Exploring the role of Bcl-2 family members & B-cell targeted therapies

From CLL to B-IMDs

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CHAPTER 7

General discussion

This thesis examined different chronic B-cell disorders: i.e. chronic lymphocytic leukemia (CLL), and two distinct systemic B-cell immune-mediated disorders (B-IMDs), namely systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA) with the purpose to find a common base to test the efficacy of B-cell targeted therapies. In oncology, advancements in treatment options and the discovery of new small molecule inhibitors, have made great strides towards better outcome, even though these three chronic B-cell disorders studied are still incurable. A significant challenge in CLL treatment is the development of acquired drug resistance as a response to therapy. Although the influence of the CLL microenvironment in driving drug resistance has been acknowledged, the precise mechanisms through which CLL cells translate signals from the microenvironment, resulting in an anti-apoptotic and proliferative phenotype, are not fully understood. B-IMDs often have a complex genetic composition that, combined with unclear environmental triggers, makes treatment less effective. This often necessitates long-term or lifelong treatment to control the disease and prevent it from becoming severe.

The experience with the novel treatment approaches in CLL focusing on cell death pathways begs the question whether such approaches would be potentially beneficial in case of B-IMDs as well, since there is emerging evidence that abnormal apoptosis is also associated with autoimmune diseases[1]. Hence, gaining a comprehensive understanding of the role of BCL-2 family members in CLL and B-IMDs could provide valuable insights into the development of novel therapeutic approaches.

Here, we address the drawbacks associated with existing treatment options, present our own research findings of (pre-)clinical targeted therapies, and examine their potential for future therapeutic approaches in both CLL and B-IMDs.

BTK INHIBITORS

BTK inhibition disrupts BCR-controlled integrin-mediated adhesion to fibronectin and CD49d/VCAM-1, which is crucial for CLL cell homing to the microenvironment[2]. As a consequence, CLL cells are redistributed from the lymph node (LN) niche to the peripheral blood (PB)[2,3]. In **Chapter 2,** we analyzed the alterations in the expression levels of the key anti-apoptotic proteins BCL-XL, MCL-1, and BCL-2 following the treatment with the first-generation covalent BTK inhibitor, ibrutinib. We expanded our research on BTK inhibition in a more homogeneous study group using relapsed/refractory CLL patient samples of the HOVON141 trial in

Chapter 3 and investigated the effects of ibrutinib treatment on CD40-mediated induction of BCL-2 family members and resistance against the BCL-2 inhibitor venetoclax. In both studies, we found that ibrutinib treatment leads to decreased expression of BCL-2 family members and CD40-mediated venetoclax resistance. An intriguing finding of our study was the reappearance of BCL-2 family members upon treatment relapse, implying that CLL cells undergo strong selective pressure to return to LNs for recharging. In search of possible diagnostic applications of the findings, we determined whether BCL-2 member expression can serve as an early biomarker for treatment non-responsiveness in ibrutinib and/or venetoclax therapies. Although we did not observe such correlations, it is important to consider that with larger patient cohorts or possibly by analyzing relapse samples, predictive aspects related to this matter may be found.

In **Chapter 4**, we studied patient samples of the phase III ACE-CL-06 trial, in which relapsed CLL patients were treated either with ibrutinib or the second generation covalent BTK inhibitor acalabrutinib. Previous studies reporting on this clinical trial demonstrated that acalabrutinib showed similar progression-free survival with fewer cardiovascular adverse events compared to ibrutinib treatment[4]. In line with these studies, our own findings revealed no noticeable differences between the effects of ibrutinib and acalabrutinib, both of which resulted in decreased expression of BCL-2 family members and reduced drug sensitivity. Consequently, it is reasonable to assume that the decrease in BCL-2 family members and the reduced CD40-mediated resistance to venetoclax are likely attributed to the egress of CLL cells from the LN to the PB. This is also supported by the fact that our in vitro co-culture experiments did not demonstrate any direct effects of ibrutinib on these mechanisms.

