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Review Article

Therapeutic antibodies for mantle cell lymphoma: A brand-new era ahead

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Abstract

Mantle cell lymphoma (MCL) is a heterogeneous aggressive disease and remains incurable with current chemotherapies. The development of monoclonal antibody (mAb) has led to substantial achievement in immunotherapeutic strategies for B-cell lymphomas including MCL. Nonetheless, progress in the clinical use of mAbs is hindered by poor efficacy, off-target toxicities and drug resistance. Thus, novel mAbs engineering and approaches to improve target specificity and enhance affinity and potency are required. In this review, we highlight the latest advances of therapeutic antibodies in MCL, alone or in combination with other strategies and agents, with a particular focus on the current challenges and future prospective.

Keywords: Cancer research, Immunology, Oncology, Pharmaceutical science

1. Introduction

In the past few decades, tremendous advances in treatment have been achieved in the field of hematological malignancies, including leukemias and lymphomas, especially non-Hodgkin's lymphomas (NHL) [1]. Mantle cell lymphoma (MCL) is an aggressive B-cell NHL characterized by the t(11; 14) (q13; q32) translocation and

increased CCND1 (also termed cyclin D1) expression. The t(11; 14) (q13; q32) translocation leads to the constitutive overexpression of CCND1, which binds to cyclin-dependent kinases 4 and 6 (CDK4/6) and results in cell cycle disorder [2]. However, a subset of patients with MCL lack the t(11; 14) translocation and CCND1 expression; remarkably, CCND1 overexpression in mouse models is insufficient to induce spontaneous tumors [3], suggesting that other genetic or epigenetic events, signaling pathways, and microenvironmental alterations might be required for the initiation and progression of MCL [4, 5, 6, 7].

Due to the complex pathogenesis of MCL, there is no generally accepted standard of care specific for MCL patients at the initial stage, nor in the relapsed/refractory setting [8]. Although there are several chemotherapeutic backbones can be used in MCL, such as CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone) and Hyper-CVAD (cyclophosphamide, vincristine, doxorubicin, and dexamethasone), and many novel agents including bortezomib, ibrutinib, and lenalidomide have been approved for use, MCL remains an incurable subtype of NHL with the poorest long-term outcome [9]. Chemoresistance is one of the major explanations for poor prognosis of MCL. Thus, there is a pressing need to develop new targeted therapeutics and precision medicine for MCL, and in particular, to integrate new therapies into rational combinations to overcome drug resistance in MCL.

Monoclonal antibodies (mAbs) are gaining an increasing role in the therapy of blood cancer, especially when incorporated into other modalities. Being a rare subtype of NHL, MCL has seldom been specifically discussed in terms of the use of therapeutic antibodies. The functions and mechanisms of mAbs as well as the application of mAbs in other B-cell malignancies rather than MCL have been extensively reviewed [10, 11, 12, 13, 14], and they will not be discussed in depth here. In this article, we will go through the current and evolving therapeutic antibodies, alone or combined with other mAbs, chemotherapies, or novel agents in MCL exclusively unless otherwise specified, with a particular focus on the challenges and future directions.

2. Main text

2.1. Anti-CD20 mAbs in MCL

2.1.1. *Rituximab (RTX)*

The most important step in improving the outcome in NHL is the use of the type I chimeric anti-CD20 mAb RTX, a human/murine chimeric monoclonal antibody which has been clearly recognized as the most extensively employed treatment for NHL by inducing cytotoxicity via antibody-dependent cell-mediated cytotoxicity (ADCC), complement activation, and apoptosis (Fig. 1 and Table 1) [15, 16, 17]. RTX specifically targets the CD20 molecule on the surface of mature B cells and

