M. & Borrebaeck, C.A. (2002) Mantle cell lymphomas express a distinct genetic signature affecting lymphocyte trafficking and growth regulation as compared with subpopulations of normal human B cells. *Cancer Res,* **62,** 4398-4405.

Eskelund, C.W., Kolstad, A., Jerkeman, M., Räty, R., Laurell, A., Eloranta, S., Smedby, K.E., Husby, S., Pedersen, L.B., Andersen, N.S., Eriksson, M., Kimby, E., Bentzen, H., Kuittinen, O., Lauritzsen, G.F., Nilsson-Ehle, H., Ralfkiaer, E., Ehinger, M., Sundström, C., Delabie, J., Karjalainen-Lindsberg, M.L., Workman, C.T., Garde, C., Elonen, E., Brown, P., Grønbaek, K. & Geisler, C.H. (2016) 15-year follow-up of the Second Nordic Mantle Cell Lymphoma trial (MCL2): prolonged remissions without survival plateau. *British journal of haematology,* **175,** 410-418.

Faron-Górecka, A., Szlachta, M., Kolasa, M., Solich, J., Górecki, A., Kuśmider, M., Żurawek, D. & Dziedzicka-Wasylewska, M. (2019) Understanding GPCR dimerization. *Methods Cell Biol,* **149,** 155-178.

Fernàndez, V., Salamero, O., Espinet, B., Solé, F., Royo, C., Navarro, A., Camacho, F., Beà, S., Hartmann, E., Amador, V., Hernández, L., Agostinelli, C., Sargent, R.L., Rozman, M., Aymerich, M., Colomer, D., Villamor, N., Swerdlow, S.H., Pileri, S.A., Bosch, F., Piris, M.A., Montserrat, E., Ott, G., Rosenwald, A., López-Guillermo, A., Jares, P., Serrano, S. & Campo, E. (2010) Genomic and gene expression profiling defines indolent forms of mantle cell lymphoma. *Cancer Res,* **70,** 1408-1418.

Fischer, K. & Hallek, M. (2017) Optimizing frontline therapy of CLL based on clinical and biological factors. *Hematology Am Soc Hematol Educ Program,* **2017,** 338-345.

Flores-Sanchez, I.J. & Verpoorte, R. (2008) Secondary metabolism in cannabis. *Phytochemistry Reviews,* **7,** 615-639.

Flygare, J., Gustafsson, K., Kimby, E., Christensson, B. & Sander, B. (2005) Cannabinoid receptor ligands mediate growth inhibition and cell death in mantle cell lymphoma. *FEBS letters,* **579,** 6885-6889.

Fong, H.K., Yoshimoto, K.K., Eversole-Cire, P. & Simon, M.I. (1988) Identification of a GTP-binding protein alpha subunit that lacks an apparent ADP-ribosylation site for pertussis toxin. *Proceedings of the National Academy of Sciences of the United States of America,* **85,** 3066-3070.

Fredriksson, R., Lagerström, M.C., Lundin, L.G. & Schiöth, H.B. (2003) The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. *Molecular pharmacology,* **63,** 1256-1272.

Freund, P., Porpaczy, E.A., Le, T., Gruber, M., Pausz, C., Staber, P., Jager, U. & Vanura, K. (2016) Cannabinoid Receptors Are Overexpressed in CLL but of Limited Potential for Therapeutic Exploitation. *PloS one,* **11,** e0156693.

Freund, T.F., Katona, I. & Piomelli, D. (2003) Role of endogenous cannabinoids in synaptic signaling. *Physiol Rev,* **83,** 1017-1066.

Förster, R., Mattis, A.E., Kremmer, E., Wolf, E., Brem, G. & Lipp, M. (1996) A putative chemokine receptor, BLR1, directs B cell migration to defined lymphoid organs and specific anatomic compartments of the spleen. *Cell,* **87,** 1037-1047.

Förster, R., Schubel, A., Breitfeld, D., Kremmer, E., Renner-Müller, I., Wolf, E. & Lipp, M. (1999) CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell,* **99,** 23-33.

Gagnon, A.W., Manning, D.R., Catani, L., Gewirtz, A., Poncz, M. & Brass, L.F. (1991) Identification of Gz alpha as a pertussis toxin-insensitive G protein in human platelets and megakaryocytes. *Blood,* **78,** 1247-1253.

Galiegue, S., Mary, S., Marchand, J., Dussossoy, D., Carriere, D., Carayon, P., Bouaboula, M., Shire, D., Le Fur, G. & Casellas, P. (1995) Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *European journal of biochemistry,* **232,** 54-61.

Galve-Roperh, I., Sánchez, C., Cortés, M.L., Gómez del Pulgar, T., Izquierdo, M. & Guzmán, M. (2000) Anti-tumoral action of cannabinoids: involvement of sustained ceramide accumulation and extracellular signal-regulated kinase activation. *Nat Med,* **6,** 313-319.

Ganju, R.K., Brubaker, S.A., Meyer, J., Dutt, P., Yang, Y., Qin, S., Newman, W. & Groopman, J.E. (1998) The alpha-chemokine, stromal cell-derived factor-1alpha, binds to the transmembrane G-protein-coupled

CXCR-4 receptor and activates multiple signal transduction pathways. *The Journal of biological chemistry,* **273,** 23169-23175.

Gantz, I., Muraoka, A., Yang, Y.K., Samuelson, L.C., Zimmerman, E.M., Cook, H. & Yamada, T. (1997) Cloning and chromosomal localization of a gene (GPR18) encoding a novel seven transmembrane receptor highly expressed in spleen and testis. *Genomics,* **42,** 462-466.

Gasperi, V., Evangelista, D., Chiurchiu, V., Florenzano, F., Savini, I., Oddi, S., Avigliano, L., Catani, M.V. & Maccarrone, M. (2014) 2-Arachidonoylglycerol modulates human endothelial cell/leukocyte interactions by controlling selectin expression through CB1 and CB2 receptors. *Int J Biochem Cell Biol,* **51,** 79-88.

Geisler, C.H., Kolstad, A., Laurell, A., Andersen, N.S., Pedersen, L.B., Jerkeman, M., Eriksson, M., Nordström, M., Kimby, E., Boesen, A.M., Kuittinen, O., Lauritzsen, G.F., Nilsson-Ehle, H., Ralfkiaer, E., Akerman, M., Ehinger, M., Sundström, C., Langholm, R., Delabie, J., Karjalainen-Lindsberg, M.L., Brown, P. & Elonen, E. (2008) Long-term progression-free survival of mantle cell lymphoma after intensive front-line immunochemotherapy with in vivo-purged stem cell rescue: a nonrandomized phase 2 multicenter study by the Nordic Lymphoma Group. *Blood,* **112,** 2687-2693.

Ghia, P., Nadel, B., Sander, B., Stamatopoulos, K. & Stevenson, F.K. (2017) Early stages in the ontogeny of small B-cell lymphomas: genetics and microenvironment. *Journal of internal medicine*.

Ghosh, S., Preet, A., Groopman, J.E. & Ganju, R.K. (2006) Cannabinoid receptor CB2 modulates the CXCL12/CXCR4-mediated chemotaxis of T lymphocytes. *Mol Immunol,* **43,** 2169-2179.

Glass, M. & Felder, C.C. (1997) Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors augments cAMP accumulation in striatal neurons: evidence for a Gs linkage to the CB1 receptor. *J Neurosci,* **17,** 5327-5333.

Grimsey, N.L., Goodfellow, C.E., Scotter, E.L., Dowie, M.J., Glass, M. & Graham, E.S. (2008) Specific detection of CB1 receptors; cannabinoid CB1 receptor antibodies are not all created equal! *J Neurosci Methods,* **171,** 78-86.

Gustafsson, K., Christensson, B., Sander, B. & Flygare, J. (2006) Cannabinoid receptor-mediated apoptosis induced by R(+)-methanandamide and Win55,212-2 is associated with ceramide accumulation and p38 activation in mantle cell lymphoma. *Molecular pharmacology,* **70,** 1612-1620.