BTK inhibitors have also been found to be beneficial in recent clinical trials for treating B-IMDs such as multiple sclerosis (MS) and pemphigus vulgaris[5]. There is also a possibility of their effectiveness in treating RA, although only the non-covalent/reversible BTK inhibitor fenebrutinib has exhibited positive outcomes in RA among the various inhibitors tested[5,6]. However, it appears that BTK inhibitors do not provide therapeutic benefits for diseases like SLE and Sjogren's disease[5]. Experimental models of human autoimmune diseases have provided evidence that inhibiting BTK can effectively hinder the pro-inflammatory functions of B cells. Notably, this inhibition does not carry the risk of complete B cell depletion, which is commonly achieved through the use of anti-CD20 antibodies[7]. This suggests a potential therapeutic intervention for some autoimmune diseases without the drawbacks of B cell depletion.

MALT1 INHIBITORS; SOLUTIONS FOR ACQUIRED BTK RESISTANCE

Despite the effectiveness of targeting BCR-associated kinases as a therapeutic approach, a substantial proportion of patients eventually develop resistance to BCR inhibitors, leaving limited treatment options for progressive disease. Resistance to covalent BTK inhibitors can develop in CLL patients due to acquired mutations in BTK and/or PLCG2[8-10]. In response to this issue, more recent developed non-covalent BTK inhibitors, such as pirtobrutinib, provide a promising strategy. These inhibitors interact with alternative sites that are separate from the C481 residue, which is specifically targeted by covalent BTK inhibitors[11]. However, recent studies demonstrated that CLL patients may also develop resistance to pirtobrutinib as a result of the emergence of mutations in BTK or PLCG2[12,13].

In **Chapter 5**, we explored MALT1 as a promising novel target for treating CLL. MALT1 is critical in activating NF-κB in response to antigen receptor signaling, including the BCR and TCR[14]. Since MALT1 operates downstream of PI3K and BTK, it represents a valuable target, especially for patients who have become resistant to idelalisib and ibrutinib or those who have had to discontinue treatment with these inhibitors due to toxicity[15]. Our results in **Chapter 5** showed that MALT1 inhibition affected several signaling pathways including CD40, BCR and TLR, which prime CLL cells for venetoclax resistance. In addition, the inhibition of MALT1 resulted in a decrease in BCL-XL levels and enhanced the sensitivity of CLL cells to BH3 mimetics. Although we did not specifically examine the impact of MALT1 inhibition on BFL-1 expression, it is possible that it could be affected given that BFL-1 expression is regulated by NF-κB signaling[16], and we observed decreased NF-κB activation following MALT1 inhibition. This could have promising implications for the treatment of CLL, as there are currently no selective BFL-1 inhibitors available. Furthermore, in **Chapter 5** we have noticed the impact of inhibiting MALT1 on the activation, proliferation, and cytotoxicity of T cells, along with a notable decrease in regulatory T cell (Treg) population. These findings suggest that MALT1 inhibition exerts immunomodulatory effects on the functioning of lymphocytes and potentially enhance anti-tumor immune responses. Previous studies have demonstrated that MALT1, especially its protease activity, is crucial in maintaining homeostasis and function[17,18]. Inhibition of the CBM complex, including MALT1, could trigger an intratumoral autoimmune response through IFNγ production by destabilized Tregs. This immune response is further characterized by activation of macrophages and increased expression of MHC class I on tumor cells[19]. The pro-inflammatory properties associated with destabilized Tregs exhibited a

greater impact than the potential immune cell attenuation resulting from MALT1 inhibition[19]. Consequently, the tumor microenvironment was primed for effective immune checkpoint therapy or increased anti-tumor immunity, while minimizing the risk of systemic autoimmune toxicity[18,19]. Another study suggested that MALT1 inhibition can achieve anti-inflammatory efficacy without impacting the number or function of Tregs, supporting the potential use of MALT1 inhibitors in treating autoimmune diseases[20].

Taken together, these findings provide a rationale for considering the combination of MALT1 inhibitors with venetoclax, PD-1 or BTK inhibitors as a potential therapeutic approach in B cell malignancies. Additionally, this approach may also be applicable in B-IMDs, where there is often a concurrent dysregulation of B cells, an imbalanced adaptive immune system, and the appearance of autoreactive T and B cells that require control. However, it is important to note that disrupting tolerance carries a potential risk in treating B-IMDs, unless the precise impact of the treatment has been thoroughly evaluated through solid experimental data.