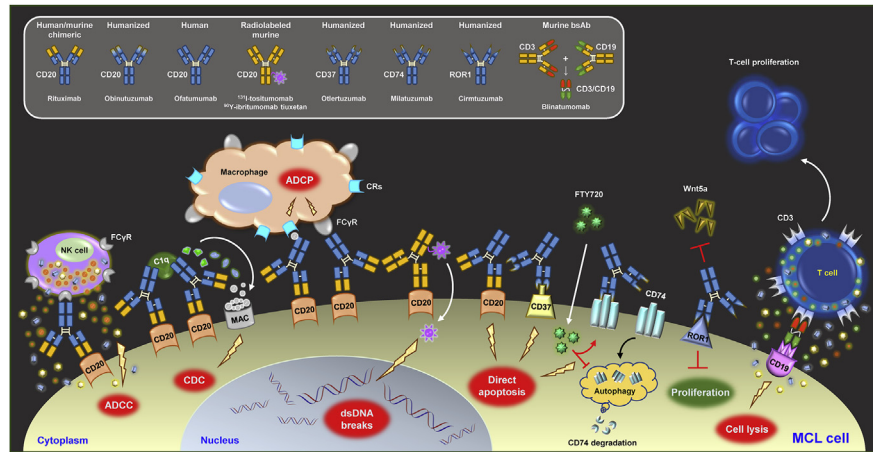


Fig. 1. Mechanisms of mAbs-mediated cell death in MCL. RTX is a human/murine chimeric anti-CD20 mAb, which leads to cell death via at least four mechanisms. Compared to RTX, humanized anti-CD20 mAb obinutuzumab and fully human anti-CD20 mAb ofatumumab have similar mechanisms, but each has its own distinct properties (not shown). To simplify the diagram, RTX is taken as an example to illustrate the mechanisms: a) RTX links natural killer (NK) cells to MCL cells through Fc γ R, resulting in ADCC by releasing cytolytic granules; b) the crosslinking of RTX and the CD20 antigen activates the complement cascade, forming the membrane attack complex (MAC) and promoting cell lysis via CDC; c) RTX that binds to CD20 can be recognized by the complement receptors (CRs) and Fc γ R expressed on macrophages, leading to antibody-dependent cellular phagocytosis (ADPCP); d) multiple binding of RTX to CD20 can cause direct apoptosis. Radiolabeled anti-CD20 mAbs, ^{131}I -tositumomab and ^{90}Y -ibritumomab tiuxetan, generate dsDNA breaks by particle emissions. Both humanized anti-CD37 olertuzumab and anti-CD74 milatuzumab induce direct apoptosis in MCL. The pro-death activity of milatuzumab is enhanced when combined with an autophagy inhibitor FTY720 by preventing degradation of CD74 in an autophagy-dependent manner. Blinatumomab is a murine bsAb targeting both the CD19 and CD3 antigens. Blinatumomab links CD3 $^{+}$ polyclonal T cells to CD19 $^{+}$ target cells, which activates T cells followed by serial cell lysis of CD19 $^{+}$ MCL cells and induces a robust T-cell proliferation as well as a transient release of cytokines.

B-cell lymphomas. In MCL, RTX has moderate single-agent efficacy [18]. In 2000, a European multicenter phase II study was conducted to assess the toxicity, overall response rate (ORR), and complete remission (CR) rate to RTX. Seventy-four patients with newly diagnosed MCL (MCL1, $n = 34$) and previously treated MCL (MCL2, $n = 40$) received RTX for four weeks via intravenous infusion. Upon treatment, 38% of MCL1 and 37% of MCL2 cases achieved ORR, and approximately 15% of patients achieved CR [19]. Similarly, a retrospective analysis from 81 evaluable MCL patients treated with RTX showed 37% of patients achieved ORR with 14% of patients achieving CR; however, the median time to disease progression (TTP) was only seven months, suggesting that single-agent RTX is active in MCL but fails to cure patients [20].

Compared to its single-agent activity, RTX combined with chemotherapeutic regimen CHOP achieved 96% ORR including 48% CR in newly diagnosed MCL, however 70% of patients finally occurred relapse or developed progressive disease,

Table 1. Therapeutic mAbs in MCL.

mAbs	Target	Origin	Isotype and format	Mechanisms
RTX	CD20	Murine-human chimeric	Human IgG1κ	ADCC, CDC, ADCP, direct apoptosis
Ofatumumab	CD20	Human	Human IgG1κ	ADCC, potent CDC, ADCP, direct apoptosis
Obinutuzumab	CD20	Humanized	Human IgG1	Enhanced ADCC, reduced CDC, ADCP, potent direct apoptosis
¹³¹ I-tositumomab	CD20	Murine	¹³¹ I-radiolabeled IgG2a	Double-strand DNA breaks caused by beta and gamma emissions
⁹⁰ Y-ibritumomab tiuxetan	CD20	Murine	⁹⁰ Y-radiolabeled IgG1κ	Double-strand DNA breaks caused by beta emission
Otlertuzumab	CD37	Humanized	Human IgG1	Direct apoptosis
Milatuzumab	CD74	Humanized	Human IgG1	Enhanced pro-death activity when combined with FTY720 by preventing degradation of CD74 in an autophagy-dependent manner
Galiximab	CD80	Primate-human chimeric	Human IgG1λ	ADCC and apoptosis in NHL, but unknown in MCL
Cirmtuzumab	ROR1	Humanized	Human IgG1	Inhibit Wnt5a-enhanced cell proliferation
Blinatumomab	CD19/CD3	Murine, bispecific	scFv-scFv	Robust T-cell activation to exert cytotoxic activity on target cells
HexAbs	CD20/CD74	Bispecific	DNL conjugation	Direct potent cytotoxicity

scFv, single-chain variable fragment.