Gustafsson, K., Wang, X., Severa, D., Eriksson, M., Kimby, E., Merup, M., Christensson, B., Flygare, J. & Sander, B. (2008) Expression of cannabinoid receptors type 1 and type 2 in non-Hodgkin lymphoma: growth inhibition by receptor activation. *International journal of cancer,* **123,** 1025-1033.

Gutkind, J.S. (2000) Regulation of mitogen-activated protein kinase signaling networks by G protein-coupled receptors. *Sci STKE,* **2000,** re1.

Hadzidimitriou, A., Agathangelidis, A., Darzentas, N., Murray, F., Delfau-Larue, M.H., Pedersen, L.B., Lopez, A.N., Dagklis, A., Rombout, P., Beldjord, K., Kolstad, A., Dreyling, M.H., Anagnostopoulos, A., Tsaftaris, A., Mavragani-Tsipidou, P., Rosenwald, A., Ponzoni, M., Groenen, P., Ghia, P., Sander, B., Papadaki, T., Campo, E., Geisler, C., Rosenquist, R., Davi, F., Pott, C. & Stamatopoulos, K. (2011) Is there a role for antigen selection in mantle cell lymphoma? Immunogenetic support from a series of 807 cases. *Blood,* **118,** 3088-3095.

Hallek, M. (2019) Chronic lymphocytic leukemia: 2020 update on diagnosis, risk stratification and treatment. *Am J Hematol,* **94,** 1266-1287.

Hallek, M., Shanafelt, T.D. & Eichhorst, B. (2018) Chronic lymphocytic leukaemia. *Lancet,* **391,** 1524-1537.

Hamblin, T.J., Davis, Z., Gardiner, A., Oscier, D.G. & Stevenson, F.K. (1999) Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood,* **94,** 1848-1854.

Hartmann, E.M., Rudelius, M., Burger, J.A. & Rosenwald, A. (2016) CCL3 chemokine expression by chronic lymphocytic leukemia cells orchestrates the composition of the microenvironment in lymph node infiltrates. *Leukemia & lymphoma,* **57,** 563-571.

Henson, S.E., Morford, T., Stein, M.P., Wall, R. & Malone, C.S. (2011) Candidate genes contributing to the aggressive phenotype of mantle cell lymphoma. *Acta Histochem,* **113,** 729-742.

Herbert, M., Coppieters, N., Lasham, A., Cao, H. & Reid, G. (2011) The importance of RT-qPCR primer design for the detection of siRNA-mediated mRNA silencing. *BMC Res Notes,* **4,** 148.

Herishanu, Y., Pérez-Galán, P., Liu, D., Biancotto, A., Pittaluga, S., Vire, B., Gibellini, F., Njuguna, N., Lee, E., Stennett, L., Raghavachari, N., Liu, P., McCoy, J.P., Raffeld, M., Stetler-Stevenson, M., Yuan, C., Sherry, R., Arthur, D.C., Maric, I., White, T., Marti, G.E., Munson, P., Wilson, W.H. & Wiestner, A. (2011) The lymph node microenvironment promotes B-cell receptor signaling, NF-kappaB activation, and tumor proliferation in chronic lymphocytic leukemia. *Blood,* **117,** 563-574.

Herkenham, M., Lynn, A.B., Little, M.D., Johnson, M.R., Melvin, L.S., de Costa, B.R. & Rice, K.C. (1990) Cannabinoid receptor localization in brain. *Proceedings of the National Academy of Sciences of the United States of America,* **87,** 1932-1936.

Hernandez, L., Fest, T., Cazorla, M., Teruya-Feldstein, J., Bosch, F., Peinado, M.A., Piris, M.A., Montserrat, E., Cardesa, A., Jaffe, E.S., Campo, E. & Raffeld, M. (1996) p53 gene mutations and protein overexpression are associated with aggressive variants of mantle cell lymphomas. *Blood,* **87,** 3351-3359.

Herrera, B., Carracedo, A., Diez-Zaera, M., Guzman, M. & Velasco, G. (2005) p38 MAPK is involved in CB2 receptor-induced apoptosis of human leukaemia cells. *FEBS letters,* **579,** 5084-5088.

Herrmann, F., Lochner, A., Philippen, H., Jauer, B. & Rühl, H. (1982) Imbalance of T cell subpopulations in patients with chronic lymphocytic leukaemia of the B cell type. *Clin Exp Immunol,* **49,** 157-162.

Hilger, D., Masureel, M. & Kobilka, B.K. (2018) Structure and dynamics of GPCR signaling complexes. *Nature Structural & Molecular Biology,* **25,** 4-12.

Hinton, D.R., Blanks, J.C., Fong, H.K., Casey, P.J., Hildebrandt, E. & Simons, M.I. (1990) Novel localization of a G protein, Gz-alpha, in neurons of brain and retina. *J Neurosci,* **10,** 2763-2770.

Hirt, C., Schüler, F., Dölken, L., Schmidt, C.A. & Dölken, G. (2004) Low prevalence of circulating t(11;14)(q13;q32)–positive cells in the peripheral blood of healthy individuals as detected by real-time quantitative PCR. *Blood,* **104,** 904-905.

Ho, M.K. & Wong, Y.H. (2001) G(z) signaling: emerging divergence from G(i) signaling. *Oncogene,* **20,** 1615-1625.

Honczarenko, M., Le, Y., Swierkowski, M., Ghiran, I., Glodek, A.M. & Silberstein, L.E. (2006) Human bone marrow stromal cells express a distinct set of biologically functional chemokine receptors. *Stem Cells,* **24,** 1030-1041.

Houghton, A.N. & Guevara-Patiño, J.A. (2004) Immune recognition of self in immunity against cancer. *J Clin Invest,* **114,** 468-471.

Howlett, A.C. & Abood, M.E. (2017) CB1 and CB2 Receptor Pharmacology. *Adv Pharmacol,* **80,** 169-206.

Howlett, A.C., Barth, F., Bonner, T.I., Cabral, G., Casellas, P., Devane, W.A., Felder, C.C., Herkenham, M., Mackie, K., Martin, B.R., Mechoulam, R. & Pertwee, R.G. (2002) International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev,* **54,** 161-202.

International CLL-IPI working group., I.C.-I.w.g. (2016) An international prognostic index for patients with chronic lymphocytic leukaemia (CLL-IPI): a meta-analysis of individual patient data. *Lancet Oncol,* **17,** 779-790.

Islam, T.C., Asplund, A.C., Lindvall, J.M., Nygren, L., Liden, J., Kimby, E., Christensson, B., Smith, C.I. & Sander, B. (2003) High level of cannabinoid receptor 1, absence of regulator of G protein signalling 13 and differential expression of Cyclin D1 in mantle cell lymphoma. *Leukemia,* **17,** 1880-1890.

Jacob, M.C., Agrawal, S., Chaperot, L., Giroux, C., Gressin, R., Marc'Hadour, F.L., Favre, M., Sotto, J.J., Bensa, J.C. & Plumas, J. (1999) Quantification of cellular adhesion molecules on malignant B cells from non-Hodgkin's lymphoma. *Leukemia,* **13,** 1428-1433.

Jain, P. & Wang, M. (2019) Mantle cell lymphoma: 2019 update on the diagnosis, pathogenesis, prognostication, and management. *Am J Hematol,* **94,** 710-725.

Jares, P., Colomer, D. & Campo, E. (2012) Molecular pathogenesis of mantle cell lymphoma. *J Clin Invest,* **122,** 3416-3423.

Jean-Gilles, L., Braitch, M., Latif, M.L., Aram, J., Fahey, A.J., Edwards, L.J., Robins, R.A., Tanasescu, R., Tighe, P.J., Gran, B., Showe, L.C., Alexander, S.P., Chapman, V., Kendall, D.A. & Constantinescu, C.S. (2015) Effects of pro-inflammatory cytokines on cannabinoid CB1 and CB2 receptors in immune cells. *Acta Physiol (Oxf),* **214,** 63-74.

