Review Article

The role of novel immunotherapies in non-Hodgkin lymphoma

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Abstract: Immunotherapy is an evolving modality in the treatment of non-Hodgkin lymphoma. Vaccinations with patient-specific tumor-derived antigens have been developed to strengthen immune response to tumor. The success of rituximab, a monoclonal antibody for CD20 on malignant B-cells, fueled further immunotherapy research. The power of the immune system to fight hematologic malignancies is seen in allogeneic stem cell transplant, where donor T cells attack residual malignant cells in the recipient. Now, three innovative therapeutic immunotherapy classes (I) adoptive cellular therapy; (II) immune-checkpoint inhibitors; and (III) novel antibody therapies show promising results in non-Hodgkin lymphoma. Genetically engineered T cells, CAR T cells, obtained remissions in lymphomas refractory to conventional chemotherapy. Immune-checkpoint inhibitors, such as nivolumab and pembrolizumab revolutionized the treatment of many solid tumors, and unprecedented results are now reported in relapsed/refractory lymphoma. Building on the success of rituximab, additional therapeutic monoclonal antibodies were developed for lymphoma treatment. Antibodies have recently been further engineered with multiple binding sites to directly engage both tumor and T cells. There are exciting early clinical trial results for the first bispecific T-cell engager (BiTE), blinatumomab, as well as promising ongoing studies for dual antibody molecules, Dual-Affinity Re-Targeting (DART) proteins. This review highlights these three immunotherapy classes for relapsed/refractory non-Hodgkin lymphomas and discusses the mechanism of action, clinical efficacy, and toxicities of each.

Keywords: Adoptive immunotherapy; antibodies; bispecific; lymphoma; non-Hodgkin; programmed cell death 1 receptor; antagonists; inhibitors

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Introduction

The native immune system prevents and combats malignancy. The intact immune system recognizes tumor cells as foreign and inhibits progression by signaling CD4+/CD8+ T cells to target tumor cells (1,2). Unfortunately, tumors escape immune surveillance through alterations in surface antigen expression or presentation leading to decreased immune recognition. Tumors may convert naive/effector T cells into senescent T cells, which induce immune tolerance (3). Additional tumor evasion mechanisms include the expression of immunosuppressive cytokines or up-regulation of negative co-stimulatory molecules for T cells (1).

A paradigm shift has occurred in cancer research from using cytotoxic chemotherapy to strategies aimed at amplifying and targeting immune response. Among the first therapies to demonstrate the power of the immune system was allogeneic stem cell transplant for hematologic malignancy. The donor provides non-malignant

hematopoietic cells, and additionally T-cells. The donor T-cells target the recipient's residual malignant cells and produce a beneficial graft versus tumor (GVT) effect. Depletion of these T cells from the donor product prior to transplant increases rates of relapse (4). An effective strategy to prevent early relapse following allogeneic hematopoietic stem cell transplant is withdrawal of immunosuppression, which unleashes the functionality of donor T cells (5). Donor lymphocyte infusions (DLIs) can successfully treat relapsed disease and further demonstrate the potency of the GVT effect (6). The benefit of allogeneic transplants is limited by the lack of specificity of donor T cells for residual malignant cells. Donor T cells also target healthy recipient tissue, leading to serious multi-organ toxicity known as graft-versus-host disease (GVHD) (7).

To utilize the power of the immune system without the adverse effects of GVHD, work has been ongoing for 20 years in vaccinations against specific tumor antigens to bolster the immune system. The proposed technique uses a cancer vaccine as adjuvant therapy to lower the risk of relapse by boosting immune response to tumor (8). In follicular lymphoma (FL), patient-specific tumor-derived antigens in first remission may improve disease-free survival (9,10).

