

Attività di Ricerca Agosto-Novembre 2022

Martina di Trani

### Identificazione di biomarcatori di risposta all'immunoterapia nel Linfoma Diffuso a Grandi Cellule B attraverso l'analisi personalizzata del DNA tumorale circolante

I pazienti di nuova diagnosi di Linfoma Diffuso a grandi Cellule B (DLBCL) sono curabili nel 50% dei casi, con la chemio-immunoterapia di prima linea (R-CHOP); il restante 50% dei pazienti sono refrattari o recidivano (R/R) entro 12-24 mesi dalla fine della terapia di prima linea e rispondono poco alle terapie di salvataggio convenzionali, incluso il trapianto autologo di cellule staminali.

Recentemente sono state studiate nuove forme di immunoterapia che impiegano anticorpi bi-specifici in grado di legare contemporaneamente le cellule tumorali e le cellule T stimolando la loro azione citotossica. Ipotizziamo che i biomarcatori di risposta e di resistenza all'immunoterapia siano legati in buona parte al tumore (es. alterazioni genetiche e/o profili mutazionali paziente specifico) e che attraverso l'analisi del DNA tumorale circolante (ctDNA) si possa in modo efficace caratterizzare il profilo molecolare specifico di ogni paziente e si possa identificare marcatori in grado di predire la risposta o la resistenza all'immunoterapia. L'analisi del ctDNA viene eseguita attraverso un approccio di sequenziamento ultrasensibile e personalizzato, Cancer Personalized Profiling by deep Sequencing (CAPP-seq), di un target di geni ricorrentemente alterati nelle patologie a cellule B.

Nell'ambito di questo progetto, abbiamo come prima fase del lavoro aggiornato il pannello di geni originale (320Kb), implementando geni legati all'interazione tra tumore e sistema immunitario (IL4R, CTSS, CD37), geni oncosoppressori (RB1, PTEN), geni regolatori dell'espressione genica (DNMT3A, IRF2BP2), geni codificanti antigeni di superficie utilizzati per il binding di anticorpi bi-specifico (CD19), per un totale di 154 geni (372kb). Il pannello è stato realizzato attraverso il portale Hyper Design Tool (V 6.0.0.180) costruito sul genoma di riferimento hg19 (NCBI Build 37.1/GRCh37). Il target di geni è stato disegnato al fine di identificare le regioni codificanti, siti di splicing e geni affetti dal fenomeno di ipermutazione somatica (ASHM), nei geni ricorrentemente mutati nelle patologie a cellule B. Una volta realizzato il nuovo pannello è stato necessario testarlo, al fine di verificare che le regioni d'interesse fossero catturate e sequenziate secondo l'atteso. Per questo motivo sono stati individuati 11 campioni di Linfoma di Hodgkin precedentemente sequenziati con il pannello originale. Il sequenziamento col pannello arricchito ha ottenuto risultati sovrapponibili a quelli del pannello originale. Ad esempio, è stata ottenuta una copertura media dei campioni superiore a 5000X garantendo che più del 80% del target fosse letto >2000X, aspetto necessario per l'analisi somatica del ctDNA. L'analisi somatica ha confermato che più del 95% delle varianti identificate attraverso il primo pannello di geni target, sono state adeguatamente identificate anche con il nuovo pannello. Inoltre vengono identificate varianti aggiuntive nelle regioni d'interesse implementate. Verificata l'adeguatezza del pannello con campioni di Linfoma di Hodgkin, lo abbiamo utilizzato su una selezione di 10 campioni di DLBCL R/R. Per ognuno dei campioni è stato sequenziato il cfDNA e il DNA germinale, ottenendo una profondità media di lettura del target di geni, rispettivamente di 4300X e 4800X. Mediamente è stato utilizzato 1 ml di plasma (range 0,5-1), ottenendo un quantitativo medio di cfDNA di 42 ng/ml (range 6-251). La genotipizzazione dei pazienti con DLBCL mostra la presenza di un valore medio di 16 varianti somatiche non sinonimiche per paziente (range 5-45). Le varianti non sinonimiche identificate sono SNVs e Indels, in particolare varianti Missense, Frameshift, 3' o 5' UTR, Splicing e Start Lost o Stop Gain. I geni ricorrentemente mutati sono BCL2, BCL6, KMT2D, MYC, CREBBP, TNFRSF14. È possibile apprezzare che i geni mutati, identificati attraverso la genotipizzazione del cfDNA, sono tipici della malattia come descritto in letteratura (Rossi et al. Blood 2017). Questi risultati ci permettono di concludere che il pannello di geni è adeguato ai fini del progetto.



