Review article

# Modulation of the innate immune system in the treatment of non-Hodgkin lymphoma – literature review

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## ABSTRACT

Non-Hodgkin lymphoma is a common, fast-growing malignancy. A novel strategy in its treatment is the use of modulators of the innate immune system, and here, we intended to investigate their role. To evaluate their efficacy and safety, we reviewed the PubMed database. The use of new therapeutic strategies reduces side effects and positively affects the treatment process. The effects of targeting some molecules, particularly CD47, are promising.

Their use is associated with side effects. Many of them are transient and can be alleviated. Current advances in non-Hodgkin lymphoma treatment are promising. The efficacy and safety of innate immune system modulators have been demonstrated for some of the non-Hodgkin lymphoma subclasses. Nevertheless, there is a need for further carefully designed studies.

**Key words:** non-Hodgkin lymphoma, immunotherapy, immune system, innate immune system, lymphoma

## CHARACTERISTICS OF NON-HODGKIN LYMPHOMA

Non-Hodgkin lymphoma (NHL) is one of the most common malignant tumors originating from the basal cells of lymphoid tissue, lymphocytes, and histiocytes at any stage of their development with the fastest growing rate [1–3]. The most common NHL subtypes in developed countries are diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL), contributing to about 30% and 20% of total NHL cases, respectively. All other NHL subtypes have an incidence of 10% at most [3], including the entire group of T-cell NHL (T-NHL) [4]. NHL is the 6$^{th}$ leading cause of cancer deaths in the U.S. after prostate, breast, lung, colorectal, and bladder cancer [5]. According to the Northern California Cancer Center in the United States, the incidence of NHL remains stable in children but continues to rise among Caucasians aged 15–24 years (2–3% per year), women aged 25–54 years (1–6% per year), and African Americans over 55 years (2–4% per year) [6]. There are many seemingly imperceptible differences between NHL subtypes, which is why accurate histopathologic classification of lymphomas has become one of the most difficult challenges for hematologists and pathologists, as well as the development of computer vision methods [7]. In addition, NHL is a heterogeneous group of lymphoproliferative malignancies that are far less predictable in terms of location than Hodgkin's lymphoma (HL) – almost 25% of NHL cases are extranodal, with most of them affecting both nodal and extranodal sites [8]. Genetic alterations as well as activation of certain signaling pathways and viral infections, often lead to mechanisms that evade immune surveillance through cytokine and chemokine signaling, as well as abnormal expression of checkpoint proteins such as programmed cell death protein 1 (PD-1) and its ligands [9]. Recent advances in next-generation sequencing strategies have revealed the genetic aspect of B-cell NHL (B-NHL), but the tumor microenvironment is increasingly recognized as critical for the survival and growth of malignant B-cells, subclonal evolution, and drug resistance. The cancer niche consists of an organized and dynamic network of highly heterogeneous subsets of immune cells and framework cells, characterized by specific phenotypic features [10]. Lymphomas are extremely radiosensitive, but most patients are not candidates for advanced radiotherapy [11]. Although response rates are high in some groups, relapses can be difficult to treat, and newer approaches are needed for this patient population. An innate immune system plays a key role in inhibiting lymphomagenesis. Its modulation can increase the cytotoxic activity of the acquired and innate immune systems and improve the killing of cancer cells [12].

## MODULATORS OF THE INNATE IMMUNE SYSTEM

Despite the intensive research into oncological immunology is a matter of recent years, some links between the immune system and neoplasms were observed a long time ago. In the 18$^{th}$ century, some researchers hypothesized that the presence of certain infections in oncologic patients could lead to therapeutic benefits [13]. William B. Colley, a New York surgeon, was a pioneer of therapy based on immunologic stimulation in oncology. In the treatment of inoperative sarcomas, he used a vaccine containing *Serratia marcescens* and *Streptococcus pyogenes*, later named: 'a Coley's toxin' [14].

Innate immune system modulators are a group of drugs used in modern oncological immunotherapy, along with immune checkpoint inhibitors. 'Immune checkpoints' is a collective term for certain molecules expressed by T lymphocytes and antigen-presenting cells (APCs), that modulate the activation of naïve T lymphocyte. As more and more therapies targeting the innate immune system have emerged, the term 'immune checkpoint' sometimes also includes molecules expressed by other cells [15]. Several innate immune system molecules are suggested as potential targets of NHL immunotherapy [16], however, their role is yet to be established.

