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Beginning of a novel frontier: T-cell directed immune manipulation in lymphomas

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Summary

Checkpoint inhibitors with monoclonal antibodies targeting the CTLA-4 or PD-1 axis have revolutionized treatment in some solid tumours, especially melanoma and lung. The role of the CTLA-4 and PD-1 pathways and their inhibition in lymphoma may be different compared to solid tumours. In heavily pre-treated Hodgkin lymphoma, PD-1 directed treatment has led to high remission rates. Several studies are now conducted also including diffuse large B-cell and follicular lymphoma. Beside antibody-based immunotherapy, treatment with chimeric antigen receptor (CAR) T-cells has also come back to the focus of recent studies. Clinical evidence of CAR T-cell treatment in B-cell malignancies is limited to small series, because of the dedicated resources needed. However, impressive response rates have been observed, but toxicities associated with cytokine release can be very severe and fatal. We herein review background, early clinical evidence and future perspectives of T-cell directed immune manipulation for lymphomas including checkpoint inhibitors and CAR T-cell therapies.

Keywords

Lymphoma, Anti PD-1 treatment, Chimeric antibody receptor, CAR, T-cells, Review, Ipilimumab, Nivolumab, Pembrolizumab, Pidilizumab, Immunotherapy

Introduction

The immune system plays an important role in controlling and eradicating malignant cells. Increasing the power of the immune system to fight cancer has been a long-standing ultimate goal in oncology. Several different strategies aiming to harness the immune system for controlling and eradicated malignant cells are summarized under the umbrella of *immunotherapies*. These strategies can be subdivided into *drug-based* or *cell-based* immunotherapies.

Drug-based immunotherapy includes a broad range of strategies such as general stimulation of the immune response (e.g. interferon), targeting surface proteins with monoclonal antibodies on malignant cells to facilitate complement-mediated cytotoxicity and antibody-dependent cell-mediated cytotoxicity (e.g. CD-20 with rituximab) (1), or modification of the crosstalk between malignant cells and cytotoxic T-cells with agents such as lenalidomide (2), monoclonal antibodies (checkpoint inhibitors, e.g. monoclonal antibodies against anti-programmed death 1 [PD-1] or cytotoxic T-lymphocyte-associated antigen 4 [CTLA-1]) (3,4). Strategies in *cell-based* immunotherapies include ex-vivo stimulation and re-infusion of autologous T-cells (e.g. Sipuleucel-T) (5), ex-vivo genetic manipulation and re-infusion of autologous T-cells (chimeric antigen receptor therapy) (6) and ultimately, allogeneic hematopoietic stem cell transplantation (allo-HCT). Clearly, allo-HCT is a unique approach in itself and is very different in terms of biological action compared to the other immunotherapies listed above.

The first practice-changing breakthrough in solid tumours was only recently achieved with CTLA-4 and PD-1 directed checkpoint inhibitors in the treatment of advanced melanoma (7–10), a disease for which chemotherapy was only of very limited activity. For example, in one of the first studies, median survival in patients was improved from 9.1 to 11.2 months with ipilimumab in combination with dacarbazine versus dacarbazine alone (hazard ratio [HR] 0.72; 95% confidence interval [CI] 0.59 – 0.87), which translated into an absolute 2-year survival benefit of 10% (8). The PD-1 antibody pembrolizumab further improved survival compared to ipilimumab (HR 0.63; 95% CI 0.47 - 0.83; 1-year survival 74.1% versus 58.2%) (11).

Despite improved treatment options for indolent and aggressive lymphomas, relapsed or refractory disease is common. In diffuse large B cell lymphoma (DLBCL), one third of patients relapses after standard rituximab-containing chemotherapy; of these, only about 10% will be cured following high-dose chemotherapy and autograft (12). For follicular lymphoma (FL), conventional therapies are not curative and patients typically present with repeated relapses and a more resistant disease over

time (13–15). Particularly for patients with refractory lymphoma and for those ineligible for high-dose therapies, new therapeutic approaches are highly warranted. Unlike solid tumours, lymphomas arise from the immune system itself, therefore the role of the CTLA-4 and PD-1 pathway and their inhibition by monoclonal antibodies may be very different. In general, lymphomas offer a very interesting ground for immunotherapy. In this review, we will focus on T-cell directed immune manipulation for lymphomas including checkpoint inhibitors and chimeric antigen receptor (CAR) therapies.

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Checkpoint inhibition

The growing knowledge about the interaction between malignant cells and the immune system has led to development of new immunotherapies. One remarkable advance is the understanding of how malignant cells usurp immune checkpoint pathways to protect themselves against activated immune cells. Checkpoints of clinical relevance today are the CTLA-4 and PD-1 (also known as PDCD1) pathways (16,17).

CTLA-4 was the first immune checkpoint receptor to be clinically targeted. After T-cell activation, CTLA-4 is normally upregulated on the plasma membrane to downregulate T-cell function through a variety of mechanisms. These include preventing co-stimulation by outcompeting CD28 for its ligand, B7, and also by inducing T-cell cycle arrest (18–20). Through these mechanisms and others, CTLA-4 has a fundamental role in maintaining normal immunologic homeostasis, which has clearly been illustrated in animal models, where mice deficient in CTLA-4 died from fatal lymphoproliferation (21,22). The yet mostly investigated CTLA-4 targeting antibody is ipilimumab, which is a fully human (IgG1) monoclonal antibody that blocks CTLA-4. In pre-clinical models, ipilimumab has been shown to enhance T-cell activation, anti-tumour activity and also memory against murine tumours (23,24).

