Breakthrough therapies in B-cell non-Hodgkin lymphoma

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The last 5 years have seen significant advances in our understanding of the molecular pathogenesis of B-cell lymphomas. This has led to the emergence of a large number of new therapeutic agents exploiting precise aspects of the tumor cell’s signaling pathways, surface antigens or microenvironment. The purpose of this comprehensive review is to provide a detailed analysis of the breakthrough agents in the field, with a focus on recent clinical data. We describe agents targeting the B-cell receptor pathway, Bcl-2 inhibitors, emerging epigenetic therapies, new monoclonal antibodies and antibody drug conjugates, selective inhibitors of nuclear export, agents targeting the programmed cell death axis and chimeric antigen receptor T cells.

Key words: lymphomas, treatment, novel agents, ibrutinib, idelalisib, venetoclax
introduction

B-cell non-Hodgkin lymphomas (B-NHL) comprise collectively the most common hematologic malignancy. Although outcomes have improved, many patients are not cured using currently available therapies. Most patients requiring therapy still receive cytotoxic chemotherapy combined with the anti-CD20 monoclonal antibody (mAb) rituximab. Greater understanding of key signaling and regulatory pathways and the tumor microenvironment have led to the discovery and evaluation of a new generation of agents. Our goal is to provide an overview of emerging agents currently under development in B-NHL. A list of classes of agents, specific examples and phase of development is presented in Table 1; their mechanisms of action are summarized in Figure 1. This review does not include new data on CLL/SLL, a deserving subject covered in other recent reviews [2]. Although the immunomodulatory drug lenalidomide is now an important treatment option in B-NHL, this was recently reviewed and will not be discussed further [3].

B-cell receptor pathway inhibitors

Normal and malignant B cells rely on the B-cell receptor (BCR) for myriad cellular processes, including proliferation, apoptosis and differentiation [4]. Deregulation of the BCR pathway appears critical to the development of B-NHL [5]. The pathway includes druggable targets, including Bruton’s tyrosine kinase (BTK), spleen tyrosine kinase (Syk) and phosphatidylinositol-3-kinase (PI3K).

PI3K inhibitors

Class I PI3K are heterodimers comprising regulatory (p85) and catalytic (p110) subunits. The p110 subunit exists as four isoforms (α, β, δ, γ) with non-overlapping functions and differing expression profiles: the α and β isoforms expressed ubiquitously, while the γ and δ isoforms are expressed primarily in hematolymphoid tissues [6]. Although the importance of the pathway has been known for some time, initial attempts to target PI3K...
patients with rituximab and alkylator-refractory indolent B-NHL were treated with idelalisib 150 mg twice-daily until disease progression [8]. Despite the heavily pre-treated population, significant anti-tumor activity was observed, with ORR 57% [complete response (CR) 7%]. The median progression-free survival (PFS) and overall survival (OS) were 11.0 and 20.3 months, respectively. Kahl et al. [9] treated 40 patients with relapsed/refractory MCL, with the toxicity profile similar to prior experience with the agent; the ORR was 40% for all doses, and 69% for patients treated with ≥150 mg daily. However, the median DOR and PFS were 2.7 and 3.7 months, respectively. Eight patients experienced prolonged clinical benefit, most of whom were less heavily pre-treated. This lack of durability suggests some facet of MCL pathophysiology results in the rapid development of secondary resistance to p110δ inhibition.

The toxicities caused by this agent merit further discussion. Diarrhea is common, with grade ≥3 events in ~14% of patients. This appears minimally responsive to anti-motility agents—a lymphocytic colitis has been demonstrated on some colonoscopic biopsies and anecdotal reports indicate a potential role for steroids [10]. Intestinal perforation and fatal pneumonitis have occurred in <1% patients treated. Toxicities may be enhanced by immunomodulators: a study exploring the combination of idelalisib, lenalidomide and rituximab resulted in unacceptable rates of hepatotoxicity, with two deaths likely attributable to the treatment [11]. We suggest idelalisib be reserved as a third-line agent for patients with indolent B-NHL, with careful monitoring for treatment-emergent adverse events [12, 13].