Antibody therapies have further revolutionized the field of lymphoma, with rituximab, a monoclonal antibody to CD20, greatly improving outcomes when added to cytotoxic chemotherapy. Chemo-immunotherapy is now the first-line standard of care for many sub types of lymphoma (11). Treatment with chemo-immunotherapy for aggressive lymphomas such as diffuse large B-cell (DLBCL) obtains complete responses (CRs) in 75–80% of patients (12,13). However, the prognosis remains poor in patients who relapse or have refractory disease. The options for salvage therapy include high dose chemotherapy followed by autologous stem cell transplant (AHSCT) with response rates of only 63%. Patients who relapse within 12 months of first-line therapy have a particularly poor prognosis with a 3-year progression-free survival (PFS) of only 23% with salvage therapy (14). Indolent non-Hodgkin lymphomas, such as FL, are slowly progressive but incurable. Despite rituximab-based regimens, 20% of FL patients relapse within 2 years of treatment. These patients have substantially increased risk of death with decreasing responsiveness to conventional rituximab-based therapies (15).

Given the limited salvage options, non-Hodgkin lymphoma would benefit from the further application of immunotherapy. Adoptive cellular therapies, immune-checkpoint inhibitors, and novel antibody therapies have all demonstrated efficacy in both aggressive and indolent non-Hodgkin lymphoma. We will review the mechanism of action, clinical trial results, and toxicity management of the leading immunotherapies for treatment of non-Hodgkin lymphoma.

Adoptive cellular therapy

CAR T cells-mechanism

CAR T cells are autologous T lymphocytes genetically engineered to bind to specific antigens expressed on malignant cells. Through the CAR T cell binding to the malignant cell, the signaling domains stimulate T-cell proliferation, cytolysis, and cytokine secretion to eliminate the tumor cell. CAR T cells are generated through apheresis of patient's peripheral blood mononuclear cells at steady state. T cells are isolated from peripheral blood and activated. The T cells are then transduced with retroviral or lentiviral vector with a CAR construct, typically an antibody single chain variable fragment (scFv) or peptide (16). Second and third generation CAR T cells incorporate into the construct a domain such as CD28, which supplies a co-stimulatory signal. The modified T cells are then expanded and infused into the patient over 1–2 days (17). Patients receive lymphodepleting chemotherapy prior to CAR T cell infusion to limit the early immune-destruction of CAR T cells.

Ideally the antigen targeted by CAR T cells is present on malignant cells and absent on healthy cells. Such unique antigens are difficult to identify but targeting an antigen on a specific cell lineage has proven feasible. CD19 is a B-cell surface protein found in nearly all B-cell malignancies including B-cell ALL, chronic lymphocytic leukemia (CLL), and many non-Hodgkin lymphomas.

CAR T cells-clinical use in non-Hodgkin lymphoma

Multiple centers are investigating CAR T cells for treatment of non-Hodgkin lymphoma (18-21). CD19-specific CAR T cells have been studied in relapsed/refractory lymphomas as well as ongoing clinical trials for CD 30-specific CAR T cells (NCT01316146).

The National Cancer Institute (NCI) (Bethesda, MA, USA) first reported successful treatment of a patient with second-generation anti-CD19 CAR T cells. This patient with refractory stage IVB FL obtained partial remission of lymphoma lasting 32 weeks following CAR T-cell infusion and IL-2 (18). A subsequent study from

the NCI of 15 patients with advanced DLBCL, CLL, or indolent lymphomas found that 8 patients achieved complete remission (CR), 4 achieved partial remission, and 1 had stable disease. In the NCI study, patients received a conditioning regimen of cyclophosphamide and fludarabine prior to CAR T-cell infusion (22).

Memorial Sloan Kettering Cancer Center (MSKCC) (New York, NY, USA), studied the use of CAR T cells as consolidative therapy following AHSCT. In this phase I study, six patients with poor-risk NHL underwent AHSCT with subsequent CAR T cell infusion on day 2 and 3. All patients obtained CR at first restaging following transplant and remained in remission at the reported median follow-up of 6 months (19).

Studies performed at Fred Hutchinson Cancer Research Center (Seattle, WA, USA) and University of Pennsylvania (UPenn) (Philadelphia, PA, USA) allowed patients to receive a variety of lymphodepleting regimens. The Fred Hutchinson group treated patients with a defined composition of CD8+ central memory T cells and CD4+ T cells. Of the nine patients with NHL in their study, one experienced CR and five had partial remissions (20).