PD-1 is an inhibitory receptor expressed by activated T-cells, activated B-cells, natural killer cells, and myeloid cells. PD-1 inhibits T-cell activation when engaged by its ligands PD-L1 (also known as CD274) or PD-L2 (also known as PDCD1LG2), which are expressed on tumour cells and stromal cells (16). In contrast to CTLA-4, which is primarily believed to regulate immune responses early in T-cell activation, PD-1 is primarily believed to inhibit effector T-cell activity in the effector phase within tissue and tumours (25). Current PD-1 antibodies being approved or under investigation in lymphoma include nivolumab, pembrolizumab (previously known as lambrolizumab), and pidilizumab. Nivolumab and pembrolizumab are fully human monoclonal IgG4-kappa antibodies, while pidilizumab is a humanised IgG1-kappa monoclonal antibody; all target PD-1. In preclinical studies, all of these antibodies have shown activity against a variety of solids tumour, but also lymphoid malignancies (26–29).

Interrupting the inhibitory stimuli of malignant cells on activated T-cells with antibodies targeting these checkpoints promotes the immune system and enables an endogenous antitumor immune response (24,28–31). These pre-clinical findings

have translated into significant clinical activity, which have been practise changing especially for patients with advanced melanomas (7,10,11,32) and most recently also for squamous lung cancer (33).

Rationale for checkpoint inhibition in lymphoma

Classic Hodgkin lymphoma (HL) only includes small numbers of malignant Reed–Sternberg cells within an extensive inflammatory and immune-cell infiltrate (34,35). The 9p24.1 amplification is a recurrent genetic abnormality in the nodular sclerosis type of HL. The genes encoding the PD-1 ligands (PDL1 and PDL2) are key targets of chromosome 9p24.1 amplification (34). The 9p24.1 amplicon also includes JAK2, and gene dose–dependent JAK-STAT activity further induces PD-1 ligand transcription (34). These copy-number–dependent mechanisms and less frequent chromosomal rearrangements (36) lead to overexpression of the PD-1 ligands on Reed–Sternberg cells in patients with HL. This supports the hypothesis that HL may have genetically determined vulnerability to PD-1 blockade (37).

Regarding follicular lymphoma (FL), there is some evidence that survival may correlate with gene expression signatures of infiltrating non-malignant immune cells (38). Furthermore, an immune-surveillance pattern (CD8+ T cells) seems to correlate with good prognosis (39), whereas an immune-escape pattern (CD57+ T-cells) correlated with poor prognosis (40). CD20 positive lymphoma cells do not directly express PD1 ligands, but because tumour infiltrating T-cells probably receive suppressive signals through PD-1, targeting the PD-1 axis may reverse the exhausted/arrested T-cell phenotype by activation of tumour reactive T-cells in lymphoma organs and in the periphery, allowing for enhanced trafficking of tumour adjacent T-cells into the tumour to restore their anti-lymphoma activity (41,42). Such possible associations between immune-surveillance patterns and prognosis like in FL have yet not been shown for diffuse large B-cell lymphoma (DLBCL), but there is some evidence that host immunity as measured by the neutrophil/lymphocyte ratio has a prognostic impact (43–47). However, the rationale for testing checkpoint inhibitors in DLBCL is similar to the one as outlined for FL.

Checkpoint inhibition with monoclonal antibodies is a new approach to strengthen T-cell activity against malignant cells. Currently, the main targets include CTLA-4 and PD-1. Both are expressed on the surface of T-cells, but also other cells involved in the immune response and therefore are crucial in maintaining immunologic homeostasis. In some solid tumours, there have been practice changing successes. There is evidence that host-immunity is also relevant for development and

progression of lymphomas, which strengthens the hypothesis that manipulating T-cell dependent response, could be successful in lymphomas.

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Clinical evidence for checkpoint inhibition in lymphomas

Anti-CTLA-4 treatment in NHL

Bashey and colleagues reported on a phase I dose escalation study in which 29 patients were treated with ipilimumab at recurrence after allo-HCT (48). Of these, 14 had Hodgkin lymphoma (HL) and 1 had a mantle cell lymphoma. None of the patients developed graft versus host disease \geq grade 3. One patient with mantle cell lymphoma achieved a partial remission lasting for 2 months (48). In another phase I study, Ansell et al reported on the safety and efficacy in 18 patients with relapsed or refractory B-cell lymphomas (14 FL, 3 DLBCL, one mantle cell lymphoma) (49). All were treated with ipilimumab single agent (3mg/kg once followed by three monthly 1mg/kg doses or four doses of 3mg/kg monthly). There was one partial remission (follicular lymphoma grade 1) lasting for 19 months and one complete remission (diffuse large B-cell lymphoma) lasting for more than 31 months (49).

In summary, these 2 studies provide preliminary evidence for some clinical activity in NHL. Interestingly, there does not seem to be an excess of graft versus host disease with CTLA-4 inhibition after allogeneic stem cell transplantation, which could be an important piece of information for future studies.

Anti-PD1 treatment in NHL

In a first phase I trial in 17 patients with advanced haematological malignancies, pidilizumab as single infusion showed a favourable safety profile and clinical activity (50). Subsequently, two open-label, non-randomised phase II trials of pidilizumab in DLBCL and FL have been conducted.