Duvelisib (IPI-145) is an oral, potent dual p110δ/γ inhibitor [14]. In the phase I study in relapsed/refractory indolent NHL, among 32 patients with a median of three prior treatments, the ORR was 65% with 5 CRs seen in patients with FL. Similar to idelalisib, transaminitis (41%) and diarrhea (22%) were the most common grade ≥3 events in similar to prior experience with the other PI3K inhibitors [15]. Other PI3K inhibitors in early clinical development include TGR-1202, a PI3K p110δ inhibitor that appears to have lower rates of hepatotoxicity, with two deaths likely attributable to the treatment [11]. We suggest idelalisib be reserved as a third-line agent for patients with indolent B-NHL, with careful monitoring for treatment-emergent adverse events [12, 13].

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**BTK inhibitors**

Ibrutinib is a small molecule, irreversible inhibitor of BTK [21]. Ibrutinib is pro-apoptotic, disrupts cellular adhesion and migration [22], and has pleiotropic effects on the tumor microenvironment [23]. Briefly, ibrutinib 560 mg daily results in an ORR of 68% (CR 21%) in relapsed/refractory mantle cell lymphoma (MCL) irrespective of prior bortezomib exposure [24]. In updated results of this study, with a median observation period of 27 months, the median PFS and OS were 13 and 22 months, respectively [25]. The most common non-hematologic adverse events were diarrhea (54%), fatigue (50%), nausea (33%) and dyspnea (32%), grade ≥3 non-hematologic adverse events

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Btk, Bruton’s tyrosine kinase; PI3K, phosphatidyl-inositol-3-kinase; Syk, spleen tyrosine kinase; PD-1, programmed cell death-1; NHL, non-Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; CLL/SL, chronic lymphocytic leukemia/small lymphocytic lymphoma; WM, Waldenstrom’s macroglobulinemia.
included pneumonia (8%), urine tract infection (4%) and cellulitis (3%). Bleeding appears to be a common complication, experienced by 50% of patients: contusion (18%), epistaxis (11%), petechiae (10%) and subdural hematoma (4%). Grade ≥3 bleeding events were rare, occurring in 6% patients, most of whom were using concomitant non-steroidal anti-inflammatory medications. Atrial fibrillation (AF) was observed in 11% (grade 3 in 6%), most of whom had a history of cardiovascular disease. Discontinuation of therapy was not required in these patients. Dreyling et al. [26] recently reported the results of a phase III study in which 280 patients with relapsed/refractory MCL were randomized to either ibrutinib or temsirolimus. The ORR was higher in the ibrutinib arm (72% versus 40%, P < 0.001) and after a median follow-up of 20 months, patients receiving ibrutinib were less likely to experience disease progression or death [hazard ratio (HR) for PFS 0.43 compared with temsirolimus]. The difference in OS did not reach statistical significance, although 23% of temsirolimus-treated patients crossed over to ibrutinib. Wilson et al. [27] evaluated ibrutinib in a phase I/II clinical trial of 80 patients with relapsed/refractory DLBCL; efficacy was limited to non-germinal center B-cell cell-of-origin, in which the ORR was 17 out of 38 (40%) compared with 1 out of 20 (5%) in germinal center DLBCL. As a result of this activity, a randomized, double-blinded, placebo-controlled phase III study of R-CHOP ± ibrutinib is ongoing (NCT01855750). In patients with previous treated Waldenstrom's macroglobulinemia, ibrutinib at a lower dose of 420 mg daily resulted in an ORR of 90.5%, with higher response rates observed in patients with MYD88L265P than those with MYD88WT [28]. The 2-year PFS and OS were 69% and 95%, respectively. AF occurred in three (5%) patients, all of whom had a history of paroxysmal AF. However, ‘off-target’ inhibition of interleukin-2-inducible T-cell kinase (ITK) results in impairment of NK-cell effector function [29]. There are in vitro and in vivo data, suggesting ibrutinib may antagonize antibody-mediated cell mediate cytotoxicity (ADCC) induced by anti-CD20 monoclonal antibodies such as rituximab [30]. Despite this theoretical antagonism, data from studies evaluating the combination of ibrutinib and rituximab in previously treated MCL do not suggest antagonism; in relapsed/refractory MCL, the combination resulted in an ORR of 88% (CR 40%) [31]. Further, Fowler et al. [32] carried out a phase II study of ibrutinib (until disease progression) and four doses of rituximab in 60 patients with treatment-naive FL. The ORR was 82% (CR 30%), with 12-month PFS 86% and the toxicity profile was consistent with ibrutinib monotherapy.