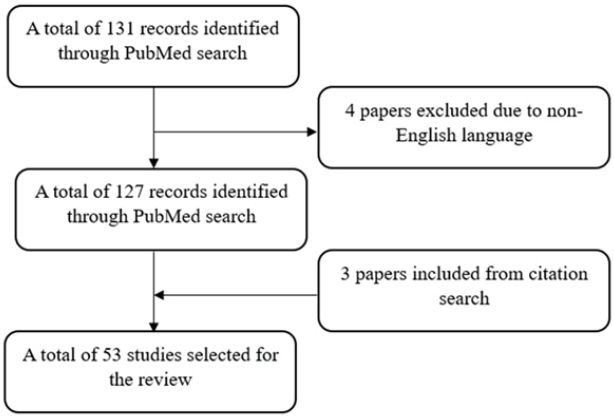
## OBJECTIVE OF THE STUDY

The main objective of this review was to investigate the role of innate immune system modulators in the immunotherapy of NHL. We intended to evaluate the efficacy and safety of the available treatment options.

## MATERIALS AND METHODS

We conducted a narrative literature review using the combination of MeSH terms: "Lymphoma, Non-Hodgkin"[Mesh], and "CD47

**Figure 1.** The flowchart illustrating the process of literature search.



Antigen"[Mesh], "NADPH Oxidase 2"[Mesh], and "Receptors, KIR"[Mesh]. : "Lymphoma, Non-Hodgkin" AND ("CD47 Antigen" OR "NADPH Oxidase 2" OR "Receptors, KIR"). Starting from 131 papers, we excluded 4 papers due to non-English language, and focused on the most recent publications. Additionally, we included 3 papers via citation search. Finally, we obtained 53 articles published between 1994 and 2025. In this review, we discuss the characteristics of NHL and report the current knowledge of the role of modulators of the innate immune system in its treatment.

## INHIBITORS OF THE INNATE IMMUNOLOGICAL SYSTEM MOLECULES IN THE TREATMENT OF NHL

Apart from molecules engaged in the activation of naïve T lymphocytes, some pathways of the innate immune system may also serve as targets for NHL immunotherapy. Here we present studies on the most frequently proposed targets in the innate immune system.

### CD47-SIRPα pathway

Cluster of differentiation 47 (CD47) is a molecule of the immunoglobulin superfamily, that is widely expressed on cell surfaces. It is involved in proliferation, adhesion, migration, apoptosis, and phagocytosis. It can interact with integrins, thrombospondin (TSP-1), and particularly signal regulatory protein alpha (SIRPα) [17].

SIRPα is a receptor of immunoglobulin superfamily molecules present on macrophages and dendritic cells. Activation of a SIRPα by its ligands, e.g. CD47, leads to a cascade that results in the inhibition of phagocytosis [17]. Up-regulated CD47 also promotes macrophage polarization towards pro-oncogenic M2 macrophages [18]. Hence, in the literature, the interaction between CD47 and its ligands is sometimes referred to as the 'do not eat me' signal [17–27]. Excessive CD47 expression has been observed for example in the cells of B-NHL [18, 23], T-NHL [24, 28], acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL) [17], breast cancer, and hepatocellular carcinoma [16]. Interestingly, Willingham et al. suggested that CD47 is expressed by all types of cancers and is required to suppress phagocytosis [29]. Indeed, modified DLBCL cell lines with knocked-out CD47 or with overexpressed CD47 are more susceptible or nearly resistant to phagocytosis, respectively [23]. Qin et al. made similar findings in NK/T-cell lymphoma (NKTCL) cell lines (NKYS and YTS) [24]. In a recent study, Masroni et al. suggested that miR-101-5p downregulates the expression of C47, and its low concentrations could indicate a favorable response to CD47 blockage [30]. SIRPα levels may also be elevated and were inversely correlated with susceptibility to tafasitamab-mediated phagocytosis, as Biedermann et al. showed using DLBCL specimens [23].

Elevated CD47 is an independent predictor of a worse prognosis [18]. However, its loss [19] or pharmacological blockade [17] leads to stimulation of macrophage-dependent phagocytosis. In addition, immunoglobulins blocking the CD47 act like opsonins and lead to antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) [31]. The CD47--SIRPα pathway is a promising target in NHL immunotherapy that is being investigated in several clinical trials (e.g. NCT05737628, NCT05025800, NCT05892718, NCT05507541, NCT05896163).