Jeong, S.W. & Ikeda, S.R. (1998) G protein alpha subunit G alpha z couples neurotransmitter receptors to ion channels in sympathetic neurons. *Neuron,* **21,** 1201-1212.

Jerkeman, M., Hallek, M., Dreyling, M., Thieblemont, C., Kimby, E. & Staudt, L. (2017) Targeting of B-cell receptor signalling in B-cell malignancies. *Journal of internal medicine.*

Jhaveri, M.D., Richardson, D., Robinson, I., Garle, M.J., Patel, A., Sun, Y., Sagar, D.R., Bennett, A.J., Alexander, S.P., Kendall, D.A., Barrett, D.A. & Chapman, V. (2008) Inhibition of fatty acid amide hydrolase and cyclooxygenase-2 increases levels of endocannabinoid related molecules and produces analgesia via peroxisome proliferator-activated receptor-alpha in a model of inflammatory pain. *Neuropharmacology,* **55,** 85-93.

Jin, H.T., Ahmed, R. & Okazaki, T. (2011) Role of PD-1 in regulating T-cell immunity. *Curr Top Microbiol Immunol,* **350,** 17-37.

Joshi, N. & Onaivi, E.S. (2019) Endocannabinoid System Components: Overview and Tissue Distribution. In: *Recent Advances in Cannabinoid Physiology and Pathology* (ed. by Bukiya, A.N.), pp. 1-12. Springer International Publishing, Cham.

Kampa-Schittenhelm, K.M., Salitzky, O., Akmut, F., Illing, B., Kanz, L., Salih, H.R. & Schittenhelm, M.M. (2016) Dronabinol has preferential antileukemic activity in acute lymphoblastic and myeloid leukemia with lymphoid differentiation patterns. *BMC Cancer,* **16,** 25.

Kaplan, B.L. (2013) The role of CB1 in immune modulation by cannabinoids. *Pharmacol Ther,* **137,** 365-374.

Karmaus, P.W., Chen, W., Crawford, R.B., Harkema, J.R., Kaplan, B.L. & Kaminski, N.E. (2011) Deletion of cannabinoid receptors 1 and 2 exacerbates APC function to increase inflammation and cellular immunity during influenza infection. *Journal of leukocyte biology,* **90,** 983-995.

Katritch, V., Cherezov, V. & Stevens, R.C. (2013) Structure-function of the G protein-coupled receptor superfamily. *Annu Rev Pharmacol Toxicol,* **53,** 531-556.

Khuja, I., Yekhtin, Z., Or, R. & Almogi-Hazan, O. (2019) Cannabinoids Reduce Inflammation but Inhibit Lymphocyte Recovery in Murine Models of Bone Marrow Transplantation. *Int J Mol Sci,* **20**.

Kishimoto, S., Gokoh, M., Oka, S., Muramatsu, M., Kajiwara, T., Waku, K. & Sugiura, T. (2003) 2-arachidonoylglycerol induces the migration of HL-60 cells differentiated into macrophage-like cells and human peripheral blood monocytes through the cannabinoid CB2 receptor-dependent mechanism. *The Journal of biological chemistry,* **278,** 24469-24475.

Kishimoto, S., Muramatsu, M., Gokoh, M., Oka, S., Waku, K. & Sugiura, T. (2005) Endogenous cannabinoid receptor ligand induces the migration of human natural killer cells. *J Biochem,* **137,** 217-223.

Kishimoto, S., Oka, S., Gokoh, M. & Sugiura, T. (2006) Chemotaxis of human peripheral blood eosinophils to 2-arachidonoylglycerol: comparison with other eosinophil chemoattractants. *Int Arch Allergy Immunol,* **140 Suppl 1,** 3-7.

Klein, C., Karanges, E., Spiro, A., Wong, A., Spencer, J., Huynh, T., Gunasekaran, N., Karl, T., Long, L.E., Huang, X.F., Liu, K., Arnold, J.C. & McGregor, I.S. (2011) Cannabidiol potentiates Delta(9)-tetrahydrocannabinol (THC) behavioural effects and alters THC pharmacokinetics during acute and chronic treatment in adolescent rats. *Psychopharmacology (Berl),* **218,** 443-457.

Klein, T.W. (2005) Cannabinoid-based drugs as anti-inflammatory therapeutics. *Nat Rev Immunol,* **5,** 400-411.

Kohno, M., Hasegawa, H., Inoue, A., Muraoka, M., Miyazaki, T., Oka, K. & Yasukawa, M. (2006) Identification of N-arachidonylglycine as the endogenous ligand for orphan G-protein-coupled receptor GPR18. *Biochemical and biophysical research communications,* **347,** 827-832.

Kolstad, A., Pedersen, L.B., Eskelund, C.W., Husby, S., Grønbæk, K., Jerkeman, M., Laurell, A., Räty, R., Elonen, E., Andersen, N.S., Brown, P.D., Kimby, E., Bentzen, H., Sundström, C., Ehinger, M., Karjalainen-Lindsberg, M.L., Delabie, J., Ralfkiær, E., Fagerli, U.M., Nilsson-Ehle, H., Lauritzsen, G.F., Kuittinen, O., Niemann, C. & Geisler, C.H. (2017) Molecular Monitoring after Autologous Stem Cell Transplantation and Preemptive Rituximab Treatment of Molecular Relapse; Results from the Nordic Mantle Cell Lymphoma Studies (MCL2 and MCL3) with Median Follow-Up of 8.5 Years. *Biol Blood Marrow Transplant,* **23,** 428-435.

Kose, S., Aerts-Kaya, F., Kopru, C.Z., Nemutlu, E., Kuskonmaz, B., Karaosmanoglu, B., Taskiran, E.Z., Altun, B., Uckan Cetinkaya, D. & Korkusuz, P. (2018) Human bone marrow mesenchymal stem cells secrete

endocannabinoids that stimulate in vitro hematopoietic stem cell migration effectively comparable to beta-adrenergic stimulation. *Exp Hematol,* **57,** 30-41.e31.

Kozela, E., Juknat, A., Kaushansky, N., Rimmerman, N., Ben-Nun, A. & Vogel, Z. (2013) Cannabinoids decrease the th17 inflammatory autoimmune phenotype. *J Neuroimmune Pharmacol,* **8,** 1265-1276.

Kurtova, A.V., Tamayo, A.T., Ford, R.J. & Burger, J.A. (2009) Mantle cell lymphoma cells express high levels of CXCR4, CXCR5, and VLA-4 (CD49d): importance for interactions with the stromal microenvironment and specific targeting. *Blood,* **113,** 4604-4613.

Küppers, R. & Dalla-Favera, R. (2001) Mechanisms of chromosomal translocations in B cell lymphomas. *Oncogene,* **20,** 5580-5594.

Köse, S., Aerts-Kaya, F., Köprü Ç, Z., Nemutlu, E., Kuşkonmaz, B., Karaosmanoğlu, B., Taşkıran, E.Z., Altun, B., Uçkan Çetinkaya, D. & Korkusuz, P. (2018) Human bone marrow mesenchymal stem cells secrete endocannabinoids that stimulate in vitro hematopoietic stem cell migration effectively comparable to beta-adrenergic stimulation. *Exp Hematol,* **57,** 30-41.e31.

Laezza, C., Pagano, C., Navarra, G., Pastorino, O., Proto, M.C., Fiore, D., Piscopo, C., Gazzerro, P. & Bifulco, M. (2020) The Endocannabinoid System: A Target for Cancer Treatment. *Int J Mol Sci,* **21**.

Lagneaux, L., Delforge, A., Bron, D., De Bruyn, C. & Stryckmans, P. (1998) Chronic lymphocytic leukemic B cells but not normal B cells are rescued from apoptosis by contact with normal bone marrow stromal cells. *Blood,* **91,** 2387-2396.

Lagneaux, L., Delforge, A., De Bruyn, C., Bernier, M. & Bron, D. (1999) Adhesion to bone marrow stroma inhibits apoptosis of chronic lymphocytic leukemia cells. *Leukemia & lymphoma,* **35,** 445-453.