The phase IIa trial of CD19-CAR T cells at the UPenn Abramson Cancer Center included patients with FL, DLBCL and mantle cell lymphoma with relapsed/refractory disease and anticipated survival of less than 2 years. Eighteen patients were evaluable for response at 3 months (12 DLBCL and 6 FL) with a reported 67% overall response rate and a 6-month PFS of 59% (21).

While multiple centers report promising results for CAR-T cells, there are important differences between institutions in their study protocols. The lymphodepleting regimen prior to CAR T cell infusion varies by study institution. The protocols for design of the CAR T cell, either lentivirus or retrovirus, for transducing CAR T cells and protocols for culturing the CAR T cells also differ by study. Different centers adjusted the timing of CAR T cell infusion either following chemotherapy alone or immediately following autologous transplantation. Further multi-center trials are required to optimize CAR T cell therapy.

Studies suggest some cases remain resistant to CAR T cells. Resistance may in part be due to the inability of the CAR T-cell to overcome the inhibition produced by the tumor cells' expression of T-cell inhibitory ligands. Thus, trials combining CAR T cell therapy with monoclonal antibody immune-checkpoint inhibitors are ongoing. A trial at Baylor College of Medicine (Houston, TX, USA) combines ipilimumab with CAR T cells (NCT00586391), and an ongoing clinical trial at the UPenn is exploring pembrolizumab following CAR T cells (NCT02650999).

Another proposed mechanism of CAR T cell failure is lack of persistence of the genetically modified T cells. The recipient's immune system may recognize the CAR-derived foreign peptides and destroy the modified T cells. Investigation is ongoing into whether co-administered cytokines improves the expansion or persistence of CAR T cell (NCT00968760).

There are significant barriers to implementing widespread use of CAR T cell therapy. There are technical challenges in manufacturing CAR T cells, which currently limits use to few centers. Multiple pharmaceutical companies are developing larger-scale production facilities of CAR T cells. Additionally, significant time is needed to expand the CAR T cells after harvesting T cells from the patient, which limits use in a patients requiring urgent therapy.

CAR T cells-toxicities and management

Cytokine release syndrome (CRS) is a potentially life-threatening toxicity of CAR T cell infusion. CRS is associated with high levels of several cytokines, including interleukin-6 (IL-6) and interferon γ. The clinical syndrome of CRS observed in response to adoptive cellular immunotherapy includes fever, hypotension, and hypoxia. Laboratory values suggestive of CRS include elevated CRP, markedly elevated ferritin, and low fibrinogen (23).

With CAR T cell therapy, symptom onset is typically within days to weeks, correlating with peak *in vivo* T cell expansion (23). The rates and severity of CRS from CAR T cell therapy in lymphoma patients are less frequent and milder than those with high levels of circulating disease from acute lymphoblastic leukemia (ALL). The CAR T cell study from Fred Hutchinson included both ALL and NHL patients, and reported severe CRS only in ALL patients (20). In the UPenn study, 15 of the 29 NHL patients treated with CAR T cells experienced some degree of CRS, but the majority (87%) were only grade 2 (21).

Treatment for CRS is dependent upon severity of symptoms as well as patient comorbidities. Experts recommend supportive care with fluids and close monitoring for grade 1 CRS. Immunosuppressive agents are generally reserved for CRS of higher grade. Given IL-6 is a key mediator in the syndrome; tocilizumab (IL-6 receptor antibody) at a dosing of 4 mg/kg is effective for treatment of CRS (23,24). A response is typically seen within hours

of administration. Corticosteroids have also be added to tocilizumab in unresponsive patients (23).

The use of immunosuppressive therapy for CRS must be balanced against the theoretical concern of extinguishing the efficacy of the CAR T cells. It is proposed that the cytokine cascade of the syndrome may be partly responsible for response to CAR T cells. Various centers have reported many of the responding patients develop some degree of CRS (25). However, patients with ALL who received tocilizumab for CRS still obtained CR (26). Porter *et al*. reported that four CLL patients who received tocilizumab had peak T-cell proliferation several days following, suggesting that the agent does not affect long-term T cell survival (27).

