Novel insights into membrane targeting of B-cell lymphoma

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Abstract

Standard therapy of patients with B-cell non-Hodgkin lymphoma (B-NHL) mostly consists of chemotherapy combined with anti-CD20 (e.g. rituximab) immunotherapy. However, relapse of aggressive B-NHL occurs frequently, which may coincide with therapy resistance. This demonstrates the urgent need for exploring new lymphoma-targeted therapies. Here, we review recent insights in the pathophysiology of B-NHL, and discuss CD20 and three alternative membrane targets (B-cell receptor, immune checkpoints PD-1/PD-L1, tetraspanin CD37) that are currently in the spotlight for treatment of B-NHL. Furthermore, we present a novel concept in which plasma membrane organization of the lymphoma B cell determines the efficacy of membrane-targeted therapies, which has consequences for treatment application and clinical outcome for patients with B-cell lymphoma.

Origin of B-cell non-Hodgkin lymphoma

B cells undergo several distinct phases of development, starting from pre-B cells in the bone marrow towards ultimately the differentiation into antibody-producing plasma cells and memory B cells. Upon encountering specific antigens, germinal centers (**GC**, see Glossary) are formed within the lymph node, where B cells undergo somatic hypermutation (**SHM**, see Glossary) and class switch recombination (**CSR**, see Glossary) in order to generate a high affinity B-cell receptor (BCR). However, SHM and CSR are error-prone mechanisms, hence the majority of B-cell non-Hodgkin lymphomas (B-NHL) arises from GC B cells [1]. These GC-derived B-cell lymphomas frequently harbor chromosomal translocations of proto-oncogenes, like *BCL2*, *BCL6* or *MYC*, that are relocated under the control of the active immunoglobulin heavy chain (IGH) locus [1,2]. Since these translocations often involve the non-productively rearranged IG loci, most lymphomas still express a functional BCR [1].

The GC-derived B-NHLs include follicular lymphoma (**FL**, see Glossary), Burkitt lymphoma (**BL**, see Glossary), and diffuse large B-cell lymphoma (**DLBCL**, see Glossary). DLBCL is the most prevalent type, accounting for approximately one third of all NHL cases. Two subtypes of DLBCL have been identified: germinal center B-cell-like (GCB-)DLBCL, arising from GC B cells, and activated B-cell-like (ABC-)DLBCL, which has a post-GC origin [3]. ABC-DLBCL is characterized by constitutively active BCR/NF-κB signaling [4], and has a worse prognosis than GCB-DLBCL [3]. The hallmark of BL is translocation of *MYC* to the IGH locus [5]. Both DLBCL and BL are aggressive cancers in contrast to FL, which is an indolent lymphoma. However, in a large proportion of FL cases the disease transforms into the more aggressive DLBCL [6]. Current first-line treatment of DLBCL consists of the combination of rituximab (anti-CD20 monoclonal antibody (mAb)) with **CHOP**-based (see Glossary) chemotherapy, frequently followed by subsequent radiotherapy. BL is treated with CHOP-based chemotherapy, sometimes combined with rituximab. For FL, a watch-and-wait policy is frequently applied. In case of treatment indication, first-line treatment consists of rituximab combined with CHOP- or **CVP**-based (see Glossary) chemotherapy with or without subsequent radiotherapy. The exact protocol depends on the diagnosis and condition of the patient [7–9].

Although immunotherapy with rituximab has significantly improved the clinical outcome of patients with B-cell malignancies [10,11], many patients with DLBCL [12], BL [11] and FL [13] suffer from treatment failure or relapse. Several novel targeted therapies are currently under (pre-)clinical investigation, including therapies targeting the tumor microenvironment (reviewed in [14]), and therapies targeting the lymphoma cells directly, either intracellular or at the membrane (reviewed in [15]). Targeting membrane proteins to treat cancer has been extensively studied by researchers and clinicians in the last two decades since the cell surface is the easiest accessible part of the cell. Rituximab is a clear example of

successful antibody-based immunotherapy targeting a membrane protein. Antibody-based membrane-targeted therapies act through different mechanisms, including direct cell death signaling, antibody-dependent cell-mediated cytotoxicity (**ADCC**, see Glossary) and complement-dependent cytotoxicity (**CDC**, see Glossary). In this review, we focus on recent developments and new membrane-targeted therapies for GC-derived B-cell lymphoma (Figure 1a). Furthermore, we propose a model illustrating the dynamic cell surface protein landscape of lymphoma B cells that influences the efficacy of membrane-targeted therapies in B-NHL.

CD20 as the first B-cell-specific membrane target to treat B-NHL

Anti-CD20 mAb rituximab represents the first mAb approved for cancer therapy by the FDA in 1997. CD20 is a four-transmembrane protein expressed on mature B cells that is involved in B-cell activation and differentiation, mainly by controlling calcium influx [16]. CD20 was selected in the early 1980's as new target for B-NHL since the majority of B-NHL cells (90%) express CD20 with high density at the cell surface [17]. Pro-B cells and antibody producing plasma cells do not express CD20, and are therefore not vulnerable to CD20 targeting. As such, the healthy B cell pool is restored after rituximab treatment and B cell immune responses are largely preserved.