Lam, K.P., Kühn, R. & Rajewsky, K. (1997) In vivo ablation of surface immunoglobulin on mature B cells by inducible gene targeting results in rapid cell death. *Cell,* **90,** 1073-1083.

Langerak, A.W., Davi, F., Ghia, P., Hadzidimitriou, A., Murray, F., Potter, K.N., Rosenquist, R., Stamatopoulos, K. & Belessi, C. (2011) Immunoglobulin sequence analysis and prognostication in CLL: guidelines from the ERIC review board for reliable interpretation of problematic cases. *Leukemia,* **25,** 979-984.

Larose, M.C., Turcotte, C., Chouinard, F., Ferland, C., Martin, C., Provost, V., Laviolette, M. & Flamand, N. (2014) Mechanisms of human eosinophil migration induced by the combination of IL-5 and the endocannabinoid 2-arachidonoyl-glycerol. *The Journal of allergy and clinical immunology,* **133,** 1480-1482, 1482.e1481-1483.

LeBien, T.W. & Tedder, T.F. (2008) B lymphocytes: how they develop and function. *Blood,* **112,** 1570-1580.

Lecluse, Y., Lebailly, P., Roulland, S., Gac, A.C., Nadel, B. & Gauduchon, P. (2009) t(11;14)-positive clones can persist over a long period of time in the peripheral blood of healthy individuals. *Leukemia,* **23,** 1190-1193.

Li, H.-L. (1974) An Archaeological and Historical Account of Cannabis in China. *Economic Botany,* **28,** 437-448.

Li, H., Ghazanfari, R., Zacharaki, D., Lim, H.C. & Scheding, S. (2016) Isolation and characterization of primary bone marrow mesenchymal stromal cells. *Ann N Y Acad Sci,* **1370,** 109-118.

Li, J.Y., Gaillard, F., Moreau, A., Harousseau, J.L., Laboisse, C., Milpied, N., Bataille, R. & Avet-Loiseau, H. (1999) Detection of translocation t(11;14)(q13;q32) in mantle cell lymphoma by fluorescence in situ hybridization. *Am J Pathol,* **154,** 1449-1452.

Lieber, M. (2009) Immunoglobulin Gene Rearrangements. In: *eLS*.

Long, T., Wagner, M., Demske, D., Leipe, C. & Tarasov, P.E. (2017) Cannabis in Eurasia: origin of human use and Bronze Age trans-continental connections. *Vegetation History and Archaeobotany,* **26,** 245-258.

López-Valero, I., Saiz-Ladera, C., Torres, S., Hernández-Tiedra, S., García-Taboada, E., Rodríguez-Fornés, F., Barba, M., Dávila, D., Salvador-Tormo, N., Guzmán, M., Sepúlveda, J.M., Sánchez-Gómez, P., Lorente, M. & Velasco, G. (2018) Targeting Glioma Initiating Cells with A combined therapy of cannabinoids and temozolomide. *Biochem Pharmacol,* **157,** 266-274.

Lutzny, G., Kocher, T., Schmidt-Supprian, M., Rudelius, M., Klein-Hitpass, L., Finch, Andrew J., Dürig, J., Wagner, M., Haferlach, C., Kohlmann, A., Schnittger, S., Seifert, M., Wanninger, S., Zaborsky, N., Oostendorp, R., Ruland, J., Leitges, M., Kuhnt, T., Schäfer, Y., Lampl, B., Peschel, C., Egle, A. & Ringshausen, I. (2013) Protein Kinase C-β-Dependent Activation of NF-κB in Stromal Cells Is

Indispensable for the Survival of Chronic Lymphocytic Leukemia B Cells In Vivo. *Cancer Cell,* **23,** 77-92.

Lwin, T., Crespo, L.A., Wu, A., Dessureault, S., Shu, H.B., Moscinski, L.C., Sotomayor, E., Dalton, W.S. & Tao, J. (2009) Lymphoma cell adhesion-induced expression of B cell-activating factor of the TNF family in bone marrow stromal cells protects non-Hodgkin's B lymphoma cells from apoptosis. *Leukemia,* **23,** 170-177.

Lwin, T., Hazlehurst, L.A., Li, Z., Dessureault, S., Sotomayor, E., Moscinski, L.C., Dalton, W.S. & Tao, J. (2007) Bone marrow stromal cells prevent apoptosis of lymphoma cells by upregulation of anti-apoptotic proteins associated with activation of NF-kappaB (RelB/p52) in non-Hodgkin's lymphoma cells. *Leukemia,* **21,** 1521-1531.

Ma, Q., Jones, D., Borghesani, P.R., Segal, R.A., Nagasawa, T., Kishimoto, T., Bronson, R.T. & Springer, T.A. (1998) Impaired B-lymphopoiesis, myelopoiesis, and derailed cerebellar neuron migration in CXCR4- and SDF-1-deficient mice. **95,** 9448-9453.

Macari, D.M., Gbadamosi, B., Jaiyesimi, I. & Gaikazian, S. (2020) Medical Cannabis in Cancer Patients: A Survey of a Community Hematology Oncology Population. *Am J Clin Oncol*.

Marchalant, Y., Brownjohn, P.W., Bonnet, A., Kleffmann, T. & Ashton, J.C. (2014) Validating Antibodies to the Cannabinoid CB2 Receptor: Antibody Sensitivity Is Not Evidence of Antibody Specificity. *J Histochem Cytochem,* **62,** 395-404.

Martell, K., Fairchild, A., LeGerrier, B., Sinha, R., Baker, S., Liu, H., Ghose, A., Olivotto, I.A. & Kerba, M. (2018) Rates of cannabis use in patients with cancer. *Curr Oncol,* **25,** 219-225.

Martinez-Pinilla, E., Varani, K., Reyes-Resina, I., Angelats, E., Vincenzi, F., Ferreiro-Vera, C., Oyarzabal, J., Canela, E.I., Lanciego, J.L., Nadal, X., Navarro, G., Borea, P.A. & Franco, R. (2017) Binding and Signaling Studies Disclose a Potential Allosteric Site for Cannabidiol in Cannabinoid CB2 Receptors. *Front Pharmacol,* **8,** 744.

Matsuda, L.A., Bonner, T.I. & Lolait, S.J. (1993) Localization of cannabinoid receptor mRNA in rat brain. *J Comp Neurol,* **327,** 535-550.

Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C. & Bonner, T.I. (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature,* **346,** 561-564.

Matsuoka, M., Itoh, H. & Kaziro, Y. (1990) Characterization of the human gene for Gx alpha, a pertussis toxin-insensitive regulatory GTP-binding protein. *The Journal of biological chemistry,* **265,** 13215-13220.

McGuinness, D., Malikzay, A., Visconti, R., Lin, K., Bayne, M., Monsma, F. & Lunn, C.A. (2009) Characterizing cannabinoid CB2 receptor ligands using DiscoveRx PathHunter beta-arrestin assay. *J Biomol Screen,* **14,** 49-58.

McHugh, D., Tanner, C., Mechoulam, R., Pertwee, R.G. & Ross, R.A. (2008) Inhibition of human neutrophil chemotaxis by endogenous cannabinoids and phytocannabinoids: evidence for a site distinct from CB1 and CB2. *Molecular pharmacology,* **73,** 441-450.

McKallip, R.J., Lombard, C., Fisher, M., Martin, B.R., Ryu, S., Grant, S., Nagarkatti, P.S. & Nagarkatti, M. (2002) Targeting CB2 cannabinoid receptors as a novel therapy to treat malignant lymphoblastic disease. *Blood,* **100,** 627-634.

McKallip, R.J., Nagarkatti, M. & Nagarkatti, P.S. (2005) Delta-9-tetrahydrocannabinol enhances breast cancer growth and metastasis by suppression of the antitumor immune response. *Journal of immunology,* **174,** 3281-3289.