In mantle cell lymphoma (MCL), in vitro and in vivo studies have demonstrated that MALT1 overexpression is linked to resistance to BTK inhibitors and that the combination of safimaltib, an oral MALT1 inhibitor, and pirtobrutinib, targeting BTK, exhibited strong anti-MCL effects in both ibrutinib-resistant MCL cell lines and patient-derived xenografts[21]. Another study recently presented that combination of ABBV-MALT1 inhibitor with venetoclax showed strong antitumor activity in patient-derived xenograft models of diffuse large B-cell lymphoma[22]. There are already clinical trials ongoing studying MALT1 inhibitors in B cell malignancies. Safimaltib (JNJ-67856633) is currently undergoing multiple clinical investigations to assess its clinical efficacy as a single agent (ClinicalTrials.gov NCT03900598) or in combination with covalent BTK inhibitors ibrutinib and JNJ-64264681 in patients with CLL or non-Hodgkin's Lymphoma (ClinicalTrials.gov NCT04876092 and NCT04657224).

LYMPH NODE MICROENVIRONMENT

Previous studies have demonstrated that venetoclax responses are less complete, likely due to the persistence of enlarged LNs[23]. Additionally, the upregulation of other BCL-2 family members in the LN, which are not directly affected by venetoclax, is likely to contribute to this incomplete response[24]. CD40-CD40L interactions within the LN are associated with the induction of those BCL-2 family

members and venetoclax resistance[24-26]. In **Chapter 3**, we identified a new role of TLR9 signaling in CLL via CpG stimulation, namely the induction of CD40 on CLL cells, specifically in the LN emigrant population. Although there is no direct proof of TLR9 signaling occurring in the LN yet, Kennedy *et al. (2021)* suggested that CLL patient plasma has elevated levels of mitochondrial DNA (mtDNA) compared to healthy controls[27]. This mtDNA harbors unmethylated CpG motifs that resemble bacterial DNA, potentially triggering TLR9 signaling. This study also showed that plasma, similarly to CpG stimulation, induced CLL cell migration through activation of p65 NF-κB and STAT3 transcription factors[27]. In relation to this, we studied whether CLL plasma could also induce CD40 expression. We did not find any induction of CD40 expression by using plasma of CLL patients (data not shown). However, we did not assess the potential of our tested plasma to activate NF-kB or STAT3, as examined in the prior study[27]. Moreover, from other B cell malignancy studies it seems that hematopoietic cell kinase (HCK), downstream of TLR signaling, serves as an NF-κB target and is also expressed in CLL cells[28]. Additionally, it was shown that the recruitment of HCK in BTK C481F and C481Y mutants resulted in the subsequent phosphorylation of PLCγ2, thereby promoting the propagation of BCR signaling and the proliferation of clonogenic cells[29]. Furthermore, HCK has been identified as a crucial driver of mutated MYD88 pro-survival signaling in lymphomas, and its inhibition could overcome ibrutinib resistance[30]. Consequently, HCK could potentially play a role in inducing CD40 expression following TLR9 stimulation in CLL cells (Figure 1). Our findings revealed that CpG triggers an increase in HCK protein levels, but we did not observe a similar induction of HCK expression upon CD40 stimulation in CLL (data not shown).

Furthermore, we tried to inhibit TLR9-induced CD40 expression by using an IRAK1/4 inhibitor but we found only minor reduction in CD40 expression measured by flow cytometry, and which were unchanged on western blot. Earlier studies have demonstrated that the activation of the tyrosine kinase SYK downstream of the BCR can be induced by CpG stimulation[31]. This can then trigger the production and secretion of autoreactive IgM and, when combined with TLR triggering, ultimately lead to the proliferation of CLL[31]. Consistently, inhibition of SYK blocked CpG/CD40L-induced CLL proliferation but not in healthy B cells[31,32]. SYK is also involved in CD40 signaling[33] and therefore, it would be interesting to investigate a potential crosstalk between SYK, CD40 and TLR signaling in CLL. Previous studies have shown that the expression of BCL-2, BCL-XL and BFL-1 are transcriptionally regulated, while it has been demonstrated that the expression of MCL-1 is regulated translationally[34]. In **Chapter 3**, we discovered