Anti-CD20 mAbs have been classified as type I and type II [18], which both induce ADCC. Type I mAbs (e.g. rituximab and ofatumumab) are regarded as the most potent antibodies because of their capacity to initiate CDC in contrast to type II mAbs (e.g. obinutuzumab). Furthermore, type II mAbs may induce direct cell death (Figure 1b), although the exact mechanism remains to be elucidated. Upon type I mAb binding, CD20 is redistributed to specific microdomains, including lipid rafts and tetraspanin-enriched

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microdomains (Box 1), which stimulates clustering of the antibody Fc regions involved in CDC. Unfortunately, Fc receptor polymorphisms and downregulation of CD20 caused by repeated exposure to rituximab [19] demonstrate the urgent need for development of alternative membrane-targeted strategies.

Emerging membrane-targeted therapies for B-cell lymphoma

Besides CD20, numerous other potential membrane targets have been investigated in lymphoma including: CD19, CD22, CD23, CD37, CD47, CD52, CD74, CD79α, CD80, HLA-DR, and the BCR (idiotype). Furthermore, immune checkpoint inhibitors that target programmed cell death protein 1 (PD-1) and programmed cell death ligand 1 (PD-L1) are currently in the spotlight for treatment of B-NHL. Targeting of some of these cell surface proteins with mAbs has shown promising therapeutic efficacy, but targeting others seems less suitable due to limited specificity (e.g. CD80 and HLA-DR are very broadly expressed) or resistance development (e.g. due to antigen shedding or internalization). A few new mAbs directed against B-cell surface proteins currently tested in clinical trials show promising results either as first-line therapy in combination with rituximab, or as monotherapy for heavily-pretreated B-NHL patients (e.g. anti-CD22: epratuzumab; anti-CD37: otlertuzumab; anti-CD74: milatuzumab) (reviewed in [15]). Future antibody engineering studies, including conjugation to radio/immunotoxins or generation of bispecific antibodies, may further improve their anti-lymphoma potency. Below we will discuss recent results and focus on novel developments in targeting membrane proteins in B-NHL, including immune checkpoints and tetraspanins (Figure 1).

Aberrant B-cell receptor function in B-NHL

The BCR, a membrane-bound immunoglobulin (Ig), can be divided in 5 classes (IgG, IgD, IgM, IgA or IgE) that are important for antigen recognition and initiation of signaling events leading to B-cell survival, proliferation and migration [20]. The BCR forms a complex with CD79α (or Igα) and CD79β (or Igβ) which both contain an immunoreceptor tyrosine-based activation motif (ITAM) to transmit intracellular signaling. Upon antigen binding, the conformational state of the BCR changes to initiate downstream signaling, but the underlying molecular mechanisms are still debated. The "conformation-induced oligomerization model", which proposes that single BCRs oligomerize upon antigen recognition [21], has recently been challenged by super-resolution studies. This has led to convincing evidence for the "dissociation-activation model", in which BCR oligomers dissociate upon antigen binding, gaining an open conformation [22,23]. This conformational change influences the distribution of BCR co-receptors CD19 and CD20 that interact with IgD oligomers on resting B cells, and relocate to IgM oligomers upon B-cell activation [23]. These changes in plasma membrane organization are controlled by a superfamily of membrane-organizing proteins, called tetraspanins (Box 2). Tetraspanin CD81 is required for reorganization of CD19 to BCR nanoclusters, which enables binding of signaling proteins to the intracellular domain of CD19 and initiation of signaling downstream of the BCR [24,25].

Two modes of BCR signaling have been described: 1) active signaling upon antigen binding to the BCR leading to NF-κB activation via Syk, phosphoinositide 3-kinase (PI3K) and Bruton's tyrosine kinase (BTK); and 2) tonic signaling, an antigen-independent mechanism required for B-cell survival which occurs in mature resting B cells via activation of PI3K and protein kinase B (AKT) (reviewed in [26]) (Figure 1c). In most GC-derived B-cell lymphomas, BCR signaling is disturbed resulting in enhanced B-cell survival and proliferation. BL shows tonic BCR signaling (Figure 1c) via constitutive activation of the PI3K pathway driven by *MYC*

translocation to the IGH locus during SHM [5,27]. Chronic active BCR signaling is seen in ABC-DLBCL which is evidenced by knockdown experiments of BTK or components of the BCR complex that induced killing of lymphoma cells [4]. In contrast, GCB-DLBCL cells were not killed, indicating they do not require BCR signaling to survive. In the majority of ABC-DLBCL cases, self-antigens present in the tumor microenvironment may drive continuous BCR stimulation (Figure 1c) [28]. The presence of BCR clusters on ABC-DLBCL cells resembles BCR clusters observed on normal B cells following antigen stimulation [4]. In 10% of ABC-DLBCL cases, gain-of-function mutations in CARMA1 (also known as CARD11) were found to result in continuous NF-κB activation [29]. Furthermore, in 20% of ABC-DLBCL cases, somatic mutations in the CD79α/CD79β ITAM domains induced chronic active BCR signaling via inhibition of BCR endocytosis and prevention of binding of Lyn to CD79β [4].