Medina, D.J., Goodell, L., Glod, J., Gelinas, C., Rabson, A.B. & Strair, R.K. (2012) Mesenchymal stromal cells protect mantle cell lymphoma cells from spontaneous and drug-induced apoptosis through secretion of B-cell activating factor and activation of the canonical and non-canonical nuclear factor kappaB pathways. *Haematologica,* **97,** 1255-1263.

Melchers, F. (2015) Checkpoints that control B cell development. *J Clin Invest,* **125,** 2203-2210.

Merolle, M.I., Ahmed, M., Nomie, K. & Wang, M.L. (2018) The B cell receptor signaling pathway in mantle cell lymphoma. *Oncotarget,* **9,** 25332-25341.

Middle, S., Coupland, S.E., Taktak, A., Kidgell, V., Slupsky, J.R., Pettitt, A.R. & Till, K.J. (2015) Immunohistochemical analysis indicates that the anatomical location of B-cell non-Hodgkin's lymphoma is determined by differentially expressed chemokine receptors, sphingosine-1-phosphate receptors and integrins. *Exp Hematol Oncol,* **4,** 10.

Miyake, K., Hasunuma, Y., Yagita, H. & Kimoto, M. (1992) Requirement for VLA-4 and VLA-5 integrins in lymphoma cells binding to and migration beneath stromal cells in culture. *The Journal of cell biology,* **119,** 653-662.

Molica, S., Dattilo, A., Mannella, A., Levato, D. & Levato, L. (1995) Expression on leukemic cells and serum circulating levels of intercellular adhesion molecule-1 (ICAM-1) in B-cell chronic lymphocytic leukemia: Implications for prognosis. *Leukemia research,* **19,** 573-580.

Montresor, A., Toffali, L., Rigo, A., Ferrarini, I., Vinante, F. & Laudanna, C. (2018) CXCR4- and BCR-triggered integrin activation in B-cell chronic lymphocytic leukemia cells depends on JAK2-activated Bruton's tyrosine kinase. *Oncotarget,* **9,** 35123-35140.

Moore, C.A., Milano, S.K. & Benovic, J.L. (2007) Regulation of receptor trafficking by GRKs and arrestins. *Annu Rev Physiol,* **69,** 451-482.

Morales, P. & Jagerovic, N. (2016) Advances Towards The Discovery of GPR55 Ligands. *Curr Med Chem,* **23,** 2087-2100.

Morales, P. & Reggio, P.H. (2017) An Update on Non-CB(1), Non-CB(2) Cannabinoid Related G-Protein-Coupled Receptors. *Cannabis Cannabinoid Res,* **2,** 265-273.

Mundt, F., Merrien, M., Nygren, L., Sutton, L.A., Christensson, B., Wahlin, B.E., Rosenquist, R., Sander, B. & Wasik, A.M. (2019) Expression of GNAZ, encoding the Gα(z) protein, predicts survival in mantle cell lymphoma. *British journal of haematology,* **185,** 708-712.

Munro, S., Thomas, K.L. & Abu-Shaar, M. (1993) Molecular characterization of a peripheral receptor for cannabinoids. *Nature,* **365,** 61-65.

Muppidi, J.R., Arnon, T.I., Bronevetsky, Y., Veerapen, N., Tanaka, M., Besra, G.S. & Cyster, J.G. (2011) Cannabinoid receptor 2 positions and retains marginal zone B cells within the splenic marginal zone. *The Journal of experimental medicine,* **208,** 1941-1948.

Müller, G., Höpken, U.E., Stein, H. & Lipp, M. (2002) Systemic immunoregulatory and pathogenic functions of homeostatic chemokine receptors. *Journal of leukocyte biology,* **72,** 1-8.

Nadeu, F., Martin-Garcia, D., Clot, G., Díaz-Navarro, A., Duran-Ferrer, M., Navarro, A., Vilarrasa-Blasi, R., Kulis, M., Royo, R., Gutiérrez-Abril, J., Valdés-Mas, R., López, C., Chapaprieta, V., Puiggròs, M., Castellano, G., Costa, D., Aymerich, M., Jares, P., Espinet, B., Muntañola, A., Ribera-Cortada, I., Siebert, R., Colomer, D., Torrents, D., Gine, E., López-Guillermo, A., Küppers, R., Martin-Subero, I., Puente, X.S., Beà, S. & Campo, E. (2020) Genomic and epigenomic insights into the origin, pathogenesis and clinical behavior of mantle cell lymphoma subtypes. *Blood*.

Nagahama, M., Usui, S., Shinohara, T., Yamaguchi, T., Tani, K. & Tagaya, M. (2002) Inactivation of Galpha(z) causes disassembly of the Golgi apparatus. *J Cell Sci,* **115,** 4483-4493.

Nagy, Á., Lánczky, A., Menyhárt, O. & Győrffy, B. (2018) Validation of miRNA prognostic power in hepatocellular carcinoma using expression data of independent datasets. *Scientific reports,* **8,** 9227.

Nassef Kadry Naguib Roufaiel, M., Wells, J.W. & Steptoe, R.J. (2015) Impaired T-Cell Function in B-Cell Lymphoma: A Direct Consequence of Events at the Immunological Synapse? *Front Immunol,* **6,** 258.

Nasser, M.W., Qamri, Z., Deol, Y.S., Smith, D., Shilo, K., Zou, X. & Ganju, R.K. (2011) Crosstalk between chemokine receptor CXCR4 and cannabinoid receptor CB2 in modulating breast cancer growth and invasion. *PloS one,* **6,** e23901.

Neves, S.R., Ram, P.T. & Iyengar, R. (2002) G protein pathways. *Science,* **296,** 1636-1639.

Nielsen, S., Germanos, R., Weier, M., Pollard, J., Degenhardt, L., Hall, W., Buckley, N. & Farrell, M. (2018) The Use of Cannabis and Cannabinoids in Treating Symptoms of Multiple Sclerosis: a Systematic Review of Reviews. *Curr Neurol Neurosci Rep,* **18,** 8.

Nishimura, H., Akiyama, T., Monobe, Y., Matsubara, K., Igarashi, Y., Abe, M., Sugihara, T. & Sadahira, Y. (2010) Expression of sphingosine-1-phosphate receptor 1 in mantle cell lymphoma. *Mod Pathol,* **23,** 439-449.

Nogueiras, R., Diaz-Arteaga, A., Lockie, S.H., Velasquez, D.A., Tschop, J., Lopez, M., Cadwell, C.C., Dieguez, C. & Tschop, M.H. (2009) The endocannabinoid system: role in glucose and energy metabolism. *Pharmacol Res,* **60,** 93-98.

Nore, B.F., Vargas, L., Mohamed, A.J., Brandén, L.J., Bäckesjö, C.M., Islam, T.C., Mattsson, P.T., Hultenby, K., Christensson, B. & Smith, C.I. (2000) Redistribution of Bruton's tyrosine kinase by activation of phosphatidylinositol 3-kinase and Rho-family GTPases. *Eur J Immunol,* **30,** 145-154.

Nugent, A. & Proia, R.L. (2017) The role of G protein-coupled receptors in lymphoid malignancies. *Cell Signal,* **39,** 95-107.

Nunes, C., Wong, R., Mason, M., Fegan, C., Man, S. & Pepper, C. (2012) Expansion of a CD8(+)PD-1(+) replicative senescence phenotype in early stage CLL patients is associated with inverted CD4:CD8 ratios and disease progression. *Clinical cancer research : an official journal of the American Association for Cancer Research,* **18,** 678-687.

Nygren, L., Baumgartner Wennerholm, S., Klimkowska, M., Christensson, B., Kimby, E. & Sander, B. (2012) Prognostic role of SOX11 in a population-based cohort of mantle cell lymphoma. *Blood,* **119,** 4215-4223.

Nygren, L., Wasik, A.M., Baumgartner-Wennerholm, S., Jeppsson-Ahlberg, A., Klimkowska, M., Andersson, P., Buhrkuhl, D., Christensson, B., Kimby, E., Wahlin, B.E. & Sander, B. (2014) T-cell levels are prognostic in mantle cell lymphoma. *Clinical cancer research : an official journal of the American Association for Cancer Research,* **20,** 6096-6104.