Armand et al. evaluated efficacy of pidilizumab in DLBCL and primary mediastinal large B-cell lymphoma patients after autologous stem cell transplantation. The antibody was given at 1.5 mg/kg every 42 days for 3 cycles (51). The primary endpoint of this study was the 16-month PFS from first treatment in eligible patients. 72 patients were treated with at least one dose of pidilizumab of which 83% completed all 3 cycles. Treatment started at a median of 2.6 months after autograft. 66 patients were eligible for outcome analyses. The study met its primary endpoint with a 16-month progression free survival (PFS) of 72% (90% CI 60% - 82%). The 16-month overall survival (OS) was 85% (90% CI 74% - 92%). In an intent-to-treat analysis, the 16-month PFS was 68% (90% CI 59% - 77%) and the 16-month OS was 84% (90% CI 77-91). 35 (53%) patients had measurable disease prior to starting pidilizumab treatment and were eligible for response assessment. In this group, 18 (51%) achieved a response (12 complete and 6 partial remission) with median

response duration of 30 weeks (range 6 - 69). However, some cases with measurable disease were PET negative and might not reflect viable disease and the authors point out that the study was not powered to compare between PET subgroups. Concomitant lymphocyte subset analyses revealed that treatment with pidilizumab increased the number of circulating PD-L1 positive activated T-helper cells which were detected for a minimum of 16 weeks, suggesting on-target effects of the agent. Expression of PD-1 on tumour material has not been assessed. 40 of 72 (56%) treated patients had adverse events \geq grade 3, most frequently neutropenia (19%) and thrombocytopenia (8%). Severe adverse events were reported in 32% of patients and 4% of patients had related severe adverse events. The most common adverse event grade 1/2 was fatigue (25%). Of note, there was no relevant autoimmune reaction seen.

In summary, this study provides a first evidence of anti-PD1 maintenance treatment after autologous stem cell transplantation. Prior attempts with rituximab as maintenance for DLBCL in this setting did not show any benefit (52) and also no other treatment has been established for maintenance treatment in DLBCL in this setting. Randomized trials need to be awaited to see whether anti-PD maintenance provides meaningful clinical benefit.

The second single-arm phase II trial assessed the activity of pidilizumab in combination with rituximab in 30 rituximab-sensitive patients with relapsed FL (53). Pidilizumab was administered at a dose of 3 mg/kg every 4 weeks for 4 cycles. Patients could receive additional 8 cycles upon clinical benefit, resulting in a median of 10 cycles (range 1 - 12). Rituximab (375 mg/m²) was given weekly for 4 cycles. The primary endpoint was the objective response rate. 66% of patients showed objective responses, with 52% complete and 14% partial remission. These results compare favourably with previously published response rates after 2nd treatment with rituximab (40% objective responses with 11% complete remissions). The time to response was long with a median of 88 days (range 53 - 392) and 6 patients showed first response after more than 4 months. The median duration of response was 20.2 months (95% CI 13.9 - not reached). The median PFS was 18.8 months (95% CI 14.7 - not reached) and the median PFS for responders has not been reached (median follow-up of 15.4 months). Interestingly, responders had higher levels of PD-L1 expressing T-cells and monocytes in the peripheral blood compared to non-responders. In addition, it was demonstrated that low expression of a T effector cell signature of 41 genes was associated with a lower response rate and inferior PFS. Again, pidilizumab was very well tolerated. No treatment-related or autoimmune

adverse events grade 3/4 were observed. The most common adverse events grade 1/2 were anaemia (47%), fatigue (43%), leukopenia (37%) and thrombocytopenia (27%) (53).

In summary, this study provides first evidence for safety and efficacy of the combination rituximab (a standard component) and anti-PD treatment. The activity observed in this single arm trial is promising and a further step to establish further chemotherapy free treatments for follicular lymphoma.

Lesokhin and colleagues reported on a phase I study investigating nivolumab in 82 patients with several relapsed or refractory haematological malignancies including: 11 (13%) DLBCL, 10 (12%) FL, 10 (12%) T-cell lymphomas (5 peripheral and 5 other), 8 (10%) other B-cell lymphomas, and 2 (2%) primary mediastinal lymphomas. The remaining entities were non-lymphoma malignancies, predominately multiple myeloma (54). Nivolumab was given 3mg/kg every 2 weeks. Most promising responses were observed in DLBCL (1 complete and 3 partial remissions) and FL (1 complete and 3 partial remissions). The safety profile was very similar compared to that known from solid tumours. Based on these findings, phase II studies including DLBCL (CheckMate 139, NCT02038933) and FL (CheckMate 140, NCT02038946) were initiated.

Clinical evidence for CTLA-4 directed treatment in NHL is scarce, however, several trials are going on especially combining ipilimumab with e.g. nivolumab, lenalidomide, or radiotherapy. Most yet available evidence primarily comprises PD-1 inhibition. There have been some promising observations with pidilizumab in FL and aggressive B-cell malignancies; however, no comparative studies have been reported so far. With respect to nivolumab, the most promising first signals were seen in FL and DLBCL leading to respective single arm phase II studies that further investigate nivolumab in the refractory or relapsed setting. No randomized study has been conducted so far and durability of remission is still uncertain.

Anti-PD1 treatment in HL

Ansell and colleagues recently reported on an interim analysis of a phase I trial enrolling 23 patients with relapsed or refractory HL. The dose of nivolumab was escalated from 1mg/kg to finally 3mg/kg every 2 weeks (37). Over 80% had more than 3 or more prior therapies, and 78% had previous treatment with brentuximab and or high-dose chemotherapy with autologous stem cell support. Drug-related adverse events of any grade and of grade 3 occurred in 78% and 22% of patients,

respectively. Most common toxicities included rash any grade (22%), decreased platelet count (17%), and fatigue (13%). All of them were rated lower than grade 3. There were two cases each with diarrhoea and hypothyroidism. Four of 23 (17%) patients achieved complete remission (confirmed on PET), 16 (70%) a partial response, and 3 had stabilized disease (13%) as best response (37).