implying that RTX with conventional chemotherapy may transiently clear tumor cells but not translate into prolonged progression-free survival (PFS) in MCL [21]. In 2004, therapeutic efficacy were reported with fludarabine, cyclophosphamide and mitoxantrone (FCM), alone or combined with RTX (R-FCM) in patients with relapsed/refractory MCL from a prospective randomized multicenter phase III trial. Of the 48 evaluable MCL patients (24 randomized for FCM, 24 randomized for R-FCM), R-FCM revealed a substantial benefit with 58% of ORR (29% CR, 29% partial remission [PR]) compared with 46% for FCM alone (0% CR, 46% PR); in addition, superior median PFS ($p = 0.038$) and overall survival (OS, $p = 0.003$) were obtained in the R-FCM arm compared with FCM alone, and no differences were found in clinically relevant side effects in both study arms [22]. Notably, RTX in combination with chemotherapy as either first-line therapy or maintenance therapy could particularly improve OS in elderly patients with MCL [23, 24]. In younger MCL patients, RTX maintenance after autologous stem-cell transplantation (ASCT) was also shown to improve PFS, event-free survival (EFS), and OS in a randomized phase III trial [25]. Very recently, a phase II study by Japan Clinical Oncology Group-Lymphoma Study Group (JCOG-LSG) showed high efficacy

and acceptable toxicity of R-High-CHOP/CHASER (cyclophosphamide, high-dose cytarabine, dexamethasone, etoposide, and rituximab)/LEED (melphalan, cyclophosphamide, etoposide, and dexamethasone) plus ASCT in younger patients with untreated advanced MCL, providing a potential standard treatment option for newly diagnosed younger MCL patients [26]. More RTX-based chemotherapies in MCL have been well documented [8, 17].

In addition to chemotherapies, newer agents in combination with RTX have also been investigated. In a phase I/II clinical trial, combining RTX with lenalidomide, an oral immunomodulator with anti-neoplastic and anti-proliferative effects against MCL [27], resulted in an ORR of 57% (36% CR, 20% PR) with a median PFS of 11.1 months [28]. The efficacy of this combination appears even higher as an initial therapy for patients with previously untreated MCL [29]. Of note, RTX plus lenalidomide enhances efficacy over what has been shown with monotherapy and improves outcomes in the RTX-resistant patients [30, 31]. In addition to lenalidomide, bortezomib, a novel proteasome inhibitor approved in the U.S for the treatment of patients with MCL [32], has been incorporated into many regimens. As a part of front-line therapy, the combination of bortezomib with R-CHOP (RTX and CHOP) [33] or R-Hyper-CVAD (RTX and Hyper-CVAD) [34] obtains a striking advance over the original regimens with less toxicity. Ibrutinib, an oral covalent inhibitor of Bruton's tyrosine kinase (BTK), is able to irreversibly inactivate the B-cell receptor signaling pathway [35]. In a single-center open-label phase II trial, ibrutinib combined with RTX is active and well-tolerated in relapsed/refractory MCL patients with 88% of ORR (44% CR, 44% PR) [36]. Interestingly, the objective response was 100% in patients with Ki-67 < 50%, whereas worse treatment outcomes were observed in patients with higher Ki-67 levels ($\geq 50\%$), suggesting that Ki-67 might serve as a predictor for this combination therapy in MCL [36]. Ibrutinib is also well tolerated when added to R-CHOP in a non-randomized phase Ib study [37]. Further combination of ibrutinib with RTX and bendamustine (R-bendamustine) achieved 94% ORR (76% CR) in newly diagnosed MCL patients [38] compared with 68% for single agent ibrutinib (21% CR) [39] and 75%–92% for R-bendamustine (41%–50% CR) in MCL [40, 41], although longer follow-ups and more clinical data such as the PFS are warranted for further evaluation. The clinical data of RTX-based studies are summarized in Table 2.

2.1.2. Ofatumumab

Despite improved outcomes with RTX, a significant proportion of patients still experience disease relapse or refractory situations. In this context, alternative therapeutic strategies that can overcome resistance with more efficiency, such as second generation anti-CD20 mAbs, have been developed [1]. Ofatumumab is one of the second generation type I anti-CD20 mAbs, which was generated from human

Table 2. Monoclonal antibody-based therapies in MCL.