O'Hayre, M., Salanga, C.L., Kipps, T.J., Messmer, D., Dorrestein, P.C. & Handel, T.M. (2010) Elucidating the CXCL12/CXCR4 signaling network in chronic lymphocytic leukemia through phosphoproteomics analysis. *PloS one,* **5,** e11716.

Ofek, O., Karsak, M., Leclerc, N., Fogel, M., Frenkel, B., Wright, K., Tam, J., Attar-Namdar, M., Kram, V., Shohami, E., Mechoulam, R., Zimmer, A. & Bab, I. (2006) Peripheral cannabinoid receptor, CB2, regulates bone mass. *Proceedings of the National Academy of Sciences of the United States of America,* **103,** 696-701.

Oka, S., Ikeda, S., Kishimoto, S., Gokoh, M., Yanagimoto, S., Waku, K. & Sugiura, T. (2004) 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand, induces the migration of EoL-1 human eosinophilic leukemia cells and human peripheral blood eosinophils. *Journal of leukocyte biology,* **76,** 1002-1009.

Okada, T., Ngo, V.N., Ekland, E.H., Förster, R., Lipp, M., Littman, D.R. & Cyster, J.G. (2002) Chemokine requirements for B cell entry to lymph nodes and Peyer's patches. *The Journal of experimental medicine,* **196,** 65-75.

Ondrisova, L. & Mraz, M. (2020) Genetic and Non-Genetic Mechanisms of Resistance to BCR Signaling Inhibitors in B Cell Malignancies. *Front Oncol,* **10,** 591577.

Orchard, J., Garand, R., Davis, Z., Babbage, G., Sahota, S., Matutes, E., Catovsky, D., Thomas, P.W., Avet-Loiseau, H. & Oscier, D. (2003) A subset of t(11;14) lymphoma with mantle cell features displays mutated IgVH genes and includes patients with good prognosis, nonnodal disease. *Blood,* **101,** 4975-4981.

Oscier, D.G., Gardiner, A.C., Mould, S.J., Glide, S., Davis, Z.A., Ibbotson, R.E., Corcoran, M.M., Chapman, R.M., Thomas, P.W., Copplestone, J.A., Orchard, J.A. & Hamblin, T.J. (2002) Multivariate analysis of prognostic factors in CLL: clinical stage, IGVH gene mutational status, and loss or mutation of the p53 gene are independent prognostic factors. *Blood,* **100,** 1177-1184.

Panayiotidis, P., Jones, D., Ganeshaguru, K., Foroni, L. & Hoffbrand, A.V. (1996) Human bone marrow stromal cells prevent apoptosis and support the survival of chronic lymphocytic leukaemia cells in vitro. *British journal of haematology,* **92,** 97-103.

Patrussi, L., Capitani, N., Martini, V., Pizzi, M., Trimarco, V., Frezzato, F., Marino, F., Semenzato, G., Trentin, L. & Baldari, C.T. (2015) Enhanced Chemokine Receptor Recycling and Impaired S1P1 Expression Promote Leukemic Cell Infiltration of Lymph Nodes in Chronic Lymphocytic Leukemia. *Cancer Res,* **75,** 4153-4163.

Pereira, J.P., An, J., Xu, Y., Huang, Y. & Cyster, J.G. (2009) Cannabinoid receptor 2 mediates the retention of immature B cells in bone marrow sinusoids. *Nature immunology,* **10,** 403-411.

Perez-Andres, M., Paiva, B., Nieto, W.G., Caraux, A., Schmitz, A., Almeida, J., Vogt, R.F., Jr., Marti, G.E., Rawstron, A.C., Van Zelm, M.C., Van Dongen, J.J., Johnsen, H.E., Klein, B. & Orfao, A. (2010) Human peripheral blood B-cell compartments: a crossroad in B-cell traffic. *Cytometry B Clin Cytom,* **78 Suppl 1,** S47-60.

Pergam, S.A., Woodfield, M.C., Lee, C.M., Cheng, G.S., Baker, K.K., Marquis, S.R. & Fann, J.R. (2017) Cannabis use among patients at a comprehensive cancer center in a state with legalized medicinal and recreational use. *Cancer,* **123,** 4488-4497.

Pertwee, R.G. (2008) The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. *Br J Pharmacol,* **153,** 199-215.

Pertwee, R.G. (2015) Endocannabinoids and Their Pharmacological Actions. *Handb Exp Pharmacol,* **231,** 1-37.

Pertwee, R.G., Howlett, A.C., Abood, M.E., Alexander, S.P., Di Marzo, V., Elphick, M.R., Greasley, P.J., Hansen, H.S., Kunos, G., Mackie, K., Mechoulam, R. & Ross, R.A. (2010) International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB(1) and CB(2). *Pharmacol Rev,* **62,** 588-631.

Peyravian, N., Deo, S., Daunert, S. & Jimenez, J.J. (2020) Cannabidiol as a Novel Therapeutic for Immune Modulation. *Immunotargets Ther,* **9,** 131-140.

Piccaluga, P.P., Navari, M., Visani, A., Rigotti, F., Agostinelli, C., Righi, S., Diani, E., Ligozzi, M., Carelli, M., Ponti, C., Bon, I., Zipeto, D., Landolfo, S. & Gibellini, D. (2019) Interferon gamma inducible protein 16 (IFI16) expression is reduced in mantle cell lymphoma. *Heliyon,* **5,** e02643.

Powles, T., te Poele, R., Shamash, J., Chaplin, T., Propper, D., Joel, S., Oliver, T. & Liu, W.M. (2005) Cannabis-induced cytotoxicity in leukemic cell lines: the role of the cannabinoid receptors and the MAPK pathway. *Blood,* **105,** 1214-1221.

Punzo, F., Manzo, I., Tortora, C., Pota, E., Angelo, V.D., Bellini, G., Di Paola, A., Verace, F., Casale, F. & Rossi, F. (2018) Effects of CB2 and TRPV1 receptors' stimulation in pediatric acute T-lymphoblastic leukemia. *Oncotarget,* **9,** 21244-21258.

Rajagopal, S., Rajagopal, K. & Lefkowitz, R.J. (2010) Teaching old receptors new tricks: biasing seven-transmembrane receptors. *Nat Rev Drug Discov,* **9,** 373-386.

Ramsay, A.G., Clear, A.J., Fatah, R. & Gribben, J.G. (2012) Multiple inhibitory ligands induce impaired T-cell immunologic synapse function in chronic lymphocytic leukemia that can be blocked with lenalidomide: establishing a reversible immune evasion mechanism in human cancer. *Blood,* **120,** 1412-1421.

Rayman, N., Lam, K.H., Laman, J.D., Simons, P.J., Löwenberg, B., Sonneveld, P. & Delwel, R. (2004) Distinct expression profiles of the peripheral cannabinoid receptor in lymphoid tissues depending on receptor activation status. *Journal of immunology,* **172,** 2111-2117.

Rayman, N., Lam, K.H., Van Leeuwen, J., Mulder, A.H., Budel, L.M., Löwenberg, B., Sonneveld, P. & Delwel, R. (2007) The expression of the peripheral cannabinoid receptor on cells of the immune system and non-Hodgkin's lymphomas. *Leukemia & lymphoma,* **48,** 1389-1399.

Rickert, R.C. (2013) New insights into pre-BCR and BCR signalling with relevance to B cell malignancies. *Nat Rev Immunol,* **13,** 578-591.

Rikitake, Y. & Takai, Y. (2011) Chapter three - Directional Cell Migration: Regulation by Small G Proteins, Nectin-like Molecule-5, and Afadin. In: *International Review of Cell and Molecular Biology* (ed. by Jeon, K.W.), Vol. 287, pp. 97-143. Academic Press.