Clinical trials also noted neurologic toxicity with CAR T cells infusion. Of the 20 patients who received CAR T cells in the UPenn trial, 3 patients experienced neurologic toxicity including delirium (grade 1 and 3) and one grade 5 encephalopathy (21). The neurologic toxicity may reflect the ability of the CAR-T cells to penetrate the blood brain barrier. Davila *et al*. reported CD-19 CAR T cells in cerebrospinal fluid of three patients with neurologic complications. Notably, CAR T cells were not identified in the CSF of all patients with neurologic complications by lumbar puncture (26). Most of the neurologic symptoms observed are reversible with use of dexamethasone, which also penetrates the blood-brain barrier. As tocilizumab is a monoclonal antibody, it does not likely penetrate the blood-brain barrier; hence, corticosteroids are favored when severe neurologic toxicity is observed (25).

B-cell aplasia also has been reported due to the depletion of non-malignant CD 19 B lymphocytes. Patients are at risk for opportunistic infections due to hypogammaglobulinemia. In a trial of CAR T cells in CLL, three patients who entered the trial with polyclonal blood B-cell counts in the normal range had B-cell depletion for at least 4 months following CAR T cell infusion (22). Hypogammaglobulinemia has been successfully managed with IV immunoglobulins and is recommended for patients who experience low immunoglobulin levels following CAR T cell infusion (27).

Antibody therapies

Monoclonal antibodies-mechanism

Antibodies targeted to tumor cell surface antigen kill tumor cells through complement and antibody-dependent cytotoxicity and induction of apoptosis. Antibodies can also work through a "vaccinal effect", through the cross-presentation of tumor antigens released by dying cells to antigen-specific T cells leading to activation (11). Additional preclinical studies suggest that monoclonal antibodies can sensitize cells to induction of apoptosis, thus accounting for their success in combination with cytotoxic therapy (28). Ideal targets for monoclonal antibodies include an antigen that is present on malignant cells only.

Monoclonal antibodies-clinical use in non-Hodgkin lymphoma

The success of rituximab raised interest in the development of novel agents targeting other surface antigens on malignant B-cells. Now, monoclonal antibodies can successfully target multiple antigens, including CD20, CD52, and CD40. Anti-CD20 antibodies have expanded from rituximab to include ofatumumab and obinutuzumab. Ofatumumab is a human monoclonal antibody that targets a novel epitope on CD20. Ofatumumab demonstrated closer binding to the B-cell surface and increased complement-dependent cytotoxicity than rituximab in preclinical models (29). A phase I/II trial of ofatumumab as a single-agent in relapsed/refractory FL reported response rates of 64%, 33%, 20% and 70% in patients treated with 300, 500, 700, and 1,000 mg doses respectively. Notably, a response was seen in three of four patients in the study who were previously refractory to rituximab (30). In a study of ofatumumab in relapsed/refractory CLL, the cohort with highest dose escalation to one 500 mg infusion followed by three 2,000 mg infusions, the response rate was 62% and remission rate was 50% (31). Another anti-CD20 humanized monoclonal antibody, obinutuzumab, has also been trialed for relapsed/refractory non-Hodgkin lymphomas. A phase III trial compared bendamustine alone to obinutuzumab and bendamustine followed by obinutuzumab maintenance therapy in rituximab-refractory patients with indolent NHL. Results suggested PFS was significantly longer with the addition of obinutuzumab versus bendamustine alone (32).

Additional targets for monoclonal antibodies include CD52 and CD40. Studies revealed efficacy of anti-CD52 antibody, alemtuzumab in CLL as well as peripheral T-cell lymphoma (33,34). However, it is currently available only on a compassionate use basis. An anti-CD40 monoclonal antibody, dacetuzumab showed efficacy as monotherapy in

a phase I trial of 50 patients. Approximately one-third had a decrease in tumor size with dacetuzumab 8 mg/kg/week for 4 weeks, including one complete and five partial responses (35). An additional CD40 monoclonal antibody, lucatumumab reported phase I data in relapsed CLL and doses were well-tolerated with 1 of the 26 patients having a partial response and 17 with stable disease (36).