### CD47-related antibodies

Hu5F9-G4 (magrolimab [20]) – humanized anti-CD47 immunoglobulin is the most widely studied CD47 antibody [24]. It was studied in the diffuse Burkitt lymphoma (BL) mice model induced by Raji cells [32]. In a study by Liu et al., benefits in survival were slightly observed with no mice cured successfully. Whereas, in the group of mice treated with Hu5F9-G4 and rituximab simultaneously, 3 of 4 mice were successfully cured [32]. Hu5F9-G4 was also studied in humans. In 2019, Sikic et al. described the first-in-human Hu5F9-G4 study including 62 patients with different advanced cancers, of which 2 had DLBCL. One of the patients with DLBCL presented mixed response, whereas the second was not treated with Hu5F9-G4. Of note, the authors stated that Hu5F9-G4 is well-tolerated on an initial dose of 1mg/kg on the first day, and maintenance doses up to 45 mg/kg weekly [33]. In another study, Advani et al. evaluated Hu5F9-G4 in combination with rituximab. The study population comprised 15 patients with DLBCL and 7 patients with FL, of which 95% were refractory to rituximab. When treated with the combination of rituximab and Hu5F9-G4, 50% of the patients presented ORR, including 14% with partial response (PR), and 36% with complete response (CR). Among the patients with DLBCL, the ORR and CRR were 40%, and 33%, respectively. Clinical response in FL patients was better with 71% ORR and 43% CRR. The authors described no clinically significant safety events [34]. Anti-CD47 antibodies were also studied by Nguyen et al. in the Granta-519 cell line. They found that CD47 blockage promoted phagocytosis, especially when combined with rituximab. Of note, they compared CD47 antibodies with silenced Fc domain (αCD47-siFc) and functional Fc domain (αCD47--fuFc) separately. When used in monotherapy, the phagocytosis induced by αCD47-fuFc was twofold greater, but the difference was reduced when used in the combination combined with rituximab [25]. Another CD47 antibody (MIAP301) promoted the antitumor properties of Accum™ intratumor injections, resulting in the most prominent effects from all tested antibodies [27].

Another agent already evaluated in a clinical trial was IMM0306 (amulirafusp α), a fusion protein comprising a CD47-binding domain of SIRPα and a CD20 antibody. It was used in 48 heavily pre-

treated B-NHL patients, of which 20 had FL, 15 had DLBCL, 7 had MCL, 3 had MCL, and 3 had other B-NHL. IMM0306 showed potential efficacy, particularly for FL and MZL patients, with ORRs of 41.2% and 33.3%, respectively. Of the FL patients, 6 reached stable disease, 3 reached PR, and 4 reached CR. It was well tolerated, with 87.5% compliance with the whole therapy. The safety profile was also satisfactory, with no drug-related deaths and no cases of cytokine release syndrome [26].