Second-generation BTK inhibitors include acalabrutinib (ACP-196) [33], BGB-3111 [34], ONO-4059 [35] and CC-292 [36]. Clinical trials evaluating these agents in lymphomas are ongoing. Some of these agents appear to spare ITK and do not appear antagonistic to anti-CD20 mAbs in vitro, suggesting they may prove more effective in combinations; however, this remains to be proven [37]. Data from phase I studies with acalabrutinib and BGB-3111 suggest that these agents do not result in major bleeding events or AF [33, 34].

Syk inhibitors

Constitutive activation of Syk plays a role in many B-cell malignancies. The Syk inhibitor fostamatinib showed activity in a phase I study that included patients with lymphoid malignancies, but diarrhea, fatigue and hypertension (partially attributed to off-target kinase inhibition) were observed [38]. In a subsequent phase II study in patients with relapsed/refractory DLBCL, the ORR was 3% with one patient developing fatal pneumonitis [39]. Entospletinib (GS-9973) is a second-generation Syk inhibitor optimized for greater selectivity [40]. In patients with prior-treated indolent B-NHL, at a dose of 800 mg twice-daily, the agent appears better tolerated with grade ≥3 adverse events, including fatigue (12%), nausea (2%), elevated serum transaminases (20%) and neutropenia (10%) [41]. The ORR was 13%, with a median PFS of 5.5 months. A phase I/II study using entospletinib and idelalisib was terminated due to unexpectedly high rates of pneumonitis, including two fatal events, further serving to highlight the importance of careful monitoring for toxicity in studies combining novel agents [42].

Bcl-2 inhibitors

The key regulators of apoptosis in cancer cells are Bcl-2 family proteins. These comprise both anti-apoptotic proteins (Bcl-2, Bcl-xl) and pro-apoptotic proteins (Bak, Bad, Bax, Noxa and Puma) [43]. BH3-only proteins interact with Bax and Bak, resulting in activation, mitochondrial permeabilization and apoptosis [44]. The development of BH3-mimetics, which bind these proteins and promote apoptosis, has been explored as an anti-cancer therapy. Early examples including ABT-737 and its derivative ABT-263 (navitoclax) had promising clinical activity, but development was hindered by dose-limiting thrombocytopenia due to Bcl-xl inhibition [45]. Venetoclax (ABT-199, GDC-0199) is a small molecule, orally administered BH3-mimetic engineered to avoid Bcl-xl inhibition, thus reducing thrombocytopenia [46]. Among 106 patients with relapsed/refractory B-NHL treated in a phase I study, grade ≥3 thrombocytopenia occurred in only 7% of patients [47]. The ORR varied between histologic subgroups, with the highest rates observed in Waldenstrom’s macroglobulinemia (4/4 all PR) and MCL [21/28 (75%)]. Activity in FL [11/29 (38%), 4 CR (14%), Richter’s (3/7, all PR) and DLBCL [6/34 (18%), CR 4 (12%)] was present but less promising than suggested by preliminary data. In a phase Ib study of venetoclax, bendamustine and rituximab efficacy appeared improved compared with venetoclax monotherapy: the ORR among patients with FL and DLBCL were 87% and 46%, respectively [48]. Studies using venetoclax in combination with CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) and either rituximab or obinutuzumab (NCT02055820) in relapsed/refractory NHL and ibrutinib in relapsed/refractory MCL (NCT02471391, NCT02419560) are in progress.