Monoclonal antibodies-toxicities and management

Infusion reactions commonly occur with monoclonal antibodies. Tumor lysis syndrome has occurred in patients with high number of circulating malignant cells. Symptoms can include fever, bronchospasm, hypoxemia, and rigors. This is most often seen during first infusion and is reversible with supportive care. Patients are often able to tolerate subsequent infusions with pre-medication with antihistamine and steroids, particularly if disease burden has been lowered by prior treatment (37). Similar infusion-related adverse events occur with the novel anti-CD20 monoclonal antibodies, ofatumumab and obinutuzumab. Infusion-related reactions occurred in 11% of patients receiving obinutuzumab (32). The majority of patients (38 out of 40 patients) who experienced an adverse event in the ofatumumab study had the event on an infusion day (30).

Infectious complications result from the hematologic toxicity of the monoclonal antibody therapy. Reports of rituximab maintenance therapy for relapsed low-grade lymphomas show profound B-cell depletion in most patients, lasting 6–12 months. Serum levels of IgG and IgA generally remained within normal range with slight decrease in IgM (38). There are reports of hepatitis B virus (HBV) reactivation with anti-CD20 therapies, necessitating screening for HBV and prophylactic antiviral therapy (39).

The phase 1–2 trial of ofatumumab in relapsed/refractory B-cell CLL, also reported non-malignant B-cell depletion, which sustained until approximately week 24 following treatment. In this trial, 51% of patients experienced at least one infection (31). In the trial ofatumumab in FL, infectious complications were noted in 32.5% of patients with two grade 3 infections (30). When anti-CD20, obinutuzumab, was combined with bendamustine, neutropenia was most frequent grade 3 or greater adverse event occurring in 33% of patients compared to 26% in the bendamustine alone group (32).

Fatigue, pyrexia and headache were the most common reported side effects of the newer anti-CD40 monoclonal antibodies. Additionally, non-infectious inflammatory eye disorders occurred in 12% of patients (35).

Bispecific T-cell engager (BiTE) mechanism

Targeting tumor-specific antigen and directly engaging T cells may amplify the efficacy of antibody therapy and limit toxicity to other cell types. A BiTE is a molecule consisting of a single polypeptide that possesses two specific antigen binding sites, one which engages a specific B-cell marker and another targeting a co-stimulatory on T cells. This allows for recruitment of T cells specifically to malignant B-cells with subsequent engagement of the two receptors leading to T-cell activation and apoptotic death of malignant cells (40). Blinatumomab was the first developed BiTE that consists of one antigen-binding site for CD19 and another for CD3. The bispecific antibody was noted to have efficacy in indolent NHL, with further more extensive studies in Philadelphia-chromosome negative acute B-cell lymphoblastic leukemia (41,42).

BiTE-clinical use in non-Hodgkin lymphoma

Blinatumomab has a half-life of 2 hours and must be administered via continuous infusion. The infusion continues over the course of a minimum of 4 weeks and requires implanted port and mini-pump system in outpatient setting. Patients in clinical trials were monitored as inpatients for at least 3–7 days at the start of therapy or dose-escalation (43).

In the initial report of blinatumomab efficacy in non-Hodgkin lymphoma, blinatumomab 15 μg/m^2/day for 4 weeks per cycle led to CR in four patients and partial responses in seven patients (41). The phase I dose-escalation trial treated 76 patients with relapsed/refractory NHL over 4 or 8 weeks at seven different dose levels (0.5–90 μg/m^2/day). In the 35 patients escalated to the target dose of 60 μg/m^2/day, an overall response rate of 69% was observed in all NHL subtypes and in 55% of patients with DLBCL. The median response duration was 404 days (44).

The phase I trial reported titration to higher doses resulted in improvement response, and thus the phase II trial was designed to achieve greater target dosing. The phase II study had two cohorts of DLBCL patients treated at either weekly step-up dosing of 9, 28 and 112 μg/day or a fixed-dose of 112 μg/day for up to 8 weeks followed by 4 treatment-free weeks. In the fixed-dose 112 μg/day cohort, two patients experienced grade 3 neurologic toxicity, resulting in the early cessation of this cohort. Patients with

step-wise dose escalation to 112 µg/day dosing had overall response of 43% with a CR in 19%. Investigators proposed that at least 1 week of treatment at target dose of 112 µg/day is required for efficacy (45). Blinatumomab monotherapy appears to be effective in relapsed/refractory DLBCL with step-wise escalation to target dose.