As CD47 is widely expressed on RBCs and platelets, its blockage may cause anemia and thrombocytopenia. Thus, a search for novel antibodies with lower affinity to RBCs and platelets could be beneficial [21, 24]. Bispecific antibodies (BsAbs) are one of the proposed solutions to such a problem, as they could limit the spectrum of targeted cells. On that, Zhu et al. created CC-96673. This BsAb with reduced CD47 affinity successfully limited phagocytosis of $CD40^{+}CD20^{-}$ cells in vitro. They also proved its ability to promote phagocytosis of Raji and OCI-Ly3 cells, outperforming rituximab and as effective as simultaneous CD20 and CD47 blockage with rituximab and anti-CD47 antibody. They also evaluated its efficacy in SCID mouse xenografts with significant inhibition of tumor growth. The safety of CC-96673 was assessed in the cynomolgus monkey model, with good tolerance of the highest tested dose (100 mg/kg QW) [21]. On a similar premise, Li et al. tested several CD38xCD47 BsAbs with significantly greater affinity to CD38 than CD47. Compared to Hu5F9, tested BsAbs presented significantly lesser affinity to RBCs and PLTs, and what is more, no hemagglutination was observed. Meanwhile, the efficacy of their anti-NHL effects was confirmed in vitro in Raji and Daudi cell lines, as they promoted ADCC and ADCP [35]. Another CD47-related agent was proposed by Piccione et al. In their study, anti-CD47xCD20 BsAbs were studied in a Raji-induced diffuse NSG mice model. Mice treated with rituximab or the anti-CD47 antibody monotherapy presented inconsiderably extended survival compared to mice with control groups. Whereas, both combination of rituximab and anti-CD47 antibody, and anti-CD47-CD20 BsAb alone eliminated detectable NHL and significantly extended disease-free survival, compared to monotherapy-treated mice. They also reported similar effects in the diffuse NHL mice model [36]. Another anti-CD47 antibody, TTI-621 (also known as maplirpacept [23]) was studied in cutaneous T-cell lymphoma (CTCL) cell lines in monotherapy and combination with durvalumab. Han et al. proved it promotes the phagocytic activity of macrophages and its repolarization toward antitumor M1-like phenotypes. This dual blockage also promoted apoptosis, autophagy, and necroptosis of lymphoma cells. Such a combination was more effective than either durvalumab or TTI-621 alone [37]. Johnson et al. investigated its efficacy in the treatment of Sézary syndrome (SS). 5 of the 25 patients were administered TTI-621. 8 days after the first dose, 4 of the 5 patients experienced a decrease in neoplastic cells in flow cytometry. Other benefits of TTI-621 administration were a decrease in serum lactate dehydrogenase **(**LDH) level, a decrease in total lymphocyte count, and improved recovery of skin lesions [38].

Another CD47 antibody, B6H12 was studied by Biedermann et al. [23] in DLBCL cell lines (Toledo, HT, U2946), samples from DLBCL patients, and animal xenograft models. In combination with tafasitamab, a CD19 antibody promoted phagocytosis in all evaluated cell lines. Moreover, phagocytosis was also promoted in selected cells with low CD19 expression, refractory to phagocytosis during tafasitamab monotherapy. In addition, all the Ramos SCID mice models were alive at the end of such combination treatment. B6H12 and AK117 (ligufalimab), a novel CD47 antibody, were also evaluated in a similar study by Qin et al. [24]. They evaluated NKTCL cell lines (NKYS and YTS) and showed that both antibodies promoted phagocytosis with no statistically significant differences. Interestingly, no impact on apoptosis, cell cycle, and proliferation of NKTCL cells was observed [24], conversely to some evidence regarding B-CLL cells [39]. They also found that AK117 treatment in SCID mouse model can significantly inhibit NKTCL growth with a good treatment tolerance [24]. The authors of the abovementioned preclinical studies documented that CD47 antibodies can effectively eliminate NHL cells in vitro and in mice models [24]. Of note, the phagocytosis mediated by anti-CD47 antibodies can be mediated by its Fc regions [25]. CD47 antibodies also promote phagocytosis induced by CD20 and may present beneficial synergism in the treatment of NHL [21, 25, 32]. In addition, Hu5F9-G4 and CC-96673 were safely used in primate models with doses significantly greater than the therapeutic range for humans [21, 32]. The majority of adverse effects (AEs) of Hu5F9-G4 were mild to moderate [34]. Among severe AEs, e.g. infections (n = 4), severe anemia, dyspnea, pyrexia, lactate acidosis, and pulmonary embolism (n = 1 for each) were reported, and no long-term toxicity of the Hu5F9-G4 therapy was observed. The authors stated that Hu5F9-G4 combined with rituximab may be safe and effective in the treatment of relapsed and refractory DLBCL and FL [34], TTI-621 may be beneficial in SS patients [38], and CC-96673 performed usefully enough to validate its use in clinical trials [21]. CD47 was also tested as a component in the simultaneous blockage of several checkpoints, as seen in the next section.

### NOX2 pathway

The family of NOX enzymes (NADPH oxidases) comprises several multicomponent transmembrane molecules. Their only known function is to catalyze the reduction of molecular oxygen [40]. One of the NOX family members particularly involved in NHL pathogenesis is NOX2, which is expressed on cell and lysosomal

membranes of monocytes, macrophages, granulocytes, B lymphocytes, and APCs. It plays a role in the destruction of phagocytized pathogens via reactive oxygen species (ROS). From an oncological point of view, ROS that originates from NOX-mediated reactions may play a role in carcinogenesis. Overexpression of the NOX2 enzyme was reported e.g. in patients with chronic myelomonocytic leukemia (CMML), melanomas, monocytic leukemias, and lymphomas [40, 41].