In parallel, the PD-1 antibody pembrolizumab is also under investigation in relapsed or refractory HL. Treatment consists of single agent pembrolizumab 10 mg/kg administered intravenously every 2 weeks until confirmed tumour progression, excessive toxicity, or completion of 2 years of therapy. Preliminary results from the 15 patients who were evaluable for response to pembrolizumab were recently reported. All patients previously failed brentuximab vedotin. There were no serious adverse events. Most common drug related adverse events were grade 1/2 respiratory events (20%) and thyroid disorders (20%). One patient discontinued study treatment because of grade 2 pneumonitis, and 3 patients ended therapy after progressive disease. Based on investigator assessment, 3 patients (20%) had a complete remission at 12 weeks. Five additional patients (33%) had partial remission as best overall response, for an overall response rate of 53%. Four patients (27%) experienced progressive disease (55%).

The observed remission rates in heavily pre-treated Hodgkin lymphoma patients are impressive and nivolumab is currently under investigation in an international phase II single arm study including patients failing autograft and/or brentuximab. The results seen with pembrolizumab are comparable and a very similar international phase II study has been launched. Anti-PD1 treatment likely becomes a treatment option for relapsed HL in the future. However, still little is known about the durability of response and longer follow-up is required to assess survival. Also, randomized trials have to be awaited.

Biomarkers for checkpoint inhibition

Although checkpoint inhibitors have changed clinical practice in some tumours, still, the majority of patients has no durable benefit from these treatments. Therefore, biomarkers reliably predicting benefit are needed for better patient selection. The yet largest body of evidence regarding this comes from melanoma trials. In the recent 3 arm randomized phase III trial investigating nivolumab, ipilimumab and their combination, the latter showed to be the most active with respect to progression free survival. Patients with PD-L1 positive tumours did not seem to derive this extra benefit of combining nivolumab with ipilimumab over nivolumab alone. This suggests that PD-L1 positivity can enrich the patient population that will likely benefit from anti-PD-1 treatment, however, no interaction or adjusted analysis was conducted to prove this observed subgroup effect and the issues of defining positivity, as further outlined below, still remains (32). Apart from measuring PD-1/PD-L1 expression profiles, determination of immune-related gene expression patterns is another approach. One small study suggested that T-cell specific, antigen presentation related, and IFN γ signalling related genes, may allow for improved selection of patients likely to respond to anti-PD-1 treatment (56). Further validation of this approach using independent cohorts is needed.

Only few data for PD-1 and PD-L1 expression in lymphoma are generated from patients; most are derived from in-vitro studies (57). PD-1 is not commonly expressed on malignant lymphoma cells, but usually more seen on the tumour-infiltrating lymphocytes of various subtypes of lymphoma; particularly the microenvironment of FL and certain types of T-cell lymphoma are enriched with PD-1/PD-L1 expressing cells (42,58–60). Exceptions include chronic lymphocytic leukaemia or small lymphocytic lymphoma where PD-1 expression is seen both on circulating malignant cells and in the tumour microenvironment (58). Lymphomas that over express PD-L1 include: primary mediastinal B-cell lymphoma harbouring a genetic mutation (9p24.1) that causes up regulation of PD-L1 and PD-L2, Epstein-Barr virus positive lymphomas in which the virus induces PD-L1 expression, T-cell histiocyte-rich DLBCL, some cases of activated B-cell DLBCL, and lymphoplasmacytoid lymphoma. PD-L2 expression in lymphoma is less well characterised, but seems to mirror PD-L1 (57).

Apart from the high variability of PD-1/PD-L1 expression in lymphomas, several issues need to be considered when evaluating prognostic or predictive implications: First, there is much heterogeneity in measuring expression of PD-L1 components. Mostly, immunohistochemistry was used with antibodies that have not been validated

in large cohorts or are not yet commercially available (42,61–63). Second, scoring techniques are also subject to great variability and there is no standardized way to define positivity and criteria for the cut-off (58,64). Third, the microenvironment and the lymphoma are in dynamic interaction, therefore, although in-vitro studies can ensure that appropriate malignant cells are being evaluated, they cannot account for the changes in tumour microenvironment induced by the disease or the effect of the host immune system's response against the malignant cells. These, and probably more limitations, have to be taken into account when investigating and interpreting PD-1/PD-L1 expression in lymphoma and surrounding microenvironment.

Reliable and practice informing predictive biomarkers for anti-PD1 treatment have not been established for lymphomas so far.

Chimeric antigen receptor (CAR) T-cell therapy

The principle

Chimeric antigen receptors (CARs) are fusion proteins incorporating antigen-recognition domains and T-cell activation domains. Basically, autologous CAR T-cells are engineered ex vivo and given back to patient as follows: 1. Peripheral blood mononuclear cells (PBMC) are usually harvested by leukapheresis. 2. PBMCs are exposed to a mitogenic stimulus, typically using beads coated with anti-CD3/anti-CD28 monoclonal antibodies. 3. Stimulated PBMCs are then exposed to a viral vector to introduce the CAR into the T-cell and cultured in the presence of cytokine. Because the mitogenic stimulus is T-cell specific, after a few days the cultures typically only contain T-cells and natural killer cells. 4. These cells are expanded for a few days. 5. The expression of CAR is checked usually by flow-cytometry and the product is cryopreserved. The cell product is typically thawed at the bedside and administered intravenously (6). Patients are usually prepared with a lymphocyte depleting chemotherapy to enhance engraftment and anti-tumour efficacy (65,66).