BiTE-toxicities and management

The adverse events reported in the phase I/II studies of blinatumomab included CRS, neurologic toxicity, and leukopenia/neutropenia. As discussed with cellular therapies, CRS includes flu-like symptoms, hypotension, multi-organ failure, fever, and hypotension. In the phase I study of blinatumomab in NHL, less than 10% of patients experience grade 3 CRS or greater (44). The phase II trial reported no adverse CRS events. Notably, the study employed prophylactic "early" dexamethasone for each blinatumomab infusion start and dose increase as well as daily for 2 days following initiation (45).

Neurologic toxicities including headache, tremor, aphasia, ataxia, disorientation, and seizure occurred in 70% of patients in the phase I trial, and 22% of these were grade 3 (44). In the phase II trial in DLBCL, grade 3 neurologic events were in 9% of patients. The vast majority of neurologic events were reversible, with 46/48 neurologic events resolving. The median time from initiation of therapy to onset of neurologic event was 18 days with symptoms resolving at a median of 4.5 days (45).

Given the risk of neurologic toxicity and CRS, blinatumomab should be administered on a stepwise schedule starting at 9 µg/day IV with up-titration on a weekly step-up dose of 9, 28 and 112 µg/day. Inpatient hospitalization is required for up-titration to maximum dose. Patients receive dexamethasone 20 mg 1 hour prior to starting the therapy, and prior to any treatment interruptions of 4 hours or more (46). In the phase II trial, patients received "early" dexamethasone prophylaxis with 20 mg orally at 6–12 hours and 1 hour prior to infusion, and then 8 mg 3 times daily for 2 days. If neurologic toxicity or CRS occurred, management was with dexamethasone orally or IV at a dose of 24 mg per day for up to 3 days, with subsequent stepwise reduction of blinatumomab infusion over 4 days (45).

Dual-affinity Re-Targeting (DART) proteins-clinical use

Using a similar mechanism as BiTE, DART proteins also co-engage CD3 on T-cells as well as CD19 on malignant cells. The DART is a novel bispecific antibody designed to overcome structural limitations of BiTE to improve stability and efficacy. The DART molecule is based on a diabody format that has two covalently linked polypeptides creating one binding site for two specific antigens. In *in vitro* studies, DART has been shown to induce cytotoxicity, and exhibited potent activity in multiple relevant tumor models. Early *in vitro* studies showed DART exhibiting greater potency than the BiTE format (47). The DART format is particularly appealing for clinical use, as it has been shown to have a half-life similar to other monoclonal antibodies, allowing for intermittent dosing. There is a first-in-human study of a CD19XCD3 DART in patients with relapsed or refractory non-Hodgkin lymphoma that is currently recruiting participants (NCT02454270).

Immune checkpoint inhibitors

Immune checkpoint inhibitors-mechanism

Tumor cells abrogate the immune system by mimicking strategies used by the healthy immune system to regulate response and allow for self-tolerance. Two of the T-cell inhibitory mechanisms involve the cytotoxic T-lymphocyte associated protein 4 (CTLA-4) and programmed death 1 (PD-1) receptors. CTLA-4 is expressed on naive T cells and up regulated during T-cell activation. In the setting of a strong stimulus by an antigen, CTLA-4 serves as a "brake" on the immune response. This allows for establishment of peripheral T-cell tolerance. PD-1 is also important in regulation of a healthy immune system. It is present on several antigen-presenting cells as well as activated T cells (48). The PD-1 ligand, PD-L1, is normally expressed on antigen-presenting cells, activated T cells and other immune cells. PDL-2 expression is typically only on macrophages, dendritic cells, and B-cells. Immediately after activation of the T-cell receptor, engagement of PD-1 with PDL-1/2 inhibits PI3K activity which blocks further T-cell activation and down regulates cytokine production. In the intact immune system, this mechanism serves to regulate immune response in setting of chronic antigenic stimulation (49).