Some of the enzymes regulating NOX function are phosphatidylinositol-4,5-bisphosphate-3 kinases (PI3Kδ) [42]. PI3Kδ pathway activation was linked to macrophage polarization toward the M2 phenotype and was related to CD47 overexpression [18].

### NOX2 and *PI3Kδ-related antibodies*

Idelalisib is a PI3K p110δ inhibitor. It was shown to inhibit the NOX2-dependent ROS production in monocytes and ROS-dependent NK cell immunosuppression and death. Idelalisib significantly decreased the number of melanoma metastasis in the wild mice model, but not in mice models with knocked NK cells nor NOX2 gene function [42]. It was suggested that the anti-metastatic features of idelalisib depend on NK cells' function and are the effects of NOX2 inhibition [42]. Idelalisib (with ofatumumab, an anti-CD20 antibody) was also evaluated in the treatment of chronic lymphocytic leukemia/small lymphocyte B lymphoma (CLL/SLL) by Lampson et al. 31 patients were enrolled in the study, of which 27 received at least one dose of idelalisib. In 27 patients, the ORR reached 88.9% (n = 24) with 21 PRs, 2 PR with lymphocytosis, and one CR. The median of progression-free survival (PFS) was 23 months. Of note, the toxicity of idelalisib forced 15 of the patients to cease the therapy, and 12 remaining patients stopped their study at the request of one of the trial sponsors (Gilead Pharmaceutics). The most common severe AEs were severe forms of elevated transaminases – 52% with at least G3 according to Common Terminology Criteria for Adverse Events 4.0 (CTCAE 4.0). The second most common AE was neutropenia – 48% of the patients with 33% of the patients with at least G3. The remaining reported AEs were e.g. colitis/diarrhea, pneumonia, and anemia. The authors stated that idelalisib combined with ofatumumab was linked to unacceptable toxicity and emphasized that future studies regarding PI3Kδ inhibitors in CLL/SLL require a novel approach aimed to reduce the toxicity of the therapy [43].

Ryu et al. investigated the efficacy of GSK2795039 (NOX2 inhibitor) in the cell culture of Epstein-Barr virus (EBV)-positive BL type Raji and EBV-negative BL type Ramos. GSK2795039 induced dose-dependent significant cell death only in the Raji cells. In the Ramos cell lines, cell death was observed only with high doses, and the results were not statistically significant. GSK2795039 showed no impact on primary B cells. Cell death in Raji cell cultures was mediated through apoptosis with the activation of caspase 3 and caspase 9, induction of proapoptotic protein Box and Noxa, and inhibition of antiapoptotic protein Mcl-1. Additionally, in Raji cells, the shift of proapoptotic Bac protein from the cytoplasm to mitochondria was observed with consecutive effusion of cytochrome C from mitochondria. The authors suggested that EBV-dependent neoplasms are linked to excessive NOX-mediated ROS production and NOX protein may be a beneficial target for EBV-positive neoplasms [44].

### KIR pathway

Killer cell immunoglobulin-like receptors (KIR) family consists of 6 inhibitory receptors and 6 stimulating receptors expressed on the surface of NK cells [45]. Inhibitory KIR receptors have an affinity to human leukocyte antigen (HLA)-I molecules present on the surface of all correct nuclear cells. When bound to HLA-I molecules, inhibitory KIR receptors block cytotoxic mechanisms in NK cells [45, 46]. KIR2DL1 and KIR2DL2/3 receptors specific to HLA-C alleles, and KIR3DL1 receptors specific to HLA-A and HLA-B alleles play a particular role in such mechanisms. Thus, cancer cells that lost surface HLA molecules can be identified and destroyed by NK cells [46]. The expression of KIR molecules can be aberrant in several malignancies. NK cells with high KIR2DS2 expression have been shown to demonstrate increased activity against malignant B cell lines, liver cancer cell lines, and primary CLL cells [47]. KIR3DL2 overexpression was detected in aggressive PTCL cell lines [48] and lymphoma-type adult T-cell leukemia (ATL) patients [49], which was linked to hypomethylation of its promoter [48, 49]. KIR3DL2 overexpression was also linked to HTLV-1 infection [49]. On the other hand, Muriuki et al. found no differences in frequencies of KIR3DL1 and KIR3DS1 alleles between 104 healthy Kenyan children and 108 patients with endemic BL [50]. MacFarlane et al. enrolled 25 patients with untreated CLL and 10 patients with untreated SLL in a prospective study. Compared to healthy controls, they found a significant decrease in the count and vitality of NK cells with KIR2DL1 and/or KIR3DL1, which progressed over time in most cases. Additionally, the authors noted extensive susceptibility to activation-induced cell death (AICD) among patients. The abovementioned abnormalities were more visible among CLL patients, compared to CLL patients. The authors suggest that mature NK cells with KIR receptors are prone to AIDC when exposed to high circulating tumor B cell burden, and CLL patients could benefit from immunotherapies that promote $KIR^+$ NK cell survival [51].