Therapeutic regimen	Study features	Number of MCL patients	ORR (CR) (%)	PFS, TTP, EFS or other parameters (months)	Median OS (months)	Ref.
Single-agent RTX (multicenter)	Phase II	74 (MCL1, n = 34; MCL2, n = 40)	38 in MCL1 and 37 in MCL2 (15)	Median response duration: 1·2 yr	—	[19]
Single-agent RTX (multicenter)	Retrospective	87 (MCL1, n = 37; MCL2, n = 50)	37 (14)	TTP: 7	—	[20]
R-CHOP	Phase II	40	96 (48)	Median PFS: 16·6	—	[21]
R-FCM vs FCM	Phase III	48	58 (29) vs 46 (0)	Median PFS: 8 vs 4	NR vs 11; 2-yr OS: 65% vs 35%	[22]
RTX + chemotherapy vs chemotherapy	—	638	—	—	37 vs 27	[23]
RTX + ASCT vs ASCT	Phase III	240 (120/group)	—	4-yr PFS: 83% vs 64%; 4-yr EFS: 79% vs 61%	4-yr OS: 89% vs 80%	[25]
R-High-CHOP/CHASER/LEED + ASCT	Phase II	45 (n = 35 for patients who completed ASCT)	96 (82) (n = 45); 100 (91) (n = 35)	5-yr PFS: 52% (n = 45) 5-yr PFS: 55% (n = 35)	5-yr OS: 71% (n = 45); 5-yr OS: 72% (n = 35)	[26]
RTX + lenalidomide	Phase I/II	52	57 (36)	Median PFS: 11·1	24·3	[28]
RTX + lenalidomide (multicenter)	Phase II	38	92 (64)	Median PFS: NR; 2-yr PFS: 85%	2-yr OS: 97%	[29]
RTX + lenalidomide	Phase II	11	55 (55)	Median PFS: 24·4	—	[31]
R-CHOP + bortezomib	Phase I/II	36	91 (72) (n = 32); 81 (64) (n = 36)	Median PFS: 23; 2-yr PFS: 44% (n = 36)	NR; 2-yr OS: 86%	[33]
RTX + ibrutinib	Phase II	50	88 (44)	Median PFS: NR	NR	[36]
R-bendamustine + ibrutinib	Phase I/Ib	17	94 (76)	Median PFS: NR	NR	[38]
Single-agent ofatumumab	Phase II	12	8·3 (0)	—	11·2	[46]
Single-agent obinutuzumab	Phase II	15	27 (13)	—	—	[56]
¹³¹ I-tositumomab + CHOP	—	25	86 (67)	—	2-yr OS: 92%	[59]
R-CHOP + ⁹⁰ Y-ibritumomab tiuxetan	Phase II	53	88 (55)	Median PFS: 31	2-yr OS: 90%	
Single-agent ⁹⁰ Y-ibritumomab tiuxetan	Phase II	34	31 (15) (n = 32)	Median EFS: 6	21	[60]
⁹⁰ Y-ibritumomab tiuxetan + bortezomib	Phase I	5	80 (60)	—	NR	[61]
Single-agent otlertuzumab	Expansion of phase I	4	0	—	—	[65]

(continued on next page)

Table 2. (Continued)

Therapeutic regimen	Study features	Number of MCL patients	ORR (CR) (%)	PFS, TTP, EFS or other parameters (months)	Median OS (months)	Ref.
Single-agent blinatumomab (multicenter)	Phase I	6 (15, 30 µg/m ² /day); 2 (60 µg/m ² /day)	16·7 (16·7) (15, 30 µg/m ² /day); 100 (50) (60 µg/m ² /day)	–	–	[94]
Single-agent blinatumomab	Phase I	7 (60 µg/m ² /day)	71 (43) (60 µg/m ² /day)	–	–	[95]

MCL1, newly diagnosed MCL; MCL2, previously treated MCL; NR, not reached.

immunoglobulin transgenic mice (Table 1) [42, 43]. Compared to RTX, the fully human mAb ofatumumab binds to a distinct epitope of the CD20 molecule, which consists of the smaller extracellular loop and the N-terminal region of the large extracellular loop [43], much closer to the cell membrane. These favorable properties lead to an outstanding potency in complement-dependent cytotoxicity (CDC) and a lower off-rate to CD20 than RTX [42, 44]. In MCL cell lines, ofatumumab exhibits more potent CDC activity compared with RTX, including in cell lines with resistance to RTX; in a murine model of MCL, ofatumumab also delays tumor progression and prolongs survival [45]. Nevertheless, an open-label phase II single-arm study of ofatumumab monotherapy for relapsed/refractory MCL was carried out in three centers from the UK [46]. Of the 12 patients enrolled in the trial, the ORR was 8·3% with a median OS of 11·2 month; only one of the 12 patients achieved PR and six patients achieved stable disease, whereas the five remaining patients had progressive disease (Table 2) [46]. Of note, ofatumumab shows significant adverse event of fatigue (75% patients affected) that is much higher than other types of lymphomas [47]. Based on this study, the response rate of single-agent ofatumumab in relapsed/refractory MCL patients is low, although another study reported a complete remission after treatment with single-agent ofatumumab in a patient with high-risk leukemic MCL [48]. A more intensive and prolonged exposure to ofatumumab, or combination of ofatumumab with other anti-CD20 mAbs or chemotherapies might increase the potential of ofatumumab in MCL [46].