In FL, constitutive BCR signaling induced by presence of (self-)antigens has been found in only 20-25% of the cases [30,31], whereas the majority of FL cases (80%) bear somatic mutations in the IgM-BCR leading to increased incorporation of N-glycosylation sites [32,33]. If this occurs in the antigen-binding side of the BCR it may prevent binding of (self-)antigens, but in turn can be recognized and stimulated by lectin receptors on myeloid cells in the tumor microenvironment [34,35]. This results in re-organization of the BCR complex (including CD19) into signaling platforms and persistent antigen-independent signaling via Syk and BTK.

Currently, different small molecule inhibitors targeting signaling proteins downstream of the BCR (Syk, Lyn, PI3K, BTK) are under investigation as novel therapy for B-NHL (reviewed in [26]). Still, increased understanding into the conformational changes in the BCR upon antigen binding may open new opportunities to directly target the BCR itself. This idea was originally explored in the 1980's, in which treatment of lymphoma patients with anti-idiotype antibodies (targeting the variable region of the BCR) revealed promising clinical results [36].

However, this approach required generation of patient-specific idiotype-antibodies hampering clinical implementation. Recently, a new tool has been developed to screen and target tumor-specific idiotypes with small peptides linked to a pre-made IgG-Fc protein, so-called anti-idiotype peptibodies [37], which directly induce cell death via BCR signaling and ADCC. Furthermore, the anti-CD79β antibody-drug conjugate polatuzumab vedotin may be promising to treat B-NHL patients [38], which is currently investigated in clinical trials (https://clinicaltrials.gov/).

Therapeutic strategies targeting the BCR co-receptor CD19 are encouraging for patients with relapsed/refractory B-NHL. CD19 chimeric-antigen receptors expressed by T cells (CD19-CAR T cells) consist of an extracellular domain specific for CD19 on B-NHL cells and intracellular domains to provide T cell stimulatory signals (e.g. CD3ζ and CD28). Adoptive transfer of CD19-CAR T cells has given complete responses in ~50-60% of B-NHL patients in phase I/II clinical trials [39]. Furthermore, blinatumomab, a CD19/CD3 bispecific T-cell engager (BiTE) generated from the antigen-binding regions of the single-chain antibodies of CD19 and CD3, is approved by the FDA for treatment of acute lymphoblastic leukemia (ALL) and clinical results are currently evaluated for treatment of other B-cell malignancies, including B-NHL [40]. Future research is required to determine the consequences of targeting the BCR complex in B-NHL patients, and in particular how this changes BCR conformation and downstream signaling.

Function and targeting of immune checkpoints PD-1/PD-L1 in B-NHL

Cancer immunotherapy has been chosen as the scientific breakthrough of 2013 by *Science* journal [41], due to promising results with immune checkpoint inhibitors. Immune checkpoints are proteins that regulate immune cell activation to maintain self-tolerance and

prevent autoimmunity (reviewed in [42]). More specifically, these checkpoints play an important role in controlling T cell priming and activation. For example, PD-1 on T cells transfers an inhibitory signal when engaged by PD-L1 expressed by tumor cells or activated T cells.

Tumor cells may exploit these checkpoints to escape or suppress the immune system by (over-)expressing PD-L1. Currently, anti-PD-1 mAbs (pembrolizumab and nivolumab) and anti-PD-L1 mAbs (atezolizumab and durvalumab) demonstrate promising clinical results in the treatment of different solid tumors, including metastatic melanoma, urothelial carcinoma and advanced non-small cell lung carcinoma (reviewed in [43]). This treatment strategy has also gained more attention in the B-cell lymphoma field (Figure 1d) (reviewed in [42]), and nivolumab has recently been approved by the FDA for the treatment of relapsed/refractory Hodgkin lymphoma due to promising clinical results [44].

For DLBCL, promising clinical results have been obtained with the anti-PD-1 mAbs pidilizumab and nivolumab [45,46], and both anti-PD-1 and anti-PD-L1 mAbs are currently tested in clinical trials (https://clinicaltrials.gov/). It has been shown that a subset of ABC-DLBCL tumors (11-31%) display high expression of PD-L1, in contrast to GCB-DLBCL tumors that virtually lack expression of PD-L1. This high PD-L1 expression has been suggested to be involved in the poorer prognosis of ABC-DLBCL patients compared to patients with GCB-DLBCL [47,48]. Since PD-L1 expression is restricted to a minority of DLBCL patients, it is likely that only this group will benefit from immune checkpoint blockade [48,49].