### *Anti-KIR antibodies*

Armand et al. in their phase 1b clinical trial investigated the efficacy of nivolumab combined with ipilimumab or lirilumab (anti-KIR antibody) in the treatment of relapsing or refractory lym-

phoid malignancies. Among 72 patients enrolled in nivolumab combined with lirilumab cohort, 21 patients had classic Hodgkin lymphoma (cHL), 32 had B-NHL, 9 had T-NHL, and 10 had multiple myeloma (MM). In the whole B-NHL group, ORR was 13%, and CRR was 3%. In the FL subgroup, ORR reached 17%, and 17% achieved CR, and for the DLBCL subgroup, the response rates were 12% and 0%, respectively. In the T-NHL group, ORR and CR reached 22%, and 9% respectively. 71% of patients with such treatment experienced at least one AE, with 15 % of at least 3$^{rd}$ grade. The most common 3$^{rd}$- and 4$^{th}$-grade AEs were increased creatinine phosphokinase (CPK; 3%), neutropenia (3%), pleural effusion (3%), and tumor flare (3%). However, no patient ceased the treatment due to its toxicity, and no treatment-related deaths were noted [52].

KIR pathway was also targeted in vitro. In a study conducted on PTCL cell lines, Decroos et al. showed that lacutamab, an anti-KIR3DL2 antibody, effectively promoted ADCC in KIR3DL2$^+$ hepatosplenic T-cell lymphoma (HSTL) cells but not in KIR3DL2$^-$ SNK6 cells and the primary KIR3DL2$^-$ cells from HSTL. Similar results were obtained with samples from angioimmunoblastic T-cell lymphoma (AITL) and PTCL-NOS. This effect was further enhanced by the addition of gemcitabine and oxaliplatin [48]. Cheminant et al. studied the same molecule in samples of ATL patients. The study enrolled some lymphoma-type ATL, but lacutamab was tested in the samples of 3 acute-type ATL in vitro. They observed enhanced ADCC towards KIR3DL2$^+$ cells, with no effects against KIR3DL2$^-$ cells [49].

High KIR2DS2 was linked to increased ADCC against malignant cells [47]. The effect was further enhanced by the addition of rituximab and obinutuzumab (anti-CD20 antibodies). The authors stated that their results suggest no significant improvement in the efficacy of nivolumab + combined agent over single nivolumab treatment in the studied diseases. Given the data from the literature, more data is still needed to address the issue of KIR blocking usefulness in the treatment of NHL [52].

## MULTIPLE BLOCKAGE COMBINATIONS

Some studies evaluated blockages of 3 or more target molecules, including separate or multi-specific agents. Some of such combinations include innate system molecules. One of the most common components targets CD20 and CD47. One such combination therapy was tested by Zeller et al. Apart from CD47 and CD20, they targeted leukocyte immunoglobulin-like receptors subfamily B (LILRB), namely LILRB1, and LILRB2. The research was conducted on B-NHL cell lines and CLL patients' cells. CD47-IgGσ (magrolimab with abrogated FcγR binding) failed to trigger ADCP in monotherapy but significantly enhanced rituximab-induced ADCP in the majority of cell lines. Antibodies binding LILRB1 and LILRB2 (LILRB1-Ig, and LILRB2-Ig, respectively) did not induce ADCP alone or with rituximab, but LILRB1-Ig significantly induced ADCP when used in combination with rituximab and CD47 antibody. On the other hand, no similar effects were shown for LILRB2-Ig. Triplet CD20, CD47, and LILRB1 blockage significantly promoted phagocytosis in Carnaval, Granta519, and MEC2 lines. Moreover, ADCP was also increased in the DG-75 line, which hardly responded to CD20 and CD47 blockage. Life cell imaging showed a dose-dependent increase in ADCP for the triplet comprising LILRB1-Ig, but not for the triplet comprising LILRB2-Ig. Moreover, such triple blockage including LILRB1-IgG significantly promoted ADCP in cells obtained from 10 of 12 CLL patients and in all cases was more effective than the CD47-CD20 combination [20].