2.1.3. Obinutuzumab

Unlike type I anti-CD20 mAbs (e.g., RTX, ofatumumab), obinutuzumab is a novel humanized and glycoengineered type II anti-CD20 antibody, which induces enhanced ADCC and stronger direct cell death activity but does not localize mAb-CD20 antigen complexes into lipid rafts, resulting in lower levels of CDC (Table 1) [49]. Despite recognizing an overlapping epitope of the CD20 extracellular domain, obinutuzumab targets CD20 in a completely different orientation than type

I antibodies [50], and exhibits superior efficacy over RTX and ofatumumab [51], especially in RTX-resistant cases [49]. One molecular mechanism is Fc-gamma receptors IIb (Fc γ RIIb), a key molecular expressed on B cells that can promote the internalization of RTX and result in reduced efficacy of RTX [52]. This finding implicates that type II anti-CD20 mAbs may enhance immune effector function by circumventing the limitations of internalization, although it is unclear why type II anti-CD20 mAbs tend not to internalize and do not engage and activate Fc γ RIIb [52].

In MCL, one study showed that drug resistance can be overcome with obinutuzumab which counteracted Bcl-x_L induction through NF- κ B inhibition; by contrast, RTX was not able to overcome drug resistance [53]. In another study, MCL cell lines Granta-519, Jeko-1, Rec-1, and Z-138 were treated with RTX or obinutuzumab, alone or in combination [54]. Obinutuzumab induced a higher reduction in cell viability in each MCL cell line compared to RTX, whereas combination experiments showed the competitive binding of these two mAbs due to overlapping epitopes on the target cells, leading to a lower cytotoxicity than obinutuzumab alone. Of interest, uniquely deregulated genes were induced after RTX and obinutuzumab monotherapy, respectively, providing more rational strategies for new combination experiments in MCL [54]. In a phase I study (GAUGUIN), 43% of 21 heavily pre-treated patients with NHL achieved disease response to 8 \times 21-day cycles of obinutuzumab [55]; Later that year, the efficacy and safety of obinutuzumab were further explored by the same research group in one randomized phase II trial in relapsed/refractory MCL patients, in which, obinutuzumab achieved early steady-state concentration and clinical activity with an acceptable safety profile (Table 2) [56]. Ongoing and future trials will provide more evidence regarding the use of obinutuzumab in MCL, and other type II anti-CD20 mAbs are also anticipated.

2.1.4. Armed anti-CD20 mAbs

The addition of a radionuclide to monoclonal antibodies was thought to be a rational approach to achieve extra cytotoxic effects, particularly due to the inherent radiosensitivity of most lymphomas including MCL. This modification, also termed radioimmunotherapy (RIT), has been reported as a safe and effective therapeutic option for NHL.

Two anti-CD20 RIT antibodies, iodine-131 (¹³¹I)-tositumomab [57] and yttrium-90 (⁹⁰Y)-ibritumomab tiuxetan [58], have been approved by the Food and Drug Administration (FDA). Both agents have been reported for the treatment of relapsed/refractory CD20⁺ lymphomas. The radionuclides delivered by these two antibodies are decayed by releasing beta particles, thereby causing double-strand DNA (dsDNA) breaks in tumor cells (Fig. 1 and Table 1) [59]. In relapsed/refractory MCL, single-agent ⁹⁰Y-ibritumomab tiuxetan earned an ORR of 31% (15% CR) and a median EFS of 28 months for patients who achieved PR or CR [60]. Sequential RIT

with ^{131}I -tositumomab followed by CHOP consolidation in MCL patients could induce molecular remission with 86% of ORR (67% CR) and 92% of OS at two years [59]. Similarly, ^{90}Y -ibritumomab tiuxetan consolidation after R-CHOP chemotherapy in newly diagnosed MCL also obtained an ORR of 88%, and CR was dramatically elevated from 13% upon R-CHOP to 55% after RIT consolidation [59]. In addition to conventional chemotherapeutic regimens, RIT plus bortezomib were also evaluated in a phase I clinical trial: ^{90}Y -ibritumomab tiuxetan preceded by bortezomib as a radiosensitizer is a safe and well-tolerated combination therapy with an ORR of 80% in relapsed/refractory MCL [61]. Given the radiosensitive feature of MCL, RIT allows for a more targeted therapeutic option in the treatment of MCL, both in the frontline and at relapse [62]. The clinical details of radioimmunotherapy in MCL are shown in Table 2.