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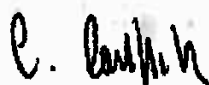
The antibody-drug conjugate loncastuximab tesirine for the treatment of Diffuse Large B-Cell Lymphoma.

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L'ipotesi di lavoro alla base del progetto proposto è che i biomarcatori di risposta e di resistenza all'immunoterapia con anticorpi siano legati in buona parte al tumore (es. alterazioni genetiche e/o profili mutazionali paziente specifico) e che attraverso l'analisi del DNA tumorale circolante (ctDNA) si possa in modo efficace caratterizzare il profilo molecolare specifico di ogni paziente e si possa identificare marcatori in grado di predire la risposta o la resistenza all'immunoterapia.

Sulla base di questa ipotesi di lavoro, gli obiettivi che ci proponiamo di raggiungere sono: (i) identificare attraverso l'analisi del ctDNA il profilo mutazionale dei pazienti con Linfoma Diffuso a Grandi Cellule B (DLBCL), ricaduti o refrattari (R/R), trattati con immunoterapia con anticorpi bispecifici; (ii) quantificare il carico di ctDNA prima e durante l'immunoterapia con anticorpi bispecifici. Useremo come gruppo di controllo il ctDNA di pazienti con DLBCL trattati con un anticorpo anti-CD19 coniugato con un potente agente alchilante. Questo anticorpo, Loncastuximab Tesirine, è stato utilizzato in uno studio clinico multicentrico di fase 2 condotto presso il nostro Istituto ed è stato recentemente approvato da FDA per l'uso clinico in pazienti con R/R DLBCL.

Il lavoro in oggetto descrive l'efficacia clinica e la tossicità del farmaco Loncastuximab Tesirine usato nello studio di fase 2 in pazienti con R/R DLBCL. Sulla base di questi risultati, i campioni di ctDNA prelevati ai pazienti trattati con Loncastuximab Tesirine verranno analizzati per caratterizzare lo stato mutazionale dei singoli pazienti e la clearance della malattia per effetto della terapia. Questi dati verranno comparati con quelli dei pazienti trattati con anticorpo bispecifico.



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# The antibody-drug conjugate loncastuximab tesirine for the treatment of diffuse large B-cell lymphoma

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**Diffuse large B-cell lymphoma (DLBCL) is a heterogenous subtype of non-Hodgkin lymphoma. Relapsed/refractory disease represents remains an unmet medical need, despite the introduction of novel cellular and targeted therapies. Loncastuximab tesirine is a cluster of differentiation 19-targeting antibody-drug conjugate approved by the US Food and Drug Administration for relapsed DLBCL after 2 lines of systemic therapy based on a trial showing a 48.3% overall response rate. The spectrum of its clinical applications is expanding and is now being tested in other B-cell malignancies.**

## Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma (NHL), accounting for 30% to 40% of all newly diagnosed NHL worldwide.<sup>1</sup> About 60% of DLBCL patients will be cured with initial standard chemotherapy (rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone [R-CHOP]). However, 10% to 15% of patients treated with R-CHOP have primary refractory disease and an additional 20% to 25% will have a relapse after an initial response.<sup>2</sup> Salvage therapy including high-dose chemotherapy and autologous stem cell transplantation can be an effective treatment for DLBCL with chemotherapy-sensitive relapse. However, more than one-half of the patients will not have long-term disease control, and a significant proportion of patients are not eligible for aggressive treatment.<sup>3</sup>