Another similar combination was studied by Aroldi et al. It comprised blockage of CD47 (with B6H12.2), CD20 (with rituximab), and CD24 (with SN3) in several combinations. In MCL cell lines, they showed significant promotion of phagocytosis for each duet combination of antibodies, with no differences between each pair, and the greatest improvement was seen in triple blockage (CD20, CD24, and CD47). Such antibodies were then tested in samples of MCL and CLL patients with similar results. Of note, one of the CLL patients was treated with rituximab before sampling. In his case, the efficacy of the current CD20 blockade was limited, but the effects of the CD24 and CD47 dual blockade were preserved [22].

Another CD47-including combination was studied by Ribeiro et al. in several NHL cell lines and BL xenograft models. The combination included TG-1801, a BsAb targeting CD47xCD19, and 2 other agents, i.e. CD20-binding ublituximab and PI3Kδ-binding umbralisib, (abbreviated as U2 if used together). The substances were tested in monotherapy and several combinations. TG-1801 monotherapy triggered a response against CD19 cells, and the U2 combination promoted ADCP and ADCC greater than ublituximab or umbralisib alone. U2 was then tested in triplet with TG-1801 in BL, DLBCL, and FL cell lines. U2 addition vastly potentiated TG-1801-mediated ADCC and ADCP in BL lines, whereas the effects in DLBCL and FL lines were slight. The TG-1801 and U2 were later evaluated in mouse BL xenografts. Both U2 and TG-1801 exerted tumor growth inhibition (TGI), (89% and 76% respectively), and TG-1801+U2 triplet showed a greater and faster response, with TGI reaching 93%. In addition, after 35 days of the last triplet dose, 40% of the mice remained tumor-free. Similar tendencies were shown in immunocompetent chicken models, with triplet therapy-induced TGI surpassing the TGI of U2 and TG-1801 apart (86%, 70%, and 70%, respectively). Moreover, the authors found that the effects of triplet treatment depended on G protein-coupled receptor 183 (GPR183), and GPR183 blockage decreased phagocytosis threefold. According to them, GPR183 could be evaluated as a predictor of immunotherapy efficacy [53].