The target

The most established CARs are designed to target the CD19 cell surface antigen. CD19 is a 95 kD transmembrane glycoprotein expressed on the B lineage from the early pro-B to mature B-cell stages and is part of the B-cell surface signal transduction complex. It can therefore be found on a range of B-cell malignancies, including B-cell NHL and chronic lymphocytic leukaemia (CLL). It is not expressed on other haematopoietic populations or non-haematopoietic cells, therefore, at least theoretically, targeting CD19 is supposed to not suppress the bone marrow or cause non-haematological toxicities. However, because it is also expressed on normal B-cells, effective CD19 CAR T-cell therapy likely results in B-cell aplasia with all associated side effects, e.g. hypogammaglobulinaemia.

Structure of CARs

The first generated CAR was reported by Eshhar and colleagues (67). They fused a single chain variable fragment (scFv) derived from an antibody with a CD3 ζ signalling domain. In this way, CARs graft the specificity of a monoclonal antibody onto the dynamic and persisting characteristics of an effector T-cell.

Common elements of all CARs include:

1. A targeting domain, typically an scFv.

2. An extracellular spacer domain, which extends the binding domain away from the T-cell membrane allowing freedom of orientation. This region is usually derived from IgG, CD8a, or CD28 molecules.
3. A transmembrane domain (e.g. from CD28).
4. An intracellular signalling domain, usually the TCR ζ chain

The importance of co-stimulation

To be effective after infusion, CAR T-cells must expand, persist, exhibit enduring antitumor cytotoxicity, withstand and/or counteract an immunosuppressive tumour microenvironment, and overcome targeted tumour antigen escape (68). In designing CAR T cells for cancer immunotherapy, all of these factors must be harmonized to generate the optimal CAR T-cell. First generation CARs triggered T-cells killing malignant B-cells, however, they did not fully activate the T-cells with respect to proliferation and cytokine secretion in response to antigen (69,70). To improve this, second generation CARs have been engineered with compound endodomains, incorporating co-stimulatory molecules such as CD28, 4-1BB and OX40 along with CD3 ζ (CD247) (71–73). T-cells expressing second generation CARs do not only kill CD19-expressing targets at lower effector/target ratios (74), but also show greater cytokine and proliferative responses (75,76). T-cells expressing second generation CARs also mediate more effective regression of acute lymphatic leukaemia in xenograft models (77). Beside this pre-clinical evidence, the importance of co-stimulation has been shown in a clinical trial, which demonstrated enhanced expansion and persistence of T-cells expressing a second generation CAR (78). However, among these second generation CARs there seems to be a difference regarding persistence between CD28 and 4-1BB containing CAR T-cells. While CD28 containing CAR T-cells were detected only up to 4 months (79), those with 4-1BB persisted up to 2 years (80). This indirect finding is also backed by the observation that B-cell aplasia after therapy with the 4-1BB CAR constructs lasts longer than in patients treated with CD28 domain constructs. This difference in CAR T cell kinetics also seems associated with the timing of cytokine release syndrome, which appears to occur earlier with CD28 containing CAR T-cells (79,80). However, these preliminary observations need further validation in future studies. Beyond this, third generation CARs with two co-stimulatory signalling domains have been developed, however, it is yet unclear whether they lead to increased clinical activity.

Clinical evidence

Clinical studies on CAR T-cells have so far mostly focused on patients with CLL or acute lymphocytic leukaemia (80–82). Regarding other B-cell malignancies, the evidence for efficacy and safety is still limited to several very small single centre series. Selected studies are summarized in **TABLE 2**. Recently, Kochenderfer and colleagues reported on a series of 15 heavily pre-treated patients with chemotherapy refractory NHL including 9 patients with DLBCL and 6 patients with indolent NHL (83). Treatment consisted of a preparation course of chemotherapy, followed by a single infusion of anti-CD19 CAR T-cells one day later. Chemotherapy included cyclophosphamide at a total dose of either 120 or 60mg/kg, followed by five daily doses of fludarabine 25 mg/m². It was administered before CAR T-cells to deplete endogenous leukocytes that can inhibit the anti tumour activity of adoptively transferred CAR T-cells. Because of toxicity, the dose of CAR T-cells was reduced from 5 to 1 x 10⁶ cells/kg bodyweight during the study. Overall, 8 patients achieved a complete remission lasting from 6 to 23 months; all but one complete remission was sustained at the time of publication. Of the 7 evaluable patients with DLBCL, 4 obtained complete remission, 2 obtained partial remission, and one had stable disease after infusion of CAR T-cells. All 6 patients with indolent B-cell malignancies obtained either a partial or complete remission (83). One female patient 30 years of age with primary mediastinal B-cell lymphoma died suddenly 16 days after infusion of anti-CD19 CAR T-cells. No cause of death was discovered at autopsy, therefore cardiac arrhythmia was considered as the most likely cause of death. Apart from this treatment related death, all patients experienced transient grade 3 or higher toxicities, which mostly occurred during the first 2 weeks after infusion. Four of 15 patients experienced grade 3 to 4 hypotension. Two patients with severe toxicities were treated with the intravenous IL-6 receptor–blocking antibody tocilizumab, but no real clinical improvement was observed. The most severe toxicities were a variety of neurologic toxicities in 5 patients including confusion and obtundation. Three of these 5 patients even developed aphasia, palsies and myoclonus. The mechanism behind these neurological side effects are unknown, but have been reported previously (79). Kochenderfer and colleagues speculate that the toxicity could be caused by some substance secreted from CAR T-cells. Importantly, all patients recovered completely from their neurologic toxicities (83).