Tumors exploit the self-regulatory mechanisms of a healthy immune system. PD-L1 expression has been found in many solid tumor types (50). There is evidence of expression of PD-L1 and/or PD-L2 in a subset of non-Hodgkin lymphomas as well as in the tumor microenvironment, making this pathway a promising target (51). The expression

of PD-L1 by immunochemistry also has been recently suggested to have diagnostic and prognostic importance. In a study of 889 lymphoma cases, PD-L1 expression was expressed in 31% of DLBCL cases, approximately 5% of FL and 10% of marginal zone lymphomas (52).

Monoclonal antibodies targeting CTLA-4 and PD-1 are now developed to decrease the down-regulation of T-cell response against tumor cells. With decreased inhibitory signals, the immune response is amplified to target tumor cells.

Immune checkpoint inhibitors-clinical use in non-Hodgkin lymphoma

Immune-checkpoint inhibitors antibodies are currently approved for treatment in a variety of malignancies, and have gained remarkable successes (53-55). In lymphoma, studies include targeting CTLA4 with ipilimumab and PD-1 with pidilizumab, pembrolizumab, and nivolumab. A phase I trial of CTLA4-blockade with ipilimumab reported durable clinical responses of 31 and 19 months respectively in two patients with relapsed/refractory DLBCL and FL. In the trial, ipilimumab was administered at 3 mg/kg and then monthly at 1 mg/kg × 3 months with subsequent escalation to 3 mg/kg monthly × 4 months (56).

Targeting PD-1 blockade in non-Hodgkin lymphoma first proved efficacious with pidilizumab. Armand *et al*. studied patients with DLBCL, primary mediastinal B-cell lymphoma (PMBCL) or transformed indolent B-cell lymphoma undergoing planned AHSCT with at least partial remission following salvage therapy. The study hypothesized pidilizumab may improve outcomes, given the low volume residual disease and remodeling of the immune system post-transplant. Three cycles of pidilizumab 1.5 mg/kg every 42 days were administered starting 30 to 90 days following AHSCT. This international phase II study reported addition of pidilizumab to AHSCT resulted in overall response rate of 51% among patients with measurable disease following transplant and CR in 34%. Most pronounced was the PFS at 16 months following AHSCT and pidilizumab of 0.72 (90% CI, 0.60–0.82). While the study did not directly compare to AHSCT alone, the investigators reported analysis of 46 patients undergoing AHSCT alone who would have met eligibility for present study and reported 18-month PFS following AHSCT of 0.52 (90% CI, 0.39–0.63) (57). Another phase II study assessed pidilizumab in patients with relapsed FL. In this trial, pidilizumab was administered at 3 mg/kg intravenously every 4 weeks for four infusions, plus eight optional infusions every 4 weeks for patients with stable disease or better. Rituximab was also given 375 mg/m^2 IV weekly for 4 weeks starting 17 days after pidilizumab therapy. Of the 29 patients treated in the study, 19 (66%) achieved an objective response: CRs were noted in 15 (52%) patients and partial responses in 4 (14%) (58).

Pidilizumab is the first PD-1 blocking antibody to show efficacy in NHL, and is now followed by agents with higher specificity for the receptor. Nivolumab and pembrolizumab are two PD-1 blocking antibodies, which show activity in lymphoma. A phase I study of nivolumab had objective response rates of 40% and 36% in FL and DLBCL respectively. Nivolumab was dosed in the phase I trial at 1 or 3 mg/kg on week 1, week 4 and every 2 weeks thereafter (59). A phase Ib study of pembrolizumab in patients with relapsed/refractory non-Hodgkin lymphoma is ongoing (60). There is an ongoing clinical trial of pembrolizumab 200 mg IV every 3 weeks up to eight cycles, administered within a few weeks after AHSCT for relapsed DLBCL, which is currently enrolling participants (NCT02362997).

Durvalumab is a selective high-affinity monoclonal antibody that blocks PD-L1 binding to PD-1 and CD80. A phase II study for durvalumab as monotherapy and in combination for lymphoma or CLL is currently recruiting participants. Data showed safety and clinical efficacy from phase I/II dose-escalation trials for durvalumab monotherapy in urothelial carcinoma (61). The durvalumab trial for lymphoma is currently ongoing with a design of a monotherapy arm, durvalumab + lenalidomide/rituximab, durvalumab plus ibrutinib and durvalumab plus bendamustine and rituximab (NCT02733042).