Rosenquist, R., Ghia, P., Hadzidimitriou, A., Sutton, L.A., Agathangelidis, A., Baliakas, P., Darzentas, N., Giudicelli, V., Lefranc, M.P., Langerak, A.W., Belessi, C., Davi, F. & Stamatopoulos, K. (2017) Immunoglobulin gene sequence analysis in chronic lymphocytic leukemia: updated ERIC recommendations. *Leukemia,* **31,** 1477-1481.

Rossi, F., Bernardo, M.E., Bellini, G., Luongo, L., Conforti, A., Manzo, I., Guida, F., Cristino, L., Imperatore, R., Petrosino, S., Nobili, B., Di Marzo, V., Locatelli, F. & Maione, S. (2013) The cannabinoid receptor type 2 as mediator of mesenchymal stromal cell immunosuppressive properties. *PloS one,* **8,** e80022.

Royo, C., Navarro, A., Clot, G., Salaverria, I., Giné, E., Jares, P., Colomer, D., Wiestner, A., Wilson, W.H., Vegliante, M.C., Fernandez, V., Hartmann, E.M., Trim, N., Erber, W.N., Swerdlow, S.H., Klapper, W., Dyer, M.J., Vargas-Pabón, M., Ott, G., Rosenwald, A., Siebert, R., López-Guillermo, A., Campo, E. &

Beà, S. (2012) Non-nodal type of mantle cell lymphoma is a specific biological and clinical subgroup of the disease. *Leukemia,* **26,** 1895-1898.

Rudelius, M., Rosenfeldt, M.T., Leich, E., Rauert-Wunderlich, H., Solimando, A.G., Beilhack, A., Ott, G. & Rosenwald, A. (2018) Inhibition of focal adhesion kinase overcomes resistance of mantle cell lymphoma to ibrutinib in the bone marrow microenvironment. *Haematologica,* **103,** 116-125.

Ryberg, E., Vu, H.K., Larsson, N., Groblewski, T., Hjorth, S., Elebring, T., Sjögren, S. & Greasley, P.J. (2005) Identification and characterisation of a novel splice variant of the human CB1 receptor. *FEBS letters,* **579,** 259-264.

Saba, N.S., Liu, D., Herman, S.E., Underbayev, C., Tian, X., Behrend, D., Weniger, M.A., Skarzynski, M., Gyamfi, J., Fontan, L., Melnick, A., Grant, C., Roschewski, M., Navarro, A., Bea, S., Pittaluga, S., Dunleavy, K., Wilson, W.H. & Wiestner, A. (2016) Pathogenic role of B-cell receptor signaling and canonical NF-kappaB activation in mantle cell lymphoma. *Blood,* **128,** 82-92.

Sadeghi, L., Arvidsson, G., Merrien, M., A, M.W., Görgens, A., Smith, C.I.E., Sander, B. & Wright, A.P. (2020) Differential B-Cell Receptor Signaling Requirement for Adhesion of Mantle Cell Lymphoma Cells to Stromal Cells. *Cancers (Basel),* **12**.

Sailler, S., Schmitz, K., Jager, E., Ferreiros, N., Wicker, S., Zschiebsch, K., Pickert, G., Geisslinger, G., Walter, C., Tegeder, I. & Lotsch, J. (2014) Regulation of circulating endocannabinoids associated with cancer and metastases in mice and humans. *Oncoscience,* **1,** 272-282.

Sait, L.G., Sula, A., Ghovanloo, M.R., Hollingworth, D., Ruben, P.C. & Wallace, B.A. (2020) Cannabidiol interactions with voltage-gated sodium channels. *Elife,* **9**.

Sander, B., Quintanilla-Martinez, L., Ott, G., Xerri, L., Kuzu, I., Chan, J.K., Swerdlow, S.H. & Campo, E. (2016) Mantle cell lymphoma--a spectrum from indolent to aggressive disease. *Virchows Archiv : an international journal of pathology,* **468,** 245-257.

Sasaki, Y. & Iwai, K. (2016) Roles of the NF-κB Pathway in B-Lymphocyte Biology. *Curr Top Microbiol Immunol,* **393,** 177-209.

Schaller, M.D. (2010) Cellular functions of FAK kinases: insight into molecular mechanisms and novel functions. *J Cell Sci,* **123,** 1007-1013.

Schwarz, R., Ramer, R. & Hinz, B. (2018) Targeting the endocannabinoid system as a potential anticancer approach. *Drug metabolism reviews,* **50,** 26-53.

Seda, V. & Mraz, M. (2015) B-cell receptor signalling and its crosstalk with other pathways in normal and malignant cells. *European journal of haematology,* **94,** 193-205.

Shanafelt, T.D., Geyer, S.M., Bone, N.D., Tschumper, R.C., Witzig, T.E., Nowakowski, G.S., Zent, C.S., Call, T.G., Laplant, B., Dewald, G.W., Jelinek, D.F. & Kay, N.E. (2008) CD49d expression is an independent predictor of overall survival in patients with chronic lymphocytic leukaemia: a prognostic parameter with therapeutic potential. *British journal of haematology,* **140,** 537-546.

Smith, C.I. (2017) From identification of the BTK kinase to effective management of leukemia. *Oncogene,* **36,** 2045-2053.

Spaargaren, M., Beuling, E.A., Rurup, M.L., Meijer, H.P., Klok, M.D., Middendorp, S., Hendriks, R.W. & Pals, S.T. (2003) The B cell antigen receptor controls integrin activity through Btk and PLCgamma2. *The Journal of experimental medicine,* **198,** 1539-1550.

Strati, P., Parikh, S.A., Chaffee, K.G., Achenbach, S.J., Slager, S.L., Call, T.G., Ding, W., Jelinek, D.F., Hanson, C.A., Kay, N.E. & Shanafelt, T.D. (2017) CD49d associates with nodal presentation and subsequent development of lymphadenopathy in patients with chronic lymphocytic leukaemia. *British journal of haematology,* **178,** 99-105.

Sugiura, T., Kishimoto, S., Oka, S. & Gokoh, M. (2006) Biochemistry, pharmacology and physiology of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand. *Prog Lipid Res,* **45,** 405-446.

Sutton, L.A., Agathangelidis, A., Belessi, C., Darzentas, N., Davi, F., Ghia, P., Rosenquist, R. & Stamatopoulos, K. (2013) Antigen selection in B-cell lymphomas--tracing the evidence. *Seminars in cancer biology,* **23,** 399-409.

Sutton, L.A., Hadzidimitriou, A., Baliakas, P., Agathangelidis, A., Langerak, A.W., Stilgenbauer, S., Pospisilova, S., Davis, Z., Forconi, F., Davi, F., Ghia, P., Rosenquist, R. & Stamatopoulos, K. (2017) Immunoglobulin genes in chronic lymphocytic leukemia: key to understanding the disease and improving risk stratification. *Haematologica,* **102,** 968-971.

Swerdlow, S.H., Campo, E., Pileri, S.A., Harris, N.L., Stein, H., Siebert, R., Advani, R., Ghielmini, M., Salles, G.A., Zelenetz, A.D. & Jaffe, E.S. (2016) The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood,* **127,** 2375-2390.

Syrovatkina, V., Alegre, K.O., Dey, R. & Huang, X.Y. (2016) Regulation, Signaling, and Physiological Functions of G-Proteins. *J Mol Biol,* **428,** 3850-3868.

Tan, W., Martin, D. & Gutkind, J.S. (2006) The Galpha13-Rho signaling axis is required for SDF-1-induced migration through CXCR4. *The Journal of biological chemistry,* **281,** 39542-39549.

Teicher, B.A. & Fricker, S.P. (2010) CXCL12 (SDF-1)/CXCR4 pathway in cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research,* **16,** 2927-2931.

Ten Hacken, E. & Burger, J.A. (2016) Microenvironment interactions and B-cell receptor signaling in Chronic Lymphocytic Leukemia: Implications for disease pathogenesis and treatment. *Biochimica et biophysica acta,* **1863,** 401-413.