An alternative approach to targeting Bcl-2 is the use of DNA interference to block transcription and initiate apoptosis. PNT-2258 is a liposomal nanoparticle containing a single-stranded 24-base DNA oligonucleotide (PNT100) designed to hybridize to the BCL2 gene (but not other Bcl-2 family members) [49]. Preliminary results from a phase I study in relapsed/refractory NHL indicate the agent has clinical activity and is well tolerated, with no tumor lysis syndrome or any grade ≥3 adverse events observed [50]. Based on these data, phase II studies in relapsed/refractory DLBCL (NCT02226965) and Richter’s syndrome (NCT02378038) are ongoing.
new mAbs and antibody drug conjugates

The most promising of the next generation of anti-CD20 mAbs is obinutuzumab (GA-101), a humanized type II IgG1 mAb. Obinutuzumab was glycoengineered with non-fucosylated Fc fragments to confer higher affinity interaction with FcγR and enhanced ADCC [51]. Other key differences from rituximab are the lack of complement-dependent cytotoxicity [52] and the ability to potently evoke direct cell death [53]. Phase II studies of single-agent obinutuzumab in relapsed/refractory indolent (ORR 55%) [54] and aggressive (ORR 28%) B-NHL have been carried out [55]. Obinutuzumab was safely combined with chemotherapy in patients with relapsed/refractory FL with excellent efficacy (ORR 93%–96%) without unexpected toxicity [56]. Sehn et al. [57] recently randomized 120 patients with previously treated indolent B-NHL to obinutuzumab or rituximab as induction, followed by maintenance. Although there was a trend toward superior ORR in obinutuzumab-treated patients, there was surprisingly no difference in PFS. In the phase III GADOLIN study, patients with rituximab-refractory indolent B-NHL were randomized to either bendamustine alone (at GALLIUM, NCT01332968) and indolent B-NHL (GALLIUM, NCT01287741) and indolent B-NHL (GALLIUM, NCT01332968). Ofatumumab monotherapy resulted in objective responses in relapsed/refractory FL (13%–62%) [59, 60] DLBCL (13%) [61] and MCL (12%) [62]. The antibody has been explored in phase II studies with chemotherapy; efficacy and tolerability seem comparable with equivalent rituximab-containing combinations [63–65]. Ublituximab is another anti-CD20 mAb targeting a differing epitope on the CD20 molecule featuring defucosylation for enhancement of ADCC [66]. In the phase I study in rituximab-treated B-cell lymphomas, the agent was well tolerated with the main adverse events neutropenia, anemia and fatigue; the ORR was 41% in a heavily pre-treated population [67]. The agent is being explored in combination with ibrutinib in a phase II study in patients with relapsed/refractory MCL (ORR 83%) without unexpected toxicities [68].

CD19 is also being explored as an alternative B-cell target. This antigen has the advantage of having expression mostly restricted to B cells, and is maintained when CD20 expression has been down-regulated [69]. The afucosylated CD19 mAb MEDI-551 has pre-clinical activity against B-cell malignancies including a suggestion of additive anti-tumor effect when combined with rituximab [70]. In phase I studies, the safety profile appears acceptable without unexpected toxicity, and encouraging single-agent activity in relapsed/refractory FL and DLBCL (ORR 24%) [71]. MOR-208 is another anti-CD19 mAb evaluated in a phase II study in relapsed/refractory NHL; among 89 patients treated, the ORR was 22% overall with responses observed in DLBCL (26%), FL (24%) but not MCL (0%) [72]. Further evaluation of both antibodies with biologic agents and chemotherapy are in progress.

Another B-cell target under investigation is the tetraspanin CD37. Otterlizumab consists of an anti-CD37 single-chain variable fragment joined to an immunoglobulin heavy and light chain fused to the Fc domain of IgG1 [73]. A phase I study among 16 patients with B-NHL (12 refractory to their previous treatment) demonstrated the agent to be well tolerated, with 2 PRs (ORR 12%) [74]. The efficacy of this agent may be enhanced when combined with chemotherapy—a phase Ib study combining the agent with bendamustine and rituximab in relapsed/refractory B-NHL indicated promising activity and no unexpected toxicity: objective responses were observed in 10 out of 12 (83%) patients, with 4 CRs [75].