Immune checkpoint inhibitors-toxicities and management

Immune-checkpoint inhibitors are particularly appealing due to their relatively low toxicity profile. The phase I trial of checkpoint inhibition with nivolumab in solid malignancies reported 41% had an adverse event, but only 6% were grade 3 or higher (62). Researchers also reported 70.9% treatment-related adverse events with pembrolizumab in solid malignancies, but only 9.5% grade 3 or higher (54).

The side effect profile of the CTLA-4 and PD-1 inhibitors is related to its mechanism of action in amplifying immune response. Studies in solid tumors report inflammatory reactions, including hepatitis, pneumonitis, colitis, thyroiditis, and hypophysitis (54). In the phase II study with pidilizumab

in lymphoma, there were no autoimmune toxicities that were grade 3 or greater (59). Investigators in the phase Ib study of nivolumab in hematologic malignancy reported immune-related adverse events in 34% of patients, but these were predominately grade 1–2. Of these events, 46% resolved without treatment or interruption of nivolumab (59).

Studies report endocrine adverse events with checkpoint inhibitors, which are autoimmune in nature. Hypothyroidism was reported in approximately 8–10% of patients in studies of PD-1 inhibitors in melanoma patients. Hyperthyroidism occurred in 3–6% of patients in the trial (63). Hypophysitis is less frequent with a rate of 1% reported in a trial with pembrolizumab (64). Patients receiving checkpoint-inhibitor therapies should have thyroid functioning monitored routinely as well as ACTH/cortisol if presenting with symptoms such as fatigue or hyponatremia. Testosterone testing should also be considered in males. With low-grade endocrine toxicity, the drugs can be continued with supportive care and close monitoring. If adrenal crisis is suspected, stress dose steroids should be administered (65).

Immune-related pneumonitis was reported at 3% of patients in a large phase III study of nivolumab in non-small cell lung cancer (NSCLC) (55). In the phase Ib nivolumab trial in hematologic malignancies, 11% experienced pneumonitis of any grade with 4% experiencing grade 3 or greater. There was one death due to fatal pneumonitis in this trial (59). Bronchoscopy and lung biopsy should be considered in patients with concern for immune-related pneumonitis. For symptoms with grade 2 mild-to moderate symptoms, withhold PD-1 and monitor. It is recommended to treat with corticosteroids 1.0 mg/kg per day for more severe symptoms. If symptoms persist or worsen after 2 days, infliximab or mycophenolate mofetil can be considered (66).

Ipilimumab treatment causes high rates of diarrhea, with grade 3 or greater colitis occurring in 28% of patients in the phase I trial of ipilimumab in non-Hodgkin lymphoma (56). This degree of gastrointestinal toxicity is rarely seen with PD-1 checkpoint inhibitors, a reported 4% in melanoma patients (63). The management of gastrointestinal toxicity includes close monitoring and prompt treatment of early symptoms to avoid more serious toxicity such as bowel perforation and consideration of infectious etiologies. Oral steroids are recommended at 1–2 mg/kg per day. In steroid-refractory cases, infliximab (TNF-α) at dose of 5 mg/kg once every 2 weeks can be used after 72 hours, but must be avoided in patients with risk of GI perforation or sepsis (66).

Conclusions-immunotherapies in non-Hodgkin lymphoma

Recent studies have added non-Hodgkin lymphoma to the rapidly expanding list of malignancies with remarkable response to immunotherapies in both indolent and aggressive subtypes. Tolerability has been demonstrated in a number of lymphoma patients, including those heavily treated with chemotherapy and rituximab. Further trials are necessary to compare responses of immunotherapy to conventional therapies for relapsed/refractory lymphoma such as AHSCT. Trials directly comparing the various immunotherapy agents and combining agents with adoptive cellular therapy are needed to delineate the most appropriate therapy for an individual patient. Technical challenges remain in cellular therapy production and side effects management of the antibody therapies. Overall the field of immunotherapy for non-Hodgkin lymphoma appears quite promising, and we await the results of many upcoming clinical trials.

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