In summary, this study shows an impressive CR rate of more than 50% in heavily pre-treated patients with NHL. The reduction of infused CAR T-cells due to toxicity mirrors the fact that the optimal balance between efficacy and safety still requires further studies to be determined.

One of the largest CLL series was reported by a group from Memorial Sloan-Kettering Cancer Centre. They treated 8 patients with CLL in 2 cohorts (84). The first cohort of 3 patients did not receive any conditioning, and did not show any objective responses. The next cohort received lympho-depleting conditioning with cyclophosphamide. Unfortunately the first patient rapidly developed multi organ failure secondary to a combination of sepsis and tumour lysis syndrome and died within 48 hours. Further 4 patients were treated with cyclophosphamide conditioning and a reduced dose of CARs; 3 out of 4 of the patients showing disease stabilization or lymph node responses (84).

Recently, Schuster and colleagues reported on a phase II study in which 29 patients with relapsed or refractory B-cell malignancies (19 DLBCL, 8 FL, 2 mantle cell lymphoma) received lymphocyte-depleting chemotherapy followed by infusion of CAR (CTL019, Novartis product) (85). The university of Pennsylvania has an exclusive global agreement with Novartis to research, develop, and commercialize CTL019. Twenty patients received CTL019 per protocol dose (12 DLBCL, 7 FL, 1 mantle cell lymphoma). Pre-infusion chemotherapy regimens were EPOCH (N=2), cyclophosphamide (N=9), radiation + cyclophosphamide (N=2), bendamustine (N=6), and cyclophosphamide-fludarabine (N=1). Cytokine release syndrome occurred in 15 patients (13 grade 2, 2 grade 3), neurologic toxicity in 3 patients: transient delirium (1 grade 2, 1 grade 3) and 1 fatal encephalopathy, which accounts for a treatment related mortality of 5%. For 18 patients evaluable for response at 3 months (12 DLBCL, 6 FL), overall response rate is 67% (DLBCL 50%, FL 100%). After a median follow-up of 6 months, progression-free survival for evaluable patients is 59% (DLBCL 37%, FL 100%) (85).

In summary, this relatively large study provides further evidence with respect to efficacy and safety in patients with chemotherapy refractory B-cell malignancies using a product, which is sought to be commercialized in the future. The known cytokine release syndrome occurred in half of the patient with one treatment associated death. These findings again emphasize that CAR T-cell treatment is effective, but definitely requires dedicated and experienced centres to manage patients with these therapies. Apart from logistical challenges, likely the safety profile needs further improvement before CAR T-cell treatment with CTL019 will be available to broader patient populations.

Major adverse events with CAR T-cells

B-cell aplasia is the most common side effect (on-target, but off-tumour effect) both seen in murine models and humans (86). It seems as if the duration of aplasia is

variable depending on the CAR used (6). Persistent B-cell aplasia can result in increased risk of infection; therefore, such patients should be considered for long-term immune globulin replacement. The cytokine release syndrome (CRS) describes a broad range of inflammatory symptoms that can range from mild flu-like symptoms to severe hypotension and multi organ failure. The frequency is not clear, because until recently there has been no standardized definition, but it appears to occur in at least 30% (6). Diagnostic criteria for CRS have now been proposed by Davila and colleagues (fever for ≥ 3 days, maximal elevation of serum cytokines [of 2 cytokines by ≥ 75 -fold, or of a single cytokine by ≥ 250 - fold] and at least one clinical manifestation of cytokine release syndrome: 1. hypotension, requiring intravenous vasopressor therapy, 2. hypoxia ($pO_2 < 90\%$), or 3. neurological disturbance including delirium, obtundation, seizures), but require further prospective validation (79). CRS typically occurs 5 to 21 days after CAR T-cell infusion. Currently available data suggests that the severity is proportional to tumour load (79,80,84), but whether it is associated with increased efficacy is unclear. Treatment of CRS includes corticosteroids, but there is concern to negatively influence T-cell function and proliferation as even short courses may limit the therapeutic efficacy (79,87). Other options include the commercially-available IL-6 receptor antibody tocilizumab (79,88). Whether interruption of the cytokine cascade leads to abrogation of anti-tumour effects remains unclear and, at present, the optimal timing of targeted therapy is not established. Currently, tocilizumab is generally only given in case of established organ dysfunction, because the cytokine storm is critical for supporting maximal T-cell expansion (6).

Future strategies to improve safety include the addition of suicide genes allowing to selectively kill CAR T-cells. Various options exist. A first attempt was to engineer T-cells expressing viral thymidine kinase, which would make them susceptible to thymidine kinase inhibition with ganciclovir (89). However, immune responses against the herpes simplex-derived thymidine kinase have been observed leading to clearance of transduced T-cells (90). More promising approaches include expression of surface proteins that render T-cells susceptible to existing therapeutic agents e.g. rituximab (91) or cetuximab (92), which are now being tested clinically. Another very interesting approach to better understand the behaviour of CAR T-cells includes visualization of their trafficking, proliferation, and retention in the body. Moroz and colleagues have recently reported on a pre-clinical comparative study of different nuclear reporter systems in mice. Human T-cells were transduced with retroviral vectors encoding for human norepinephrine transporter, human sodiumiodide symporter, a human deoxycytidine kinase double mutant, and herpes simplex virus

type 1 thymidine kinase reporter genes. Using corresponding radiolabeled probes T-cells were then visualized in vivo by sequential PET or SPECT imaging. The authors concluded that the hNET/18F-MFBG PET reporter system was most sensitive (93). These pre clinical visualization techniques could improve our understanding of CAR T-cell behaviour and therefore may contribute to further improving safety and efficacy.