antibody drug conjugates

Several attempts have been made to conjugate anti-cancer antibodies to monoclonal antibodies. An early example of this was inotuzumab ozogamicin, comprising an anti-CD22 mAb and calicheamicin, a DNA alkylating agent [76]. Although early phase clinical trials indicated promising activity [77, 78], a phase III study in relapsed/refractory aggressive NHL was terminated for futility. Polatuzumab vedotin is anti-CD79b mAb conjugated to monomethylaurostatin E (MMAE), a microtubule toxin. A phase I study in relapsed/refractory NHL defined 2.4 mg/kg as the recommended phase II dose; an additional cohort to assess the feasibility of the agent in combination with rituximab was also enrolled [79]. Patients were treated until disease progression, unacceptable toxicity or patients’ physicians’ choice. The most common grade ≥3 toxicities were hematologic and neuropathy (9%), and the ORR was 55% for assessable patients treated at the recommended phase II dose; the median PFS was 5.7 months. A subsequent study in relapsed/refractory FL explored polatuzumab vedotin in combination with rituximab at two dose levels, 2.4 and 1.8 mg/kg [80]. Treatment discontinuation rates were higher in the 2.4 mg/kg cohort (56% versus 30%) primarily due to grade 2–4 peripheral neuropathy (72% versus 40%). Ongoing studies of this agent in combination with chemotherapy and rituximab or obinutuzumab are in progress (NCT01992653, NCT02257567).

Brentuximab vedotin (BV) comprises an anti-CD30 mAb joined via protease cleavable linker to MMAE [81]. Following binding of the ADC, MMAE is cleaved, undergoes endocytosis and disrupts microtubules, resulting in cell cycle arrest and apoptosis [82]. A phase II study in DLBCL was recently carried out, in which activity was seen irrespective of CD30 staining by immunohistochemistry; the ORR was 44% overall [83]. The greater internalization of CD37 compared with CD20 has led to ADCs based on the target. The most advanced in development is IMGN529, which comprises the microtubule toxin DM1 is conjugated to an anti-CD37 mAb. Some clinical activity in relapsed/refractory B-NHL has been observed in preliminary reports of a phase I study [84].

EZH2 inhibitors

Epigenetic therapies have made greatest inroads in the treatment of patients with T-cell lymphomas, with HDAC inhibitors romidepsin [85], belinostat [86] and vorinostat [87] all approved for this indication. Vorinostat has modest activity as a single agent in B-NHL [88]; however, other epigenetic therapies in B-NHL are under investigation. Histone methyltransferases catalyze the methylation of lysine and arginine residues, and genetic alterations in these enzymes have been etiologically linked to
lymphomas. In particular, heterozygous mutations within EZH2, the catalytic subunit of the polycomb repressor 2 complex (PRC2), result in the generation of trimethylated lysine 27 on histone H3 (H3K27Me3), abnormal repression of PRC2 and lymphomagenesis [89]. These mutations have been described in a proportion of patients with germinal center DLBCL [90]. EPZ-6438 is a potent small molecule inhibitor of EZH2 that has preclinical activity in both EZH2 mutated and wild-type lymphomas [91]. A phase I study is in progress, with preliminary results in 12 patients with NHL (1 EZH2 wild-type), indicating a dose-related increase in exposure, no maximum tolerated dose and an encouraging efficacy signal, with objective responses seen in 3 of 5 patients with DLBCL, even at lower dose levels [92]. Another EZH2 inhibitor, GSK-2816126, is undergoing evaluation in a phase I study in relapsed/refractory DLBCL and transformed FL (NCT02082977) with no publically available results at the time of writing.