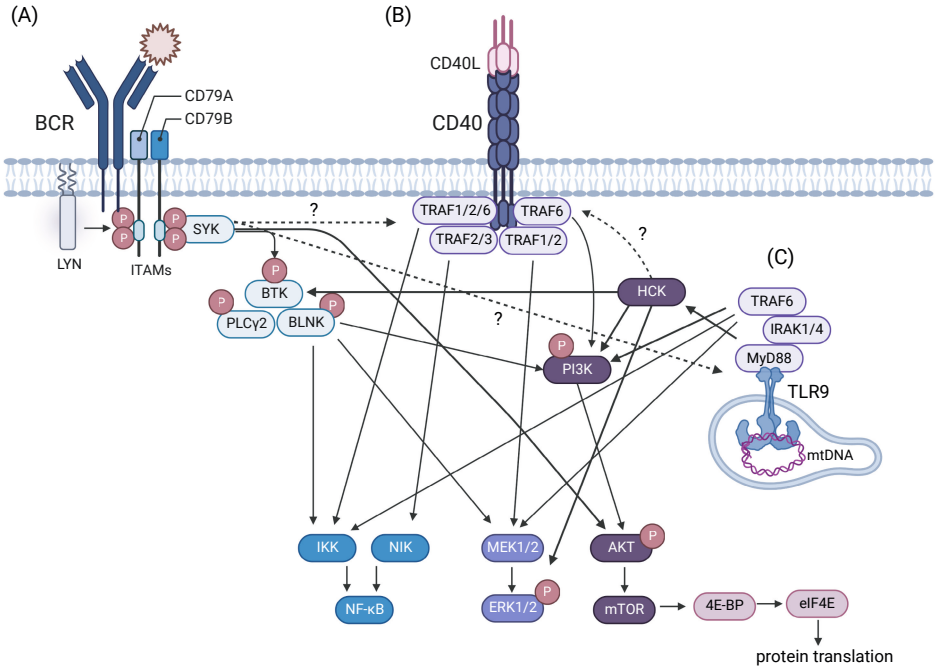

Figure 1. Schematic representation of potential crosstalk between key signaling pathways of B cell activation.
Adapted from Haselager et al. (2020)[42], Bartish et al. (2023)[40], and Yang et al. (2021)[30]. Activation of BCR, CD40 and TLR through upstream triggers results in similar downstream pathway activation, including the NF-kB, MAPK/ERK, and PI3K/AKT/mTOR pathways. **(A)** The BCR complex consists of an antigen-specific surface immunoglobulin and the CD79 heterodimers. Upon antigen binding to the BCR, the kinase LYN phosphorylates ITAMs on the cytoplasmic tail of CD79. As a result, SYK kinase is recruited and triggers the activation of BTK. Activated BTK then stimulates the downstream activation of PLCγ2 and the BLNK adaptor, which ultimately activates various downstream signaling pathways. **(B)** When CD40L binds to the CD40 receptor on B cells, the receptor trimerizes and recruits TRAFs to its cytoplasmic domain. The TRAFs can then cooperate to activate diverse downstream signaling pathways. **(C)** Once TLR9 is activated within the endosomal compartment of B cells, its TIR domain interacts with the TIR domain of the adaptor protein MyD88. MyD88 has an IRAK1 domain that can activate TRAF6, ultimately resulting in the activation of downstream pathways. Following TLR9 stimulation, HCK potentially plays a role in initiating the expression of CD40 and its recruitment and activation could enhance BCR signaling in BTK C481 mutants by phosphorylating PLCγ2. Additionally, TLR9 stimulation induces global protein translation, potentially mediated via the mTORC1–4E-BP–eIF4E axis.

that CpG has the capacity to induce global protein translation and reversed the effects of ibrutinib on TME-induced venetoclax resistance. Consistent with this, we found that especially CpG induced MCL-1 protein expression as demonstrated in **Chapter 5**. Since previous studies have established that MCL-1 expression is regulated by the mTOR/Akt/PI3K pathway[34], there could be a potential link between TLR9 stimulation and this signaling pathway, which was confirmed by previous studies[35-39]. This was also supported by our finding that TLR9 stimulation induced global protein translation, potentially mediated via the mTORC1–4E-BP–eIF4E axis[40] (Figure 1). Additional research has also demonstrated the significant involvement of TLR signaling in the development of CLL and in sustaining the survival of CLL cells during ibrutinib treatment[27,41]. These findings imply potential superior anti-tumor activity by combining ibrutinib with drugs targeting TLR signaling. In relation to this, it would be interesting to explore whether there is a correlation between TLR activity and the emergence of drug resistance in CLL. Therefore, examining the potential impact of TLR inhibitors, such as IRAK1/4 inhibitors, and/or SYK inhibitors on venetoclax resistance could be beneficial.