Armoured CAR T-cells could be another option to enhance CAR T-cell efficacy. The immunosuppressive tumour or lymphoma microenvironment plays a central role in limiting the activity of CAR T-cells (94). This environment includes endogenous immunosuppressive cells, immunosuppressive soluble ligands, and cytokines such as interleukin-10 (95). Armoured CAR T-cells that secrete the pro-inflammatory interleukin-12 could overcome these inhibitory stimuli. By this, CAR T-cells could be protected from regulatory T-cell mediated inhibition while delivering the IL-12 cytokine within the tumour microenvironment, thus potentially reversing the anergic state of endogenous tumour-infiltrating tumour-targeted T-cells (95).

Clinical evidence for effectiveness of CAR T-cell treatment is still restricted to very small case series from single centres. Results seen in terms of response in pre treated patients are impressive among various kinds of B-cell malignancies. However, the associated cytokine release syndrome is a very serious safety issue. There was one treatment related death in each of the most recently reported prospective studies, which translates in to a treatment related mortality rate of at least 5%. Further research on balancing effectiveness and safety is highly warranted. Because CAR T-cell therapy requires dedicated resources and facilities it remains unclear, whether this approach can really be an option for a larger population of patients with B-cell malignancies.

Expert Commentary

Immune checkpoint inhibitors have clearly improved prognosis in some solid malignancies and there is growing evidence that PD-1 targeted treatment could be very effective and safe in advanced HL. However, important phase II studies are still on-going and up to now there is only little information about the duration of responses and survival. No comparative studies have been conducted so far. Also, it is unclear, whether the PD-1 antibody should be given until progression or whether it can be interrupted after sustained complete remission. Bearing in mind the still limited data on NHL (indolent and aggressive), so far, it seems as if the effects are not as impressive as in HL, especially for aggressive NHL. With the introduction of checkpoint inhibitors, immunotherapy in its whole experiences a renaissance. This also includes treatment with CAR T-cells. Evidence with respect to efficacy and safety is still limited to small patient series at single centres, which is owed to the fact that engineering and production of these CAR T-cells require dedicated facilities and know-how. However, although numbers are small, there are impressive treatment results mostly for leukaemia and also heavily pre treated lymphoma patients, but severe toxicity and treatment related mortality remain a serious concern. These toxicities are mostly caused by the CRS that can lead to e.g. severe fever and hypotension, but also stroke-like symptoms have recently been reported. The optimal CAR design and effector T-cell populations still need to be defined, as does the durability of clinical responses. The biggest barrier to implementation of CAR T-cell therapy is the complexity and prohibitive cost of generating patient-specific cellular therapies. Although localized production is feasible, capacity is limited and even with considerable investment in this area, it will take time to build the infrastructure required to make this therapy available to large numbers of patients (6). However, several companies have programs on investigating and commercializing CAR therapy (96) (in example: NCT01840566 or NCT02348216). Apart from CD-19 directed CARs, there are also studies investigating CD 30 (NCT02259556) or CD 33 (NCT01864902) directed CAR T-cells.

Five year view

In 5 years, we think that commercially available PD-1 antibodies will be standard treatment in pre-treated HL. However, they will not be the final answer to sustain remission, but just another option bridging to either autologous or allogeneic stem cell transplantation or as a treatment option afterwards. Large trials will be ongoing as well to investigate whether addition of anti PD-1 antibodies to first or second-line chemotherapy improves outcome, especially in high-risk patients. Chemotherapy likely leads to an increased presentation of neo-antigens may potentiate the effect of PD-1 inhibition. However, proven survival benefit from randomized trials would still not be available in 5 years. Regarding FL, anti PD-1 antibodies will in particular be combined with CD-20 targeting antibodies or lenalidomide for first-line and maintenance treatment; probably, a large proportion of FL patients will not require any classical chemotherapy anymore. In the relapse or refractory setting we might see survival benefits. Regarding DLCL, PD-1 inhibition with single agents will not be of a large value; however, there may be a role in combination with chemotherapy or lenalidomide. Besides PD-1 targeting, other T-cell activating agents will be under investigation or available e.g. urelumab, which is a monoclonal antibody targeting CD 137 and enhancing T-cell activity. Urelumab is currently under investigation in several studies in combination with rituximab for B-cell lymphoma (NCT01775631) and CLL (NCT02420938) or in combination with nivolumab for solid tumours and B-cell lymphoma (NCT02253992).

Regarding CAR T-cells, treatment with this approach will still be limited to a small number of dedicated centres, although private companies will increasingly spend efforts to further develop this treatment approach. Controlling the severe side effects will still be an ongoing issue, but with the successful introduction of suicide genes, they will be better manageable. Regarding efficacy, armoured CAR T-cells are under clinical investigation, but there will also be further trials investigating the combination of checkpoint inhibition and CAR T-cells. However, in summary, we speculate that in 5 years, data from randomized trials on CAR T-cells will still not have the maturity to show survival benefits.