Although PD-L1 is rarely expressed in FL cells [42,47], FL patients may still benefit from immune checkpoint blockade, since inhibition of PD-1-expressing tumor-infiltrating T cells by PD-L1-expressing non-tumor cells in the tumor microenvironment can be reversed [50]. This has been supported by clinical studies demonstrating good responses in FL patients treated

with anti-PD-1 mAbs (nivolumab and pidilizumab) [46,51]. Trials with pembrolizumab in FL

patients are currently ongoing (https://clinicaltrials.gov/). Several studies have reported that

BL cells do not express PD-L1 [47,49], and PD-1/PD-L1 checkpoint inhibitors have therefore

not yet been studied in BL. Still, the promising results of immune checkpoint inhibition in FL,

which rarely expresses PD-L1, may be translated to BL. Taken together, targeting the immune

checkpoint membrane proteins PD-1/PD-L1 seems a promising novel treatment option for a

selected group of ABC-DLBCL and FL patients. However, it is still debated whether expression

of PD-1/PD-L1 is of prognostic value for immune checkpoint inhibition therapy in B-NHL

[52,53].

CD37 rediscovered as B-cell membrane target in the treatment of B-NHL

Tetraspanin CD37 recently regained attention as promising membrane target for mature B-

cell malignancies (Box 2). CD37 expression is restricted to the immune system with highest

abundance on mature B cells, and is absent in earlier stages of B-cell development and

decreased on plasma cells [54,55]. This pattern is also reflected in different B-cell lymphomas:

CD37 is mostly expressed on B-cell malignancies derived from mature B cells (although ~50%

of DLBCL patients lack CD37 [56,57], discussed below), but not in acute lymphoblastic

lymphoma and multiple myeloma [58]. Comparable to CD20, this distinct expression pattern

makes CD37 an interesting target in GC-derived B-cell lymphomas. Already in 1989, the first

CD37-targeting antibody (labeled with the radioactive isotope iodine 131) was tested in 10

refractory NHL patients with promising clinical results [59]. However, as anti-CD20 treatment

(rituximab) was introduced at the same time, anti-CD37 therapy was forgotten for almost two

decades, until the development of a novel CD37-targeting mAb-derived polypeptide re-

established the interest in this target [60].

Nowadays several different antibody-based CD37-targeting approaches are under investigation in phase I and II trials for chronic lymphocytic leukemia (CLL) and refractory or relapsed NHL patients (reviewed in [61] and see https://clinicaltrials.gov/) (Figure 1e). Otlertuzumab (TRU-016), a humanized, antibody-derived CD37-targeting peptide, is closest to general clinical application in B-cell malignancies. In a randomized phase II study, relapsed CLL patients treated with the chemotherapeutic bendamustine plus otlertuzumab showed increased progression free survival compared to patients treated with bendamustine alone [62].

The anti-tumor mechanisms by which anti-CD37 agents act include ADCC, CDC and direct apoptosis signaling [60,63,64]. Additionally, CD37-antibody complexes are known to be internalized, which has led to the generation of novel anti-CD37 agents coupled to toxins (IMGN529, an anti-CD37 antibody conjugated to an anti-microtubule agent) [64] and radioactive labels (^{177}Lu-tetulomab) [65]. Both agents showed promising results in NHL xenograft mouse models [64,65]. Moreover, ^{177}Lu-tetulomab was internalized with higher efficiency than ^{177}Lu-rituximab complexes *in vitro* [65], and IMGN529 showed increased activity compared to rituximab or CVP chemotherapy in a FL-derived xenograft mouse model [64]. A phase I clinical trial completed in July 2016 studied IMGN529 in relapsed/refractory NHL and CLL patients (NCT01534715), and several phase I/II clinical studies to test ^{177}Lu-tetulomab (Betalutin) are currently including NHL patients (https://clinicaltrials.gov/).

Furthermore, new insights into the function of CD37 have prompted the interest in combination strategies. CD37 is important for the survival of IgG1-secreting plasma cells through the membrane organization of α4β1 integrins, and subsequent activation of the AKT signaling pathway [66]. The intracellular tails of CD37 can be tyrosine phosphorylated, leading to the initiation of apoptotic signaling via the N-terminal immunoreceptor tyrosine-based

inhibition motif (ITIM)-like domain, and opposite survival signaling via its C-terminal ITAM domain [63]. Both pathways are induced by the CD37-targeting mAb-derived polypeptide SMIP-016, which was the basis for development of TRU-016. Although signaling to apoptosis is the most prominent, the efficiency of CD37 targeting could be improved using combination therapy with pro-survival PI3K inhibitors [63].

Importantly, recent studies revealed that ~50% of DLBCL tumors are negative for CD37 expression, and its loss is a potential risk factor for R-CHOP resistance and poor survival rates independent of the International Prognostic Index (IPI) [56,57]. The underlying mechanism involves enhanced activation of the IL-6 signaling pathway in CD37-negative lymphomas [56]. IL-6 is known to be involved in the development of many cancers, including hematological malignancies, and several IL-6 targeting strategies have been developed (reviewed in [67,68]). Based on these studies, inhibition of IL-6 signaling (using mAb anti-IL-6 siltuximab or anti-IL-6R tocilizumab) may represent a potential new treatment strategy for patients with CD37-negative DLBCL (Figure 1e).