BTK INHIBITOR AND VENETOCLAX-REFRACTORY CLL

In our small study of four BTK inhibitor-refractory patients in **Chapter 4**, one acalabrutinib-treated patient developed mutations in BTK, TP53 and POT1, while another patient treated with ibrutinib demonstrated mutations in both BTK and PLCG2, as well as mutations in TP53 and POT1. Further next-generation sequencing analyses still need to be performed for all tested refractory patient samples. However, previous genetic analyses conducted on both covalent and non-covalent BTK inhibitors did not reveal consistent mutations[11,43-45]. Surprisingly, about 50% of patients who demonstrated resistance to these inhibitors showed no identifiable mutations in BTK and 29% did not develop any mutations in the tested panel including TP53, ATM, NOTCH1, SF3B1, and PLCG2[44].

Prolonged administration of venetoclax may give rise to treatment resistance due to the acquisition of mutations, particularly in the BCL2 gene, or the upregulation of BCL-XL and/or MCL-1[46,47]. One of the two tested venetoclax-refractory patients in **Chapter 4** did not acquire mutations in the BCL2 gene, but in TP53 and EZH2, which are associated with aggressive clinical outcomes in CLL patients[48,49]. Possible correlations between TP53 mutations and venetoclax resistance have been found in acute myeloid leukemia[50-53]. In CLL, in vitro studies showed that TP53-mutated CLL cells are highly responsive to venetoclax[54]. However, in clinical settings, it

has been observed that TP53-mutated CLL patients may experience a reduced duration of response to venetoclax compared to wild-type TP53[55-59], potentially due to increased genetic vulnerability and clonal heterogeneity in TP53-mutated patients.

Consistent with our findings in **Chapter 2**, we found in **Chapter 4** that BCL-2 family member expression reappear once patients relapse after prolonged BTK inhibition. Furthermore, we found increased CD40 expression in BTK refractory samples compared to baseline, which needs to be further investigated with a larger patient number. We also analyzed two patient samples that relapsed on venetoclax treatment. Surprisingly, we observed increased resistance to in vitro venetoclax and AZD4320 in unstimulated CLL cells of both BTK inhibitor and venetoclax-refractory patients.

These findings suggest that refractory disease may involve other BCL-2 family members, in addition to BCL-2 and BCL-XL. Furthermore, the absence of mutations in frequently mutated genes in approximately 50% of refractory patients, such as specific mutations in BTK (C481) and PLCG2 (R665W, S707P, S707F, R742P, and L845F) genes strongly associated with resistance to BTK inhibitors[44,60], or the G101V mutation in BCL2 gene specific to venetoclax resistance[61], suggests that factors beyond genetic mutations may contribute to the development of resistance as well. Reprogramming or rewiring of signaling pathways during therapy[42,62], as well as the formation of a multiprotein supercomplex involving MYD88, TLR9, and the BCR, are potential factors that could contribute to this resistance[63] (see also Figure 1). Additionally, a novel mechanism of uncontrolled gene expression in diffuse large B-cell lymphoma was identified. This mechanism involved hypermutated super-enhancers associated with the BCL6, BCL2, and CXCR4 proto-oncogenes, which prevented the binding of transcriptional repressors and subsequent downregulation of the target genes[64].

It would be also interesting to test a MCL-1 inhibitor in refractory disease. However, from our in vitro studies in **Chapter 2**, we have observed that single agent MCL-1 inhibitor (S63845) was only partially effective in inducing cell death and combination therapy with venetoclax might be more successful[26]. However, as MCL-1 is present in various human tissues, such as cardiac tissue, and plays a crucial role in hematopoietic stem cells[65], targeting it as a treatment option may lead to considerable side effects, particularly cardiotoxicity[66]. Consequently, its clinical applicability is currently not feasible.