Patients who do not achieve a response to second-line treatment or who relapse after high-dose therapy and autologous stem cell transplantation had limited options before the approval of cluster of differentiation 19 (CD19)-specific chimeric antigen receptor (CAR) T-cell therapy, which allows to achieve durable complete response rates of ~30% to 40%.<sup>4-6</sup> However, prognosis for patients who display an unsatisfactory response to CAR T cells or experience disease relapse remains poor.<sup>7</sup> In addition, several novel immunotherapies and targeted therapies have been approved for use in relapsed or refractory (*r/r*) DLBCL, as monotherapy agents or in combination with chemotherapy, with encouraging results.<sup>8-10</sup>

However, most of these studies included few patients with adverse prognostic factors. Further, an effective therapy is still lacking for certain subgroups such as elderly patients, those with biological markers of aggressiveness (double-hit/triple-hit), and

those failing CAR T therapy and/or other novel agents. CD19-targeting antibody-drug conjugates (ADCs) have the potential to improve the clinical outcomes of these patients.

## The CD19 antigen as a therapeutic target

The surface glycoprotein CD19 is a B cell-specific member of the immunoglobulin superfamily expressed during most stages of lymphopoiesis, from immunoglobulin gene rearrangement (pre-B-cell stage) to terminal differentiation into plasma cells.<sup>11</sup> CD19 is crucial for the development and activation of B cells because it is involved in B-cell receptor-dependent and independent signaling.<sup>12-17</sup> Moreover, CD19 expression and interaction with the B-cell receptor is necessary for B-cell differentiation, including early events occurring in the marrow and late events in the spleen and secondary lymphoid tissues.<sup>18-20</sup>

Most B-cell malignancies retain expression of CD19, although its role in their development is still unclear. It has been suggested that CD19 expression promotes the upregulation of MYC, a potent oncogenic protein in aggressive B-cell lymphomas.<sup>21</sup>

Several characteristics make the CD19 antigen a very attractive therapeutic target. First, its expression is specific to B cells and B-cell malignancies, with no expression in hematopoietic stem cells. Second, it is not present as a soluble, circulating isoform, allowing for the drug to be delivered safely to target cells without competitive binding. Third, it has rapid rates of internalization on antibody binding and reexpression.

Autologous CAR T cells and bispecific T-cell engagers represent 2 successful means of targeting the CD19 antigen and have been approved by the US Food and Drug Administration (FDA) for the treatment of different types of NHL and B-cell acute

lymphoblastic leukemia.<sup>4-6,22</sup> Additionally, the combination of lenalidomide and the Fc-modified anti-CD19 monoclonal antibody tafasitamab has been granted accelerated approval by the FDA for the treatment of *r/r* DLBCL after 1 line of therapy.<sup>6</sup> These results suggest that targeting CD19, in particular with combined strategies, can become the cornerstone of targeted therapy for aggressive lymphomas.

### CD19-targeting ADCs with a special focus on loncastuximab tesirine

ADCs are immunoconjugates comprising an engineered monoclonal antibody chemically attached to a cytotoxic drug (the payload) via a stable chemical linker.<sup>23</sup> The result is a molecule capable of precise delivery of a cytotoxic drug to the desired target cell, resulting in an enhanced on-tumor effect, while minimizing off-target activity. Before engagement on the target cell, the conjugated monoclonal antibody circulates in the bloodstream, neither releasing its cytotoxic payload into circulation nor binding to nontarget cells. On antigen binding, the ADC is internalized via receptor-mediated endocytosis, followed by lysosomal degradation of the linker and release of the cytotoxic molecule.<sup>24</sup> Cell death then occurs through direct DNA damage by the payload or through disruption of cellular processes such as tubulin polymerization and polypeptide synthesis.<sup>25</sup> Seven ADCs have been approved for clinical use in various hematological disorders and have paved the way for the development of novel targets as well as the technological upgrade of the structure of the ADC itself.<sup>10,26-32</sup>

Common payloads include auristatin derivatives, calicheamicin, indolinobenzodiazepines, and duocarmycins.<sup>33</sup> More recently, pyrrolobenzodiazepines (PBDs) have been successfully tested in numerous preclinical and clinical studies. PBDs are interstrand minor groove DNA cross-linking agents (Figure 1), which present numerous advantages compared with other cytotoxic payloads.