Terol, M.J., López-Guillermo, A., Bosch, F., Villamor, N., Cid, M.C., Campo, E. & Montserrat, E. (1999) Expression of beta-integrin adhesion molecules in non-Hodgkin's lymphoma: correlation with clinical and evolutive features. *J Clin Oncol,* **17,** 1869-1875.

Tham, M., Yilmaz, O., Alaverdashvili, M., Kelly, M.E.M., Denovan-Wright, E.M. & Laprairie, R.B. (2019) Allosteric and orthosteric pharmacology of cannabidiol and cannabidiol-dimethylheptyl at the type 1 and type 2 cannabinoid receptors. *Br J Pharmacol,* **176,** 1455-1469.

Thomas, A., Baillie, G.L., Phillips, A.M., Razdan, R.K., Ross, R.A. & Pertwee, R.G. (2007) Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists in vitro. *Br J Pharmacol,* **150,** 613-623.

Till, K.J., Lin, K., Zuzel, M. & Cawley, J.C. (2002) The chemokine receptor CCR7 and alpha4 integrin are important for migration of chronic lymphocytic leukemia cells into lymph nodes. *Blood,* **99,** 2977-2984.

Till, K.J., Pettitt, A.R. & Slupsky, J.R. (2015) Expression of Functional Sphingosine-1 Phosphate Receptor-1 Is Reduced by B Cell Receptor Signaling and Increased by Inhibition of PI3 Kinase δ but Not SYK or BTK in Chronic Lymphocytic Leukemia Cells. **194,** 2439-2446.

Tsou, K., Brown, S., Sanudo-Pena, M.C., Mackie, K. & Walker, J.M. (1998) Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience,* **83,** 393-411.

Vallejo-Gracia, A., Bielanska, J., Hernández-Losa, J., Castellví, J., Ruiz-Marcellan, M.C., Ramón y Cajal, S., Condom, E., Manils, J., Soler, C., Comes, N., Ferreres, J.C. & Felipe, A. (2013) Emerging role for the voltage-dependent K+ channel Kv1.5 in B-lymphocyte physiology: expression associated with human lymphoma malignancy. *Journal of leukocyte biology,* **94,** 779-789.

Wang, L., Qian, J., Lu, Y., Li, H., Bao, H., He, D., Liu, Z., Zheng, Y., He, J., Li, Y., Neelapu, S., Yang, J., Kwak, L.W., Yi, Q. & Cai, Z. (2013) Immune evasion of mantle cell lymphoma: expression of B7-H1 leads to inhibited T-cell response to and killing of tumor cells. *Haematologica,* **98,** 1458-1466.

Wasik, A.M., Almestrand, S., Wang, X., Hultenby, K., Dackland, A.L., Andersson, P., Kimby, E., Christensson, B. & Sander, B. (2011) WIN55,212-2 induces cytoplasmic vacuolation in apoptosis-resistant MCL cells. *Cell death & disease,* **2,** e225.

Wasik, A.M., Nygren, L., Almestrand, S., Zong, F., Flygare, J., Wennerholm, S.B., Saft, L., Andersson, P., Kimby, E., Wahlin, B.E., Christensson, B. & Sander, B. (2014) Perturbations of the endocannabinoid system in mantle cell lymphoma: correlations to clinical and pathological features. *Oncoscience,* **1,** 550-557.

Wasik, A.M., Priebe, V., Lord, M., Jeppsson-Ahlberg, A., Christensson, B. & Sander, B. (2015a) Flow cytometric analysis of SOX11: a new diagnostic method for distinguishing B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma from mantle cell lymphoma. *Leukemia & lymphoma,* **56,** 1425-1431.

Wasik, A.M. & Sander, B. (2015b) Cannabinoid receptors in mantle cell lymphoma. *Cell cycle,* **14,** 291-292.

Wasik, A.M., Wu, C., Mansouri, L., Rosenquist, R., Pan-Hammarström, Q. & Sander, B. (2018) Clinical and functional impact of recurrent S1PR1 mutations in mantle cell lymphoma. *Blood Adv,* **2,** 621-625.

Weis, W.I. & Kobilka, B.K. (2018) The Molecular Basis of G Protein-Coupled Receptor Activation. *Annu Rev Biochem,* **87,** 897-919.

Velasco, G., Sánchez, C. & Guzmán, M. (2015) Endocannabinoids and Cancer. *Handb Exp Pharmacol,* **231,** 449-472.

Woyach, J.A., Furman, R.R., Liu, T.M., Ozer, H.G., Zapatka, M., Ruppert, A.S., Xue, L., Li, D.H., Steggerda, S.M., Versele, M., Dave, S.S., Zhang, J., Yilmaz, A.S., Jaglowski, S.M., Blum, K.A., Lozanski, A., Lozanski, G., James, D.F., Barrientos, J.C., Lichter, P., Stilgenbauer, S., Buggy, J.J., Chang, B.Y., Johnson, A.J. & Byrd, J.C. (2014) Resistance mechanisms for the Bruton's tyrosine kinase inhibitor ibrutinib. *The New England journal of medicine,* **370,** 2286-2294.

Yuan, M., Kiertscher, S.M., Cheng, Q., Zoumalan, R., Tashkin, D.P. & Roth, M.D. (2002) Delta 9-Tetrahydrocannabinol regulates Th1/Th2 cytokine balance in activated human T cells. *Journal of neuroimmunology,* **133,** 124-131.

Zenz, T., Vollmer, D., Trbusek, M., Smardova, J., Benner, A., Soussi, T., Helfrich, H., Heuberger, M., Hoth, P., Fuge, M., Denzel, T., Häbe, S., Malcikova, J., Kuglik, P., Truong, S., Patten, N., Wu, L., Oscier, D., Ibbotson, R., Gardiner, A., Tracy, I., Lin, K., Pettitt, A., Pospisilova, S., Mayer, J., Hallek, M., Döhner, H. & Stilgenbauer, S. (2010) TP53 mutation profile in chronic lymphocytic leukemia: evidence for a disease specific profile from a comprehensive analysis of 268 mutations. *Leukemia,* **24,** 2072-2079.

Zhang, J., Medina-Cleghorn, D., Bernal-Mizrachi, L., Bracci, P.M., Hubbard, A., Conde, L., Riby, J., Nomura, D.K. & Skibola, C.F. (2016) The potential relevance of the endocannabinoid, 2-arachidonoylglycerol, in diffuse large B-cell lymphoma. *Oncoscience,* **3,** 31-41.

Zhang, L., Yang, J., Qian, J., Li, H., Romaguera, J.E., Kwak, L.W., Wang, M. & Yi, Q. (2012) Role of the microenvironment in mantle cell lymphoma: IL-6 is an important survival factor for the tumor cells. *Blood,* **120,** 3783-3792.

Zhang, W. & Liu, H.T. (2002) MAPK signal pathways in the regulation of cell proliferation in mammalian cells. *Cell Res,* **12,** 9-18.

Zhao, X., Lwin, T., Silva, A., Shah, B., Tao, J., Fang, B., Zhang, L., Fu, K., Bi, C., Li, J., Jiang, H., Meads, M.B., Jacobson, T., Silva, M., Distler, A., Darville, L., Zhang, L., Han, Y., Rebatchouk, D., Di Liberto, M., Moscinski, L.C., Koomen, J.M., Dalton, W.S., Shain, K.H., Wang, M., Sotomayor, E. & Tao, J. (2017) Unification of de novo and acquired ibrutinib resistance in mantle cell lymphoma. *Nat Commun,* **8,** 14920.

Zou, S. & Kumar, U. (2018) Cannabinoid Receptors and the Endocannabinoid System: Signaling and Function in the Central Nervous System. *Int J Mol Sci,* **19**.

Zucchetto, A., Tripodo, C., Benedetti, D., Deaglio, S., Gaidano, G., Del Poeta, G. & Gattei, V. (2010) Monocytes/macrophages but not T lymphocytes are the major targets of the CCL3/CCL4 chemokines produced by CD38(+)CD49d(+) chronic lymphocytic leukaemia cells. *British journal of haematology,* **150,** 111-113.