Furthermore, it is important that future experiments still investigate the involvement of BFL-1 and the potential of MALT1 inhibitors in refractory patient samples.

Concerning experimental detection of BTK inhibitor and/or venetoclax refractoriness; our group has recently developed a 3D in vitro culture system. This novel approach results in formation of spheroids comprised of CLL cells derived from PB, aiming to replicate the CLL microenvironment. The data so far obtained showed clear effects of BTK inhibition on spheroid formation and proliferation, which were strongly attenuated using ibrutinib refractory samples (manuscript currently undergoing revision)[67]. Since the spheroid system also allows to measure response to venetoclax, this model offers valuable novel applications for both BTK inhibitor and venetoclax-refractory CLL.

ROLE OF BCL-2 FAMILY MEMBERS IN B-IMDS

Emerging evidence suggests a correlation between autoimmune diseases and dysregulation of apoptosis[1]. However, the specific involvement of the intrinsic apoptosis pathway and BCL-2 family members in SLE and RA is still not well comprehended. In **Chapter 6**, we focused on examining the expression levels of three significant BCL-2 family members, namely BCL-2, BCL-XL, and MCL-1, in lymphocyte subsets of patient samples diagnosed with SLE or RA, in comparison to samples obtained from healthy controls. Although we observed no disparities in BCL-2 family member ex vivo, we detected notable variations in the expression of BCL-2 family members in B cells from SLE patients following in vitro stimulation with CpG. Conversely, no such differences were observed in RA patients compared to healthy controls. It should be noted that our study did not examine potential differences in the activation or proportions of differentiated B cells following CpG stimulation within the patient groups. Additionally, we also cannot disregard the potential influence of patients' medication on the expression of BCL-2 family members and subset distributions. Since we did not find any differences in BCL-2 family members in lymphocyte subsets of RA patients compared to healthy controls, future experiments should include lymphocytes from synovial fluid/tissue or specifically autoreactive B cells expressing anti-cyclic citrullinated peptide (anti-CCP) antibodies, as this approach holds the potential for a more accurate representation of the disease. Furthermore, we focused only on studying three dominant BCL-2 family members, namely BCL-2, BCL-XL and MCL-1. It would be also interesting to investigate a complete set of BCL-2 family members

consisting also of pro-apoptotic proteins in SLE/RA. Previously, we conducted multiplex ligation-dependent probe amplification (MLPA) analysis of apoptotic genes in B cells and discovered elevated expression of pro-apoptotic NOXA and BIM in SLE patients compared to healthy controls (data not shown). In our BH3 mimetic experiments in **Chapter 6**, we observed a decrease in B cell survival in SLE patient samples following a six-day stimulation with CpG. This reduction in cell viability could potentially be linked to the aforementioned elevated expression of NOXA and BIM. However, we did not evaluate any potential alterations in the expression of NOXA and BIM following stimulation with CpG. In addition, previous studies have reported a decrease in proliferation and activation of B cells from patients with active SLE following stimulation with a TLR9 agonist[68]. Consequently, it is recommended that future experiments include alternative B cell stimulations, such as α-IgM/IL-21/α-CD40.

COMBINATION THERAPIES AND FUTURE PERSPECTIVE

Despite the availability of several effective targeted therapies for CLL, significant challenges persist. Single therapeutics like ibrutinib and venetoclax lack curative potential and require continuous treatment, leading to toxicity and the emergence of drug resistance[69].

A promising strategy to overcome drug resistance and improve the prognosis of CLL patients involves the use of combination therapies that target both the CLL cells and their surrounding microenvironment. Combining therapies offers several advantages, including the potential to reduce overall costs by discontinuing certain treatments, minimize treatment side effects, and potentially overcome resistance mechanisms. Clinical trials have already tested and approved several drug combinations in CLL. While the combination of CD20 antibodies with ibrutinib did not improve progression-free survival in relapsed or treatment-naïve high-risk CLL patients[70], the combination of venetoclax with CD20 antibodies demonstrated significantly higher rates of progression-free survival[55,71,72]. The combination of ibrutinib and venetoclax may have synergistic anti-tumor effects as ibrutinib forces CLL cells from the LN to the PB, where they become highly dependent on BCL-2 and more susceptible to venetoclax[26,73]. Time-limited combination therapy with ibrutinib and venetoclax as a first-line or relapsed/refractory CLL treatment has substantially extended progression-free survival, although relapse can occur eventually[73-78]. Additionally, recent follow-up studies have provided evidence that CLL patients who achieve undetectable minimal residual disease (uMRD) after