**Table 1.** Summary of the inhibitors of the innate immunological system molecules and the multiple blockages

| Substance | Molecular target | NHL types | Most common adverse effects | Notes | Reference |
|---|---|---|---|---|---|
| Hu5F9-G4 (magrolimab) | CD47 | BL mice model, DLBCL, FL patients | chills, anemia, headache | Hu5F9-G4 + rituximab was successful in Raji-BL mice model treatment and may be safe and effective in the treatment of relapsed and refractory DLBCL and FL. | [32, 34] |
| αCD47-siFc and αCD47-fuFc | CD47 | MCL cell lines | N/A | Promotes phagocytosis in monotherapy, presumably dependent on Fc region function. Promotes of rituximab-induced phagocytosis. | [25] |
| CD47-IgGσ | CD47 | cell lines: MCL, DLBCL, CLL | N/A | Promotes rituximab-mediated ADCP, particularly with LILRB1 blockage. | [20] |
| MIAP301 | CD47 | T-NHL cell line | N/A | Promoted antitumor effects of Accum™. | [27] |
| Several CD38xCD47 BsAbs | CD38xCD47 (BsAbs) | cell lines including BL cell lines | N/A | Promoted ADCC and ADCP. | [35] |
| Anti-CD47xCD20 BsAb | CD47-xCD20 (BsAb) | BL mice model | N/A | Anti-CD47-CD20 BsAbs significantly extended disease-free survival in Raji-BL-induced mice models. | [36] |
| IMM0306 (amulirafusp α) | CD20xCD47 binging domain of SIRPα (fusion protein) | FL, DLBCL, MZL, MCL, CLL/SCL patients | WBC decrease, RBC decrease, lymphocyte count decrease, PLT decrease | The first such fusion protein in the clinical trial. | [26] |
| TG-1801 | CD47xCD19 (BsAb) | BL cell line | N/A | Promoted ADCC and ADCP, synergism with ublituximab and umbralisib. | [53] |
| CC-96673 | CD47xCD20 (BsAb) | cell lines: BL, DLBCL, FL; mice models: BL, FL | N/A | Promoted phagocytosis more effectively than CD20 and CD47 blockage in monotherapy. | [21] |
| B6H12 | CD47 | DLBCL patients' cells, NHL cell lines, mice xenografts, NKTCL cell lines, xenografts, MCL and CLL cell lines, CLL patient samples | N/A | Promoted phagocytosis in monotherapy and combination with tafasitamab or rituximab or SN3 antibody blockage. Reduces tumor weight and volume. | [22–24] |
| AK117 (ligufalimab) | CD47 | NKTCL cell lines, xenografts | N/A | Promoted phagocytosis, reduced tumor weight and volume. | [24] |
| TTI-621 (maplirpacept) | CD47 | SS patients and CTCL cell lines | N/A | Inhibition of CD47 resulted in decreased LDH and lymphocyte burden in SS patients. It synergized with durvalumab in antitumor effects on CTCL cell lines. | [37, 38] |
| SN3 | CD24 | MCL cell lines, MCL and CLL patient samples | N/A | Promoted phagocytosis in combination with rituximab and tafasitamab. | [22] |
| Idealisib | PI3K p110δ | CLL/SLL | elevated transaminases, neutropenia, colitis/ diarrhea | The toxicity of idealisib + ofatumumab resulted in the cessation of the study. | [43] |
| Lacutamab | KIR3DL2 | many cell lines including PTCL and ATL | N/A | Promoted ADCC in KIR3DL2$^+$ cells, with no such effects in KIRDL2$^-$ cells. | [48, 49] |
| GSK2795039 | NOX2 | BL cell culture | N/A | GSK2795039 promoted cell death in Raji-BL cell cultures. | [44] |
| Lirilumab | KIR | DLBCL, FL | skin toxicity, IRR, diarrhea | The combination of lirilumab + nivolumab provided no improvement over single nivolumab. | [52] |

ADCC – antibody-dependent cellular cytotoxicity; ADCP – antibody-dependent cellular phagocytosis; ATL – adult T-cell leukemia; BL – Burkitt lymphoma; BsAbs – bispecific antibodies; CD – cluster of differentiation; CLL/SLL – chronic lymphocytic leukemia/small lymphocytic lymphoma; DLBCL – diffuse large B-cell lymphoma; FL – follicular lymphoma; IRR – infusion-related reaction; KIR – killer cell immunoglobulin-like receptor; LDH – lactate dehydrogenase; N/A—not available; MCL – mantle cell lymphoma; NHL – non-Hodgkin lymphoma; NKTCL – NK/T-cell lymphoma; NOX2 – NADPH oxidase 2; PI3K – phosphatidylinositol-4,5-bisphosphate-3 kinase; SS – Sézary syndrome.

The authors suggested that simultaneous blockage of CD47 and LILRB1 may improve lymphoma therapy through ADCP enhancement [20]. CD24 is presumably another potential target, as its blockage can significantly promote cell eradication in relapsed and refractory MCL and CLL samples [22]. Another novel CD20-CD47--PI3Kδ blockage may also contribute to NHL immunotherapy [53].

## CONCLUSIONS

Current progress in the search for treatment methods for patients with NHL provides many effective solutions. Immunotherapy aiming the innate immune system, is beginning to play an important role. The use of new therapeutic strategies reduces side effects and positively affects the NHL treatment process.

Potential therapeutic targets for the treatment of NHL in are mainly the CD47-SIRPα pathway, NOX2 pathway, and KIR pathway. More data are still needed to address the usefulness of KIR blocking in the treatment of NHL.

Immunotherapy raises great hopes among NHL patients. There is a need for further carefully designed studies of available methods to improve treatment outcomes in the near future. More data still needs to be collected to determine the effectiveness of the latest methods.

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