Dynamic protein interactions shape the organization of the B-cell membrane

Proteins in the plasma membrane are not randomly distributed, but instead they are localized to specific microdomains (Box 1). One of the first models of membrane protein organization on the B-cell membrane was published two decades ago [69]. Using flow cytometry energy transfer (FCET) experiments, it was shown that CD20 interacts with tetraspanins CD53, CD81, CD82 on a lymphoma B-cell line. In addition, confocal microscopy [70] and co-immunoprecipitation experiments [71] revealed that CD20 also clusters with the BCR in specialized microdomains, indicating that they are localized in lipid rafts or tetraspanin microdomains (Box 1). The BCR dissociates from CD20 to distinct lipid rafts upon stimulation,

and is subsequently internalized [70]. A direct interaction between CD20 and the BCR was recently confirmed using proximity-ligation assay [23]. It was shown that CD20 (similar to CD19) interacts with different BCR classes (IgD/IgM) on resting and antigen-activated human B cells, demonstrating redistribution of CD20 upon BCR stimulation.

CD20 has been shown to interact with several tetraspanins (CD53, CD81, CD82) [69], although CD37 was not studied. Recently, we discovered that both protein and mRNA expression of CD20 and CD37 on lymphoma B cells are correlated [57]. Although this is in line with the inferior outcome of CD37-negative DLBCL patients upon R-CHOP therapy, the prognostic significance of CD37 seems independent of CD20 mRNA levels [57]. Further research is necessary to verify a possible direct interaction and co-expression patterns of CD37 and CD20 in B-NHL cases.

These studies indicate that the organization of the B-cell membrane is shaped by dynamic protein-protein interactions that are subject to change upon B-cell activation. This protein organization is not only important for B-cell function but also for the efficacy of membrane-targeted therapies, as illustrated by clustering of CD20 upon rituximab binding [18]. Likewise, CD37-targeting using SMIP-016 resulted in translocation into microdomains which was required for efficient tumor cell apoptosis [63]. On lymphoma B cells, changes in membrane protein expression (e.g. absence of CD37) or protein clustering (e.g. conformational changes of the BCR upon binding of self-antigens), will change the protein organization and interactions at the plasma membrane. Based on these studies we propose that the changed cell surface protein landscape of lymphoma B cells has consequences for the efficacy and application of membrane-targeted therapies (Figure 2). Moreover, targeted therapies may alter dynamic protein-protein interactions affecting plasma membrane organization and downstream signaling.

Concluding remarks

Many patients with B-NHL still face treatment failure or relapse upon standard therapy emphasizing the urgent need for new treatment options. The plasma membrane is the most easily accessible part of the tumor cell, and exposes a variety of different proteins which may serve as treatment target. Here, we discussed the widely studied target CD20, and three alternative membrane targets (BCR, PD-L1 and CD37) that are currently under investigation in clinical trials to treat B-NHL. Furthermore, patients with CD37-negative lymphoma B cells have a dismal prognosis and may benefit from anti-IL-6(R)-targeted therapy.

Membrane proteins interact with each other and thereby facilitate various cell biological processes, including initiation of downstream signaling. B-cell activation will affect these protein interactions through induction of new interactions, more clustering, or alternatively by preventing specific interactions. Evidence is accumulating that lymphoma B cells contain aberrant cell surface protein expression and organization which has important consequences for both application and outcome of membrane-targeted therapy for B-NHL patients.

We are only at the beginning of understanding membrane organization of normal and malignant B cells and further research is required to address the outstanding questions in this new and exciting field (see Outstanding Questions). In particular, recent advances in super-resolution microscopy enables imaging the membrane composition of lymphoma cells at the nanoscale level [72]. Taking into account that lymphomas are heterogeneous tumors [2], targeting membrane proteins as monotherapy will probably not be sufficient for complete tumor eradication. Thus, the use of combination therapies, like immuno-chemotherapy and

the additional use of small molecule inhibitors, will be important in the future treatment of B-cell non-Hodgkin lymphoma.