completing 15 treatment cycles of venetoclax and ibrutinib combination can safely discontinue treatment and, if necessary, reinitiate it based on MRD guidance[79]. Ongoing clinical trials are investigating the combination of acalabrutinib with venetoclax in CLL, but no results have been reported yet. Despite advancements in treatment, some patients may develop refractory disease, particularly those with double-refractory disease involving both BTK and BCL-2 inhibitors, requiring alternative treatments[80].

It is important to note that the mentioned combination therapies have not yet been tested in B-IMDs.

Within lymphoid organs, CLL cells upregulate other BCL-2 family members, including BCL-XL, MCL-1 and BFL-1, that are not targeted by the BCL-2 inhibitor venetoclax. Therefore, there is a critical need for the development of BH3 mimetics capable of targeting multiple BCL-2 family members. In **Chapter 4**, we investigated a recent developed BH3 mimetic drug, AZD4320, targeting both BCL-2 and BCL-XL in CLL. We found that AZD4320 promoted cell death at lower concentrations than navitoclax or venetoclax in CLL. However, AZD4320 has not been tested in clinical trials yet, and possible resistance due to the upregulation of other BCL-2 family members, including MCL-1 and BFL-1, cannot be excluded in CLL[46,47].

BH3 mimetics have been developed to treat hemato-oncology patients but may also be beneficial in autoimmune diseases. Therefore, we tested various BH3 mimetics, including the dual BCL-2/BCL-XL inhibitor AZD4320, in B-IMDs in activated B and T cells of RA and SLE patients in **Chapter 6**. While we found only minor effects of AZD4320 treatment in inducing cell death of plasmablasts and T cells, MCL-1 inhibition was more potent compared to venetoclax or AZD4320. However, these effects were more prominent in healthy controls than in SLE/RA. Moreover, as stated earlier, our MLPA data revealed elevated levels of NOXA in SLE compared to healthy controls. This increase in NOXA levels specifically opposes the activity of MCL-1[24], suggesting a possible imbalance between NOXA and MCL-1 in SLE.

Furthermore, given the limited therapeutic window, it is worth exploring the effects of BH3 mimetics on autoreactive B cells directly. Additionally, it is important to contemplate the development of specific cytotoxic drugs that selectively target autoreactive B cells while preserving the integrity of healthy B cells. Consequently, it is crucial to prioritize the identification of unique surface markers that are specific to autoreactive cells in order to address this matter effectively.

Other studies showed that peripheral and intrathecal lymphocytes derived from MS patients exhibit reduced expression levels of pro-apoptotic BCL-2 family proteins in comparison to anti-apoptotic members, including BCL-2 and BCL-XL[81]. Hence, investigating the impact of AZD4320 in samples from MS patients could provide valuable insights for potential therapeutic approaches in the future.

CONCLUDING REMARKS

Our findings emphasize the significant impact of the microenvironment on treatment resistance in CLL, as it triggers multiple signaling pathways. Specifically, we discovered a potential interaction between CD40 and TLR9 signaling in the LN, which was disrupted by administering ibrutinib. This suggests that it is crucial to simultaneously target various critical signaling pathways. Combination therapies with time-limited durations which reduce the risk of emergence of resistant cell clones, have shown effectiveness with the possibility of reinitiating treatment.

In the context of B-IMDs, the efficacy of combination therapies remains unexplored.

By comprehensively studying the complete repertoire of BCL-2 family members, we anticipate gaining valuable insights for developing future treatment strategies. Finally, based on our findings in CLL, it would be also interesting to explore the combination of BTK or MALT1 inhibitors with BH3 mimetics in B-IMDs to potentially find effective strategies to control and regulate the imbalanced adaptive immune system in B-IMDs.

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