2.2. Other potential mAbs in MCL

CD37 is a heavily glycosylated cell surface tetraspanin expressed on normal and malignant B cells [63, 64]. Due to its absence or weak expression on blood cells except B cells, CD37 is considered as a lineage-specific marker of human B cells, representing a key therapeutic target for B-cell malignancies [65]. In MCL, hypomethylated and overexpressed *CD37* gene is revealed as a novel target for drug development from a genome-wide DNA methylation analysis, suggesting that distinct epigenetic changes could be targeted for therapeutic benefit in MCL [66].

Otlertuzumab is a humanized anti-CD37 protein therapeutic, and it triggers cell apoptosis directly by up-regulation of a proapoptotic protein BCL2 like 11 (BCL2L11, also termed BIM) in B-cell malignancies (Fig. 1 and Table 1) [67]. In a SCID mouse model of leukemia/lymphoma, significant *in vivo* therapeutic efficacy of otlertuzumab is revealed [68]. More importantly, otlertuzumab could offer an alternative therapeutic regimen when CD20 is blocked or even lost on the targeted B cells [69]. Therefore, it is unsurprised that otlertuzumab in combination with RTX or other chemotherapeutics leads to an enhanced anti-tumor activity in NHL models [65]. Nonetheless, the use of otlertuzumab in MCL has been rarely reported. In 2015, the clinical activity of otlertuzumab in patients with advanced MCL was firstly evaluated [65]. Among four patients with MCL, all had received prior RTX therapy and chemotherapy; in fact, otlertuzumab activity as a single agent in such a heavily pre-treated population was not satisfactory in MCL, because none of the four MCL patients had a response (Table 2) [65]. Given these results, further studies with otlertuzumab in combination with RTX or other chemotherapeutic agents should be carried out in MCL.

Another potential target is CD74 antigen, which is an integral membrane protein with diverse functions. Enhanced expression of CD74 has been implicated in malignant B cell growth and survival, making it a potential target for immunotherapy

[70]. Milatuzumab is a humanized mAb specific for CD74 (Table 1) [71]. Unlike RTX, milatuzumab does not induce ADCC or CDC [72], implying that milatuzumab targets distinct cell death signaling pathways via different cellular mechanisms. Thus, the preclinical combination of RTX and milatuzumab shows both *in vitro* and *in vivo* activity in MCL, and the same is true when incorporating milatuzumab into either ofatumumab or velutuzumab (anti-CD20 IgG) treatment [73]. Interestingly, the same research group also found FTY720, an immunosuppressive drug that serves as an autophagy-inhibiting agent in MCL cell lines; after treatment, FTY720 enhanced the pro-death activity of milatuzumab by preventing the degradation of CD74 in an autophagy-dependent manner (Fig. 1) [74, 75]. Afterwards, this group further reported OSU-2S, a novel non-immunosuppressive FTY720 derivative that could increase the surface expression of CD74. Therefore, OSU-2S in combination with milatuzumab could enhance cytotoxicity in MCL cells [76].

In addition to CD37 and CD74, CD80 is a member of the B7 family of immune coregulatory proteins that mediate both immune activation and suppression. CD80 has demonstrated a key role in regulating immune inhibitory processes [77], and in particular, it has been identified as a therapeutic target in NHL based on limited immunohistochemical studies [78, 79]. CD80 expression has been evaluated by flow cytometry analysis using primary lymphoma cells from patients with NHL, among which, 75% of MCL cases are CD80 positive [80]. Galiximab, a primate-human chimeric anti-CD80 antibody, has shown safety and efficacy in patients with relapsed/refractory follicular lymphoma (FL) (Table 1) [81]. Despite no report of galiximab in MCL yet, targeting CD80 could probably provide a clue in the treatment of MCL in near future.

Other than CD (cluster differentiation) molecules, a receptor tyrosine kinase-like orphan receptor 1 (ROR1) was identified as a dominant cell surface marker in chronic lymphoblastic leukemia (CLL) from two independent gene expression studies [82, 83]. ROR1 functions as a receptor for Wnt5a which induces noncanonical Wnt signaling, leading to activation of Rac1 and enhanced proliferation of CLL cells [84]. Intriguingly, ROR1 expression is not restricted to CLL, but is more prevalent in NHLs, especially in MCL where ROR1 is expressed intensely [85]. In this context, a humanized anti-ROR1 mAb cirmtuzumab becomes a potential target against MCL. Recently, one study revealed that MCL proliferation can be inhibited by cirmtuzumab via a ROR1-dependent pathway which, however cannot be blocked by ibrutinib, suggesting that cirmtuzumab and ibrutinib may play complementary roles in the treatment of MCL patients [86]. The synergistic anti-tumor effects of cirmtuzumab and ibrutinib has been proved last year in CLL cells [87], and the combination of cirmtuzumab with ibrutinib in MCL need further evaluation.