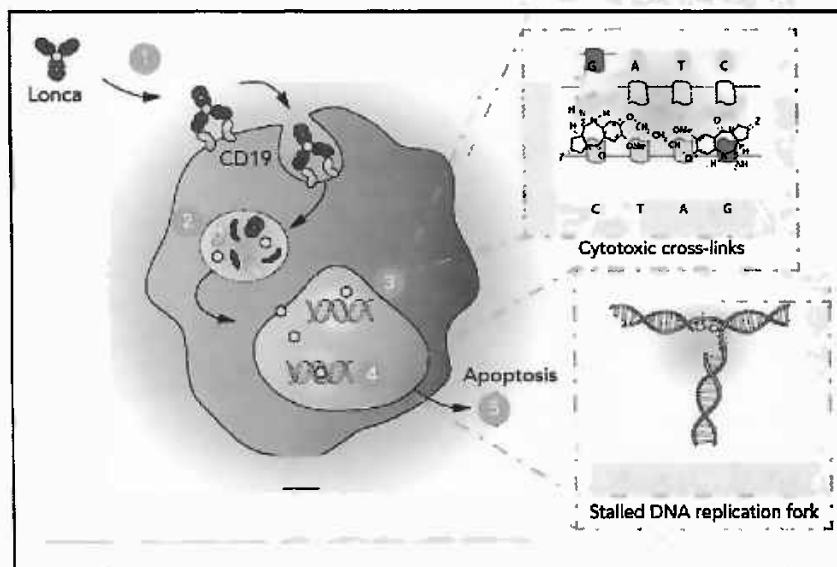
First, PBDs do not distort the structure of DNA and can therefore effectively evade mechanisms of DNA repair.<sup>34</sup> This allows for a prolonged cytotoxic activity to also eradicate slowly replicating tumor cells, which harbor clones that may promote disease resistance/recurrence. Second, PBDs maintain their cytotoxic activity in tumor cell lines expressing multidrug resistant proteins.<sup>35</sup> Last, PBD-carrying ADCs exhibit a shorter half-life compared with other ADCs, thus limiting off-target activity as well as systemic toxicity.<sup>35</sup>

Zammarchi et al were the first to document the therapeutic potential of the anti-CD19 ADC ADCT-402 (loncastuximab tesirine) containing the PBD dimer payload SG3199, in pre-clinical models. In vitro, loncastuximab tesirine had a specific activity for CD19<sup>+</sup> cells, and its killing activity was positively correlated to CD19 surface expression.<sup>36</sup> Interestingly, also CD19<sup>-</sup> cell lines exposed to loncastuximab tesirine exhibited reduced survival, likely because of a bystander effect that may be exploited to target tumor cells with negative or variable CD19 expression.

### Studies of loncastuximab tesirine in relapsed NHL

A first-in-human phase 1 study was designed to include patients with *r/r* NHL.<sup>37,38</sup> Most patients were affected by DLBCL (71.6%) and had received a median of 3 lines of therapy before enrollment. Study participants were exposed to increasing doses (15 µg/kg-200 µg/kg) of loncastuximab tesirine as intravenous injection every 3 weeks. Optimal dosing was set at 150 µg/kg, which was sufficient to obtain significant antitumor activity while avoiding excessive drug accumulation and toxicity. At doses higher than 120 µg/kg, 55% of DLBCL showed an objective response. No significant immunogenicity was observed.

Based on pharmacokinetic data from the phase 1 trial, patients enrolled in the subsequent phase 2 study were administered



**Figure 1. Mechanism of action of PBD-ADCs (loncastuximab tesirine [Lonca]).** (1) Lonca binds to CD19 on the tumor cell surface; (2) following internalization of Lonca, the protease-sensitive linker is cleaved and cytotoxic PBD dimers are released inside the cell; (3) the free PBD dimers bind in the minor groove of the cell DNA and form potent cytotoxic DNA cross-links in a sequence-selective fashion; (4) the cross-links result in a stalled DNA replication fork, blocking cell division; and (5) the cancer