Key issues

- Checkpoint inhibition with PD-1 or CTLA-4 antibodies has been practice changing in the treatment of some metastatic solid tumours.
- Hodgkin and non-Hodgkin lymphomas arise from cells of the immune system itself; however, the interplay with the host immune system is still crucial in their development and progression.
- All data on checkpoint inhibition in lymphoma are still preliminary mostly based on early phase I/II trials
- In Hodgkin-lymphoma, PD-1 antibodies such as nivolumab or pembrolizumab have shown very promising activity with up to 87% response rates in heavily pre treated patients. International phase II trials are currently ongoing to validate these preliminary findings.
- In follicular lymphoma, the combination of pidilizumab and rituximab is very active in relapsed patients leading to remission in about 66% of patients. The combination of CD 20 targeted treatments or lenalidomide with checkpoint inhibitors could be a further chemotherapy free standard in the future.
- In diffuse large B-cell lymphoma, results are not as promising, however, still a response rate of 40% was observed with nivolumab. A larger international phase II study with nivolumab in patients failing after autologous stem cell transplantation has just finished recruitment and results are awaited.
- There is some evidence that pidilizumab could be an interesting option as maintenance treatment after autologous stem cell transplantation in relapsed diffuse large B-cell lymphoma, however this approach is still considered very experimental.
- Side effects with checkpoint inhibitors mostly comprise auto-immune reactions such as rash, hepatitis, fever, diarrhoea, fatigue, and pneumonitis. So far, the safety profile of anti PD1 treatment in lymphoma is not different to that observed in solid tumours.
- With the renaissance of immunotherapies, chimeric antigen receptor (CAR) T-cell therapies have again come to the focus for treatment of haematological malignancies.
- The clinical evidence of CAR T-cell therapies is still limited to small series at dedicated centres because of the resources and know-how required to produce these constructs; however, reported response rates are impressive in heavily pre treated patients with indolent or aggressive lymphomas.
- The main safety issue with CAR T-cell therapy is the cytokine release syndrome, which can be fatal. Therefore, further developments are required to improve safety of this promising approach.
- Several companies try to commercialize this CAR T-cell treatment; however, it needs to be awaited whether these efforts translate into broader availability for larger patient populations.

Financial and competing interests disclosure

I Chau is on the advisory board for Bristol-Myers Squibb. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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TABLE 1: Selected studies on PD-1 targeted treatment in Hodgkin and Non-Hodgkin lymphoma

Reference	Year	Design	Entity/Setting	N (eligible for response)	Treatment	Outcomes
Berger	2008	Single arm, phase 1 (first in human)	advanced haematological malignancies	17 (17)	pidilizumab	Maximum tolerated dose not reached; 1 patient with FL showed a CR, 1 HL and 2 CLL showed stable disease
Armand	2013	Single arm, phase 2	diffuse large B-cell lymphoma, relapsed/refractory, post HCT-ASCT	72 (66)	pidilizumab	16 month PFS 72%; 51% overall response in 35 patients with residual disease after autologous stem-cell transplants (32% complete response)
Westin	2014	Single arm, phase 2	follicular lymphoma, relapsed/refractory	32 (29)	pidilizumab + rituximab	66% overall response, 52% complete response, 14% partial response
Lesokhin	2014	Single arm, phase 1	advanced lymphoid malignancies	82	nivolumab	B NHL: 28% overall response, 48% stable disease. DLBCL: 36% of overall response, 27% stable disease. FL: 40% overall response, 60% stable disease. T-cell NHL: 17% overall response, 43% stable disease
Moskowitz	2014	Single arm, phase 1	Hodgkin lymphoma, relapsed/refractory	15	pembrolizumab	66% overall response, 21% complete response
Ansell	2015	Single arm, phase 1	Hodgkin lymphoma, relapsed/refractory	23	nivolumab	87% overall response, 17% complete response

TABLE 2: Selected published studies reporting on chimeric antigen receptor (CAR) T-cell treatments in lymphomas

Reference	Year	Design	Entity/Setting	N	CAR design	Adjunctive therapy	Adverse events	Outcomes
Jensen	2010	single centre	NHL	2	1st generation	Fludarabine, IL-2	Grade 3-4 lymphopenia	none reported
Kochenderfer	2010	single centre	indolent NHL	7	2nd generation, CD 28 domain	Cyclophosphamide, fludarabine, IL-2	Grade \geq 3: B cell aplasia, CRS, diarrhea, fatigue, 1 treatment related death	1 CR, 6 PR
Savoldo	2011	single centre	3 DLBCL, 2 FL, 1 SLL	6	1st and 2nd generation, CD 28 domain	none	none reported	2 SD
Brentjens	2011	single centre	8 CLL, 2 ALL	10	2nd generation, CD 28 domain	Cyclophosphamide or none	Grade \geq 3: B cell aplasia, neutropenic sepsis, hypotension, 1 treatment related death	1 PR, 2 SD, 1 durable B cell aplasia
Porter	2011	single centre	3 CLL	3	2nd generation, 4-1BB domain	Pentostatin or Bendamustine +/- cyclophosphamide	Grade \geq 3: B cell aplasia, CRS	2 CR, 1 PR
Kochenderfer	2013	single centre	4 CLL, 6 NHL (all post allo-SCT)	10	2nd generation, CD 28 domain	none	Grade \geq 3: hypotensions, delirium, headache, CRS, tumour lysis syndrome	1 PR, 1 CR, 6 SD
Kochenderfer	2014	single centre	9 DLBCL, 2 indolent NHL, 4 CLL	15	2nd generation, CD 28 domain	Cyclophosphamide, fludarabine	Grade \geq 3: fevers, hypotensions, delirium; 1 treatment related death	8 CR, 4 PR, 1 SD