**selective inhibitors of nuclear export**

The transport of molecules between cytoplasm and nucleus is an essential cellular function. The export of large molecules from the nucleus to the cytoplasm is carried out via transport proteins termed exportins. One mechanism thought to play a role in the development of a number of cancers is inactivation of tumor suppressor proteins (such as p53, IκB, FOXO and p21) by transport out of the nucleus [93]. These proteins are transported by exportin 1 (XPO1), which is overexpressed in a number of hematologic malignancies including lymphomas [94]. A number of naturally occurring substances that inhibit XPO1 have been identified, including the antibiotic elactocin. Profound malaise and anorexia were dose limiting in clinical studies [95]. Several small molecule inhibitors of exportin have since been developed, with the agent under active investigation selinexor (KPT-330) showing preclinical and clinical promise in a range of hematologic malignancies [96, 97]. Chen et al. [98] recently reported on a phase I study in which patients with heavily pre-treated NHL received selinexor orally. Reminiscent of the toxicities from elactocin, the most common grade 1–2 adverse events were nausea, anorexia, fatigue and vomiting, all observed in >40% of participants. The ORR at all dose levels among patients with NHL was 33% with responses seen across indolent and aggressive histologic subtypes. Preclinical and early clinical data also suggest selinexor has activity in relapsed/refractory MYC/BCL2-rearranged double-hit lymphoma [99], a particularly challenging disease entity [100]. Because of the greater toxicity seen at higher dose levels, a modified dosing schedule of 60 mg twice-weekly was established as the recommended phase II dose for hematologic cancers.

**agents targeting the programmed cell death axis**

The programmed cell death (PD-1) axis curtails the response of activated T cells to infection and stops autoimmunity [101, 102]. Binding of PD-1 to its ligands PD-L1 and PD-L2 send inhibitory signals leading to activated T-cell apoptosis [103]. PD-1 is also present on T-regulatory (T_{reg}), B- and NK-cells and PD-1 blockade enhances thus anti-tumor cytotoxicity through increased NK-cell killing and T_{reg} suppression [104, 105]. Cancer cells may exploit the PD-1 through expression of PD-L1 on tumor-infiltrating lymphocytes [106] resulting in the impairment of anti-tumor responses [107]. Antibodies targeting the PD-1 axis promote anti-tumor cytotoxicity by ‘releasing the brakes’ from T_{effectors} [108]. ADCC of tumor cells expressing PD-1 or PD-L1 appears less important as PD-1/PD-L1 surface expression by tumor cells or the microenvironment does not correlate with anti-tumor activity [109]. Several agents targeting the PD-1 axis exist.

Pidelizumab (CT-011, MDV9300) is a humanized IgG1 anti-PD-1 mAb. Armand et al. [110] carried out an international phase II study of pidelizumab in patients with previously treated, chemosensitive de novo or transformed DLBCL or primary mediastinal B-cell lymphoma (PMBL) after autologous stem cell transplantation (ASCT). The safety profile was favorable, and among 35 patients with residual disease post-ASCT, the CR and ORR were 34% and 51%, respectively. The 16-month PFS by intention-to-treat population was 68%. Pidelizumab also has activity in relapsed/refractory rituximab-sensitive FL. Westin et al. [111] carried out a single-center phase II study with the pidelizumab and rituximab, enrolling 32 patients. There were no grade ≥3 AEs observed; the ORR was 66% (CR 52%) and after a median follow-up of 15.4 months, the median PFS was 18.8 months. Nivolumab (BMS-936558, MDX1106), a fully human IgG4 anti-PD1 mAb, has been studied in relapsed/refractory NHL. Serious events were infrequent; histologic subtypes in which the most promising efficacy signal was observed included DLBCL (n = 11, ORR 36%) and FL (n = 10, ORR 40%) [112]. Preliminary clinical data suggest promising activity for the PD-1 inhibitor pembrolizumab in patients with relapsed/refractory PMBL [113] and Richter’s syndrome [114]. On the basis of these results, phase II studies of these drugs in multiple histologic subtypes are ongoing.