2.3. Bispecific antibodies (bsAb) in MCL

Combination of antibody (Ab) therapy can be achieved by a single bsAb to avoid administering two different Abs sequentially, which is time-consuming, inconvenient, and expensive. Consequently, developing bsAbs is emerging as a new part of Ab therapies by generating various constructs differing in design, structure, and antigen (Ag)-binding properties. A desirable bsAb may serve to 1) recruit effector cells or effector molecules to target malignant cells, 2) improve the target specificity by simultaneous ligation two different Ags expressed on the same cell, or 3) involve more than one signaling pathway or mechanism controlling cell proliferation or survival. These features enable bsAbs a more potent function by overcoming single-mAb resistance and enhancing functional affinity, hence achieving a higher potency.

2.3.1. CD19/CD3 bsAb

Blinatumomab is a CD19/CD3 bispecific T cell-engaging antibody targeting both the CD3 ϵ subunit of the T-cell receptor (TCR) complex and the CD19, a B-cell Ag which is expressed on malignant B cells across all NHL subtypes (Table 1) [88, 89]. Regular antibodies cannot directly recruit T cells due to the lack of Fc-gamma receptors (Fc γ R) on these cells, whereas blinatumomab links CD3⁺ polyclonal T cells to CD19⁺ B cells, which activates T cells followed by serial lysis of CD19⁺ target cells via granzymes and perforin [89, 90], and induces a robust T-cell expansion as well as a transient release of cytokines (Fig. 1) [91, 92, 93]. In 2008, the clinical activity and safety of single-agent blinatumomab was assessed in NHLs including MCL [94]. Partial and complete tumor regressions were first observed (4/19) at a dose of 15–30 $\mu\text{g}/\text{m}^2/\text{day}$, and all patients (7/7) got a tumor regression at a dose of 60 $\mu\text{g}/\text{m}^2/\text{day}$; notably, blinatumomab doses of 15 $\mu\text{g}/\text{m}^2/\text{day}$ and higher led to clearance of tumor cells not only in peripheral blood, lymph node lesions, and spleen, but also in bone marrow and infiltrated liver (Table 2) [94]. In a following phase I dose-escalation study, a total of 24 patients with relapsed/refractory MCL were enrolled; the ORR was 71% (n = 7, 3 CR/unconfirmed CR [CR_U] and 2 PR) with blinatumomab monotherapy at a dose of 60 $\mu\text{g}/\text{m}^2/\text{day}$, which was established as the maximum tolerated dose (MTD) for administration and was finally selected as the target dose for efficacy, although one patient did not respond to retreatment at 60 $\mu\text{g}/\text{m}^2/\text{day}$ and experienced a relapse (Table 2) [95]. Future developments including the potential combination of blinatumomab with regular chemotherapies or other agents, and the extension of alternative routes of administration need to be further addressed and optimized [89].

2.3.2. CD20/CD74 bsAb

Owing to the fact that RTX and milatuzumab target different antigens and signaling pathways, one group explored a preclinical evaluation of combination strategy in

MCL: targeting both CD20 and CD74 led to rapid cell death in MCL cell lines and primary tumor cells compared with either parental Ab alone [73]. Later that year, the same team successfully generated novel bispecific hexavalent Abs (HexAbs) from veltuzumab and milatuzumab using a Dock-and-Lock (DNL) method; surprisingly, the anti-CD20/CD74 bispecific HexAbs resulted in homotypic adhesion and triggered multiple intracellular events in MCL cell lines and primary MCL cells including production of reactive oxygen species (ROS), rapid and sustained activation of ERKs and JNK-MAPKs, deactivation of the PI3K/Akt signaling pathway, and actin reorganization, etc. [96] This study indicates that such bsAb constructs are more potent than either parental mAb or even combination of these mAbs, and is likely to provide new immunotherapeutic strategies for the other CD20⁺/CD74⁺ malignancies.

2.4. Current challenges and future prospective

Over the past decades, mAbs have proven to be incredibly valuable as cancer therapeutics and have made substantial achievement in clinical outcome. Although RTX has been incorporated routinely into regimens and has made an important impact on NHL, there has been no significant therapeutic improvement for patients with MCL for many years [17].