Acknowledgements

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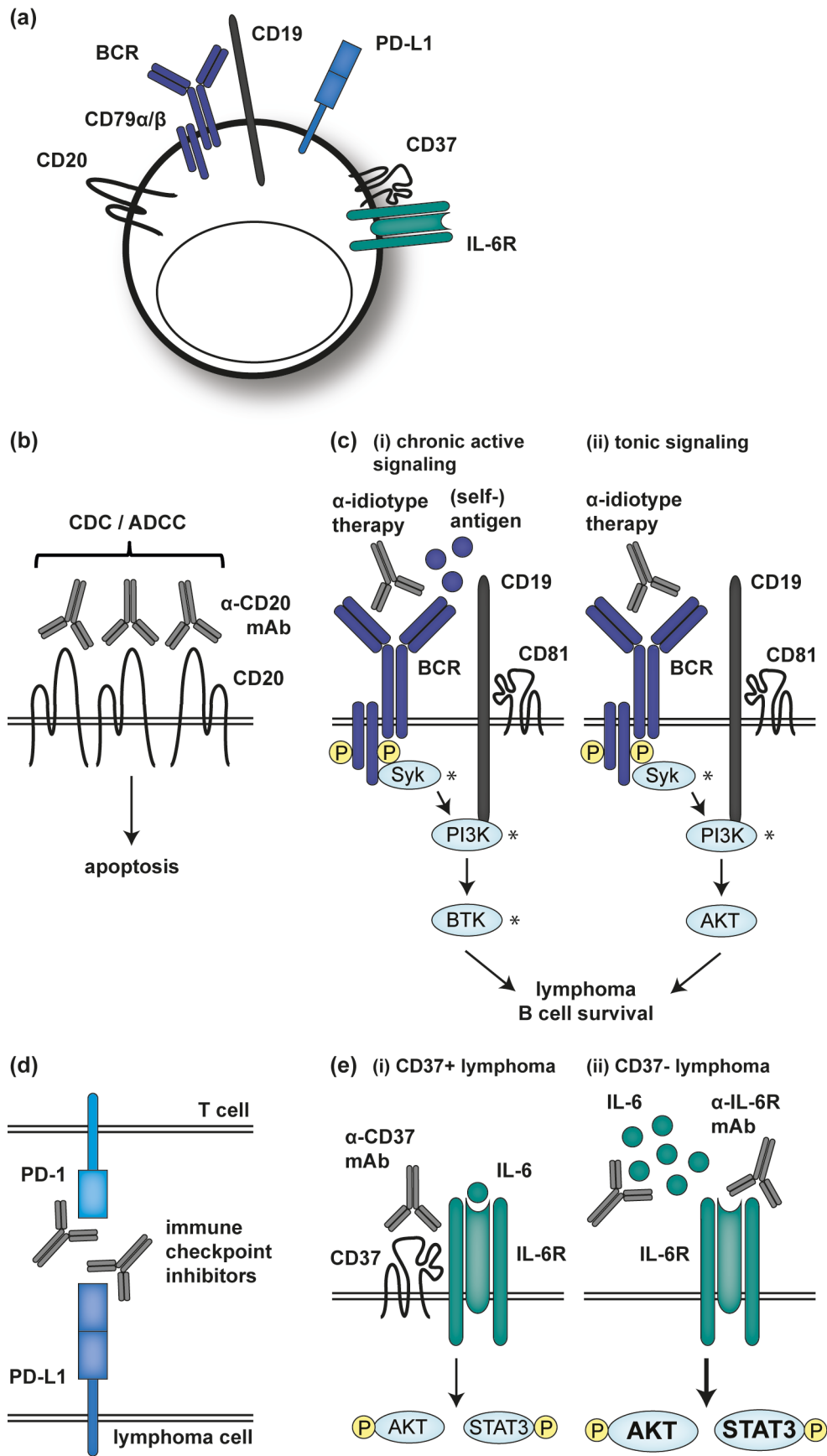
Figure 1

(a)

(b)

CDC / ADCC

α-CD20 mAb

CD20

apoptosis

(c) (i) chronic active signaling

α-idiotype therapy

(self-) antigen

CD19

BCR

CD81

P   P
Syk *

PI3K *

BTK *

(ii) tonic signaling

α-idiotype therapy

CD19

BCR

CD81

P   P
Syk *

PI3K *

AKT

lymphoma B cell survival

(d)

T cell

PD-1

immune checkpoint inhibitors

PD-L1

lymphoma cell

(e) (i) CD37+ lymphoma

α-CD37 mAb

IL-6

CD37

IL-6R

P AKT   STAT3 P

(ii) CD37- lymphoma

IL-6

α-IL-6R mAb

IL-6R

P AKT   STAT3 P

Figure 1. Current and novel membrane-targeted therapies in B-cell lymphoma. (a) Membrane proteins used in and explored for lymphoma-targeted therapy. Left to right: CD20; BCR with its signaling proteins CD79α/β; CD19; PD-L1; tetraspanin CD37 with the IL-6R. **(b)** Targeting CD20 using mAb (e.g. rituximab) will cluster CD20 proteins leading to tumor cell death via CDC, ADCC and/or direct apoptosis. **(c)** In chronic active BCR signaling **(i)**, binding of (self-)antigens to the BCR triggers recruitment of Syk to the phosphorylated (p-)ITAMs of CD79α/β, which leads to translocation of PI3K to CD19. Subsequently, BTK is activated which initiates NF-κB activation via the CARMA1-BCL10-MALT1 complex resulting in lymphoma B-cell survival. In tonic BCR signaling **(ii)**, constitutive activation of PI3K in absence of (self-) antigens results in continuous signaling via AKT pathway and lymphoma cell survival. Novel anti-idiotype therapy strategies can directly target the BCR. Syk, PI3K and BTK can be inhibited using small molecule inhibitors (*). **(d)** Immune checkpoint inhibitors (e.g. nivolumab, pembrolizumab, pidilimumab) bind to PD-L1 expressed on lymphoma B cells or to PD-1 on T cells. This inhibits the immune-escape mechanism used by ABC-DLBCL and FL cells. **(e)** In CD37-expressing (CD37+) B-cell lymphoma **(i)**, tumor cell death can be induced by targeting CD37-specific mAbs (IMGN529, Otlertuzumab). In contrast, CD37-negative (CD37-) B-cell lymphoma (~50% of DLBCL cases) **(ii)**, shows constitutive activation of the IL-6 signaling pathway (increased p-AKT and p-STAT3). These patients may benefit from α-IL-6(R) therapy.
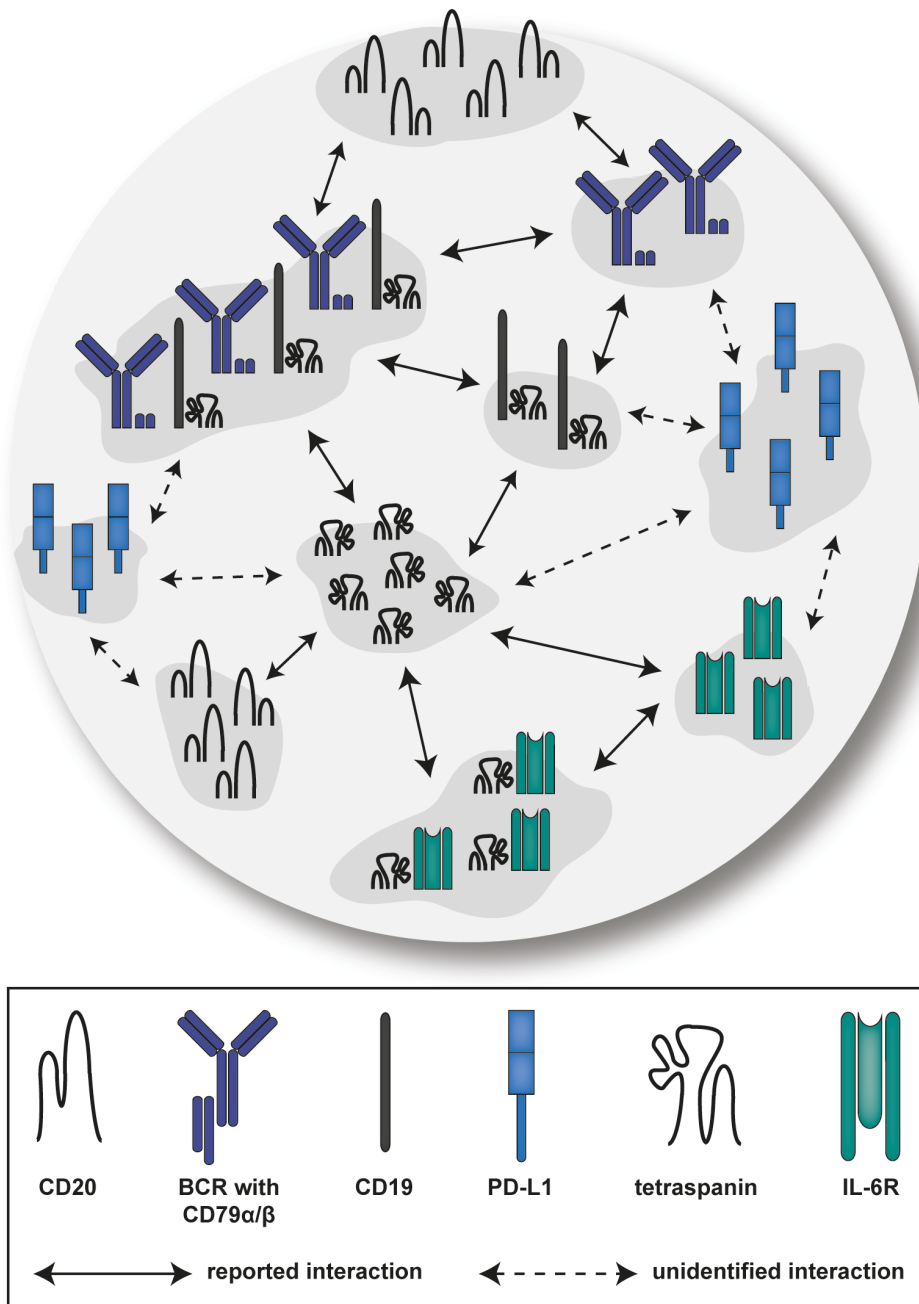
Figure 2

Figure 2. Plasma membrane organization of lymphoma B cells determines the efficacy of membrane-targeted therapies. The cell membrane (light area) is highly organized into specialized microdomains (dark area) that are formed by dynamic homotypic (e.g. CD20-CD20) and heterotypic (e.g. CD19-CD81) protein-protein interactions. Upon ligand-receptor binding (e.g. IL-6 to IL-6R), induction of a conformational change can lead to rearrangement of protein-protein interactions and reorganization of the membrane landscape. Lymphoma B cells have

a disrupted membrane protein organization, e.g. due to constant activation of the BCR by (self-)antigens, overexpression of PD-L1, or loss of tetraspanin CD37. In addition, BCR activation results in CD20 dissociation and redistribution, and anti-CD20 targeted therapy using rituximab induces CD20 clustering. These data support a model in which the microdomain organization of lymphoma cells determines the efficacy of membrane-targeted therapies, which has important implications for treatment application and clinical outcome.

Box 1. Plasma membrane microdomains

The plasma membrane is composed of multiple different proteins and lipids that are non-randomly localized into specialized areas (microdomains). Signaling molecules, lipids and proteins can cluster together in such organized domains, which is crucial for efficient signal transduction [73,74]. Disrupting membrane organization interferes with several membrane-proximal signaling processes, including those involved in tumor cell survival and metastasis (reviewed in [73]). Different types of membrane microdomains have been reported, including lipid rafts and tetraspanin-enriched microdomains (TEMs) [75]. Lipid rafts were originally identified as membrane fractions that are insoluble in the strong detergent Triton X-100 [76], hence they are also defined as detergent-resistant membrane domains (DRMs). They are enriched in densely packed sphingolipids and cholesterol, and harbor glycophosphatidylinositol (GPI)-anchored proteins among others [77]. A different type of microdomain is formed by tetraspanin proteins (Box 2) that associate with themselves and with specific partner molecules (including CD20, integrins and MHCII) in clusters known as the tetraspanin web or TEMs [78–80]. Although both TEMs and lipid rafts are detergent-resistant and present in DRMs, they are very different domains in terms of protein composition [81,82].

Box 2. Tetraspanins in cancer development

Tetraspanins are a family of four-transmembrane proteins involved in plasma membrane organization [79,82]. The mammalian tetraspanin superfamily consists of 33 members, which are expressed on almost all cells and tissues [83]. Tetraspanins consist of four transmembrane domains, a short extracellular loop (EC1), a large extracellular loop (EC2) containing a conserved CCG region, a small intracellular loop and two short intracellular tails [84], and S. van Deventer et al., in press]. Despite its similar structure, CD20 cannot be classified as a genuine tetraspanin protein, since it lacks the conserved CCG region in the large extracellular loop [85]. The EC2 and transmembrane domains are involved in the formation of tetraspanin-enriched microdomains (TEMs) by lateral associations with interaction partners, including other tetraspanins and integrins [78–80]. Their short intracellular tails can interact with cytosolic signaling molecules (e.g. PKC, Pi4K, Rac) [63,79], and S. van Deventer et al., in press]. Through their multiple interaction partners, tetraspanins are involved in different cellular processes including survival, proliferation, adhesion and migration [78]. Recently, several studies have shown that tetraspanins contribute to cancer development and metastasis, and tetraspanin expression has been associated with patient outcome [56,86]. Therefore, tetraspanins are interesting targets for cancer therapy, and different (pre-)clinical studies using tetraspanin targeting strategies are currently ongoing (https://clinicaltrials.gov/) [61,86].

Glossary

Antibody-dependent cell-mediated cytotoxicity: Induction of cell death via binding of an antibody to its cell surface target. The Fc tail of the antibody engages Fc receptor-expressing immune cells, like NK cells and macrophages, that will kill the antibody-bound target (i.e. tumor) cell.

Burkitt lymphoma: An aggressive type of B cell non-Hodgkin lymphoma characterized by deregulation of the *MYC* proto-oncogene.

CHOP: Abbreviation for Cyclophosphamide – Doxorubicin (Hydroxydaunorubicin) – Vincristine (Oncovin) – Prednisone. A chemotherapy cocktail mostly used to treat B-cell non-Hodgkin lymphoma patients. Often used in combination with anti-CD20 mAb rituximab (R-CHOP).

Class switch recombination: The process in which the isotype or class of immunoglobulin produced by the B cell is switched; e.g. from IgM to IgG. This occurs via genomic changes in the constant region of the immunoglobulin heavy chain.

Complement-dependent cytotoxicity: Induction of cell death via activation of the complement system (C1q binding to the antibody), which will lyse the target (i.e. tumor) cell membrane.

CVP: Abbreviation for Cyclophosphamide – Vincristine – Prednisone. A chemotherapy cocktail used to treat follicular lymphoma patients.

Diffuse large B cell lymphoma: The most common and most aggressive type of B-cell non-Hodgkin lymphoma, accounting for approximately one third of all non-Hodgkin lymphoma cases.

Follicular lymphoma: The second most common B-cell non-Hodgkin lymphoma. Follicular lymphoma is an indolent cancer, but can transform into the more aggressive DLBCL.

Germinal center: area in the B-cell follicles of secondary lymphoid tissues where B-cell proliferation, class switch recombination, somatic hypermutation and selection take place.

Somatic hypermutation: The process in which the immunoglobulin-encoded DNA of mature B cells is subjected to the introduction of random point mutations. These modifications occur in the variable region of the immunoglobulin gene resulting in affinity maturation (increased affinity BCR).