**chimeric antigen receptor T-cell therapy**

Another promising technology in patients with relapsed/refractory B-cell malignancies including acute lymphoblastic leukemia, chronic lymphocytic leukemia and DLBCL have emerged in the form of anti-CD19 chimeric antigen receptor (CAR) T-cell therapy [115, 116]. This therapy takes the form autologous T cells gene-modified to carry antigen specificity for CD19. Kochenderfer et al. [117] reported results from the National Cancer Institute’s CAR T-cell program, in which 15 patients with lymphoma have been treated (9 DLBCL, 6 indolent NHL) with anti-CD19 CAR T cells, with cyclophosphamide and fludarabine administered as conditioning chemotherapy. Six of seven assessable patients with DLBCL and all patients with indolent NHL achieved a response to therapy. One patient died suddenly 16 days after infusion, with cause of death attributed to cardiac arrhythmia. Most toxicities occurred within 2 weeks of T-cell administration, with hypotension and transient neurologic deficits such as aphasia observed; CAR T cells reached a peak in the peripheral blood 7–17 days after infusion and rapidly declined thereafter. Despite this, nine patients remained in ongoing remission at last follow-up, with the longest 23 months from therapy. The University of Pennsylvania group carried out a phase II study in relapsed/refractory B-NHL [118]. Of the 38 patients (21 DLBCL, 14 FL, 3 MCL) treated, the median number of prior therapies was 4; conditioning regimen
varied depending on the histologic subtype and past treatment history. Eight patients were not evaluable, mostly due to disease progression before infusion or insufficient ex vivo T-cell expansion (n = 3 each). Cytokine release syndrome and neurologic toxicity occurred in 16 and 3 patients respectively; the ORR was 68% (DLBCL 43%, FL 100%) with PFS at the median follow-up of 11.7 months 62%. Turtle et al. [119] reported on 28 patients treated with an anti-CD19 CAR T-cell product containing defined composition of CD4 and CD8 T cells at the Fred Hutchinson Cancer Research Center. Interestingly, they noted both improved CAR T-cell persistence in patients receiving fludarabine-containing (lymphodepleting) conditioning therapy and superior ORR, even in DLBCL.

**conclusion**

Although most patients with B-NHL are not currently cured, we predict this will change, given recent advances in the field. Indolent lymphomas appear highly responsive to biologic interventions and effective chemotherapy-free treatment has arrived, with rituximab and lenalidomide resulting in excellent ORR and durable disease control in FL and MCL [120, 121]. An international multicenter phase III study comparing rituximab/lenalidomide to R-chemotherapy (RELEVANCE) is ongoing (NCT01650701). In aggressive B-NHL, the reliance on conventional cytotoxic chemotherapeutics remains greater. However, the substitution or replacement of drugs with molecular targeted mechanisms may improve both efficacy and therapeutic index. This is illustrated by protocols incorporating lenalidomide and ibrutinib into frontline may improve outcomes for patients with activated B-cell DLBCL. [122]. In MCL, a phase II study using ibrutinib and rituximab in an initial window period of 4–12 months followed by abbreviated chemo-immunotherapy is in progress (NCT02427620). Preliminary data from the first 12 patients treated indicate an ORR of 100% after two cycles of ibrutinib–rituximab before the use of chemotherapy (MLW, personal communication). Ongoing collaborative efforts are required to rapidly safely and efficiently translate discoveries at the bench into breakthrough therapies in the clinic to cure more patients with B-NHL.

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**references**


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Phosphoproteomics in translational research: a sarcoma perspective

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Phosphoproteomics has been extensively used as a preclinical research tool to characterize the phosphorylated components of the cancer proteome. Advances in the field have yielded insights into new drug targets, mechanisms of disease progression and drug resistance, and biomarker discovery. However, application of this technology to clinical research has been challenging because of practical issues relating to specimen integrity and tumour heterogeneity. Beyond these limitations, phosphoproteomics has the potential to play a pivotal role in translational studies and contribute to advances in different tumour groups, including rare disease sites like sarcoma. In this review, we propose that deploying phosphoproteomic technologies in translational research may facilitate the identification of better defined predictive biomarkers for patient stratification, inform drug selection in umbrella trials and identify new combinations to overcome drug resistance. We provide an overview of current phosphoproteomic technologies, such as affinity-based assays and mass spectrometry-based approaches, and discuss their advantages and limitations. We use sarcoma as an example to illustrate the current challenges in evaluating targeted kinase therapies in clinical trials. We then highlight useful lessons from preclinical studies in sarcoma biology to demonstrate how phosphoproteomics may address some of these challenges. Finally, we conclude by offering a perspective and list the key measures required to translate and benchmark a largely preclinical technology into a useful tool for translational research.

Key words: phosphoproteomics, sarcoma, signal transduction, clinical trials, drug resistance

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