RTX resistance is a great challenge in the treatment of MCL. The mechanisms underlying resistance are still unclear. Reduction or loss of the CD20 hallmark from the lymphoma cells has been reported as a major contributor to unresponsiveness [97, 98, 99, 100, 101]. One mechanism of CD20 loss is the “shaving reaction”, in which RTX-CD20 complex is cleared from MCL cells by monocytes in the circulation mediated by FcγR [102]. Another mechanism is the CD20 internalization modulated by type I anti-CD20 mAbs, shortening mAb half-life via a rapid degradation of CD20/mAb complex in lysosomes [103]. FcγRIIb is further revealed as a key participant to promote CD20 internalization [52]. Importantly, these findings probably explain the different sensitivity of B-cell malignancies to RTX. For example, CD20 internalization is the most evident in MCL samples which express higher FcγRIIb [52, 103], consistent with the relatively poor response of MCL to RTX treatment; by contrast, significantly lower internalization and FcγRIIb expression are observed in FL and diffuse large B-cell lymphoma (DLBCL) samples which are far more resistant to CD20 loss [52, 103]. Other possible resistance mechanisms include alterations of tumor cells survival pathways (ERKs, p38 MAPK, NF-κB, etc.), leading to failure of RTX to target these signaling [104]; defective RTX-induced redistribution of CD20 in lipid rafts, failing to evoke CDC, calcium influx and apoptosis [105, 106, 107]; FcγR polymorphisms that can increase RTX affinity in FL and can predict the outcome in some studies of FL and DLBCL [108, 109, 110], but it is independent of the RTX efficacy in MCL [111]. Despite these potential

mechanisms for disparity in efficacy among different NHL subtypes, MCL continues to be a therapeutic challenge due to its unclear pathogenesis and very limited research.

Owing to poor response to RTX, advances in the design of other anti-CD20 mAbs, such as ofatumumab and obinutuzumab, have offered alternative options for the treatment of MCL. However, it is hard to make a clear comparison between RTX and other types of anti-CD20 mAbs, because most of patients have already received prior RTX therapy. Therefore, well-designed and -executed clinical trials are needed to determine the true clinical benefit of each mAb and its long-term safety. Apart from CD20, other Ags including CD37, CD74, CD80, and ROR1 also exhibit great potentials in MCL, although very limited studies are reported.

Step forward, multiple newer drugs have incorporated into the standard regimens. In addition to bortezomib, ibrutinib, and lenalidomide, agents that are being explored or under investigation comprise PI3K inhibitor idelalisib, BCL2 inhibitors, HDAC inhibitors, and CDK4/6 inhibitors etc., and we are still in a “watching and waiting” stage. It is beyond the scope of this article to discuss these agents in detail, however we can anticipate that over the next decades, we will be very likely to embrace a brand-new chemo-free model of treatment with mAbs and target agents in MCL. Nevertheless, the management of relapsed/refractory MCL with high tumor burden still requires a long duration to be fully realized, and chemotherapy-free regimens must be cautiously considered and performed.

It is noteworthy that most current reports focus on either basic research of mAbs engineering or clinical trials regarding the use of mAbs in MCL, and both are seriously disconnected to each other. Remarkably, the mechanism of mAb action rather than therapeutic efficacy in MCL patients are lacking, resulting in many un-addressed questions, for example, how multiple agents work together and interact in the human body? Why they do not work in some selected MCL patients? Compared to other types of NHLs, what is the unique mechanisms in MCL underlying the resistance to mAbs? How to stimulate effector cells appropriately without an overshooting immune response upon the treatment of bsAbs? It is necessary to go deeper into the research, not solely to assess clinical efficacy of mAbs, but also to figure out why or why not mAbs work, how they work, and which mechanism is to be favored when adding adjuvants, thereby providing evidence-based guidelines for treatment.

Another major challenge is how to collect solid clinical data with very limited population of MCL patients. Few studies have specifically targeted MCL, instead, most scientific and clinical data are along with other types of NHL. It seems hard and insufficient to draw a conclusion with only a small number of MCL cases enrolled in clinical trials. Therefore, a multicenter study from several groups around the world is probably the best way to solve this problem. Furthermore, off-target effects of

mAbs therapy is also a big issue that should not be underestimated. Despite high potency and favorable cytotoxicity obtained by mAbs, they are not toxicity-free for normal tissues and cells, and potential side effects might be caused, especially when incorporated into other strategies.

Last but not the least, mounting evidence from earlier clinical trials suggests that initial therapy is very likely the best time to incorporate novel agents in order to minimize drug resistance and improve overall outcome [112]. Thus newly designed clinical trials in untreated MCL patients instead of heavily pretreated patients are warranted.

3. Conclusions

Therapeutic mAbs alone or combined with conventional chemotherapies or newer agents provide alternative potentials for the treatment of MCL. Despite great progress of utilizing mAbs in MCL, there is still much leeway for improvement. A better understanding of the tumor-specific mechanisms and pharmacokinetics, tailoring new anti-CD20 mAbs, developing novel mAbs and antibody-drug conjugates (ADC), designing bsAbs with brand-new concept, and identifying predictors for therapeutic response are urgently needed and will offer new opportunities to make better therapeutic mAbs or make mAbs better. The advances even breakthroughs in the treatment of MCL in the future is worth looking forward to.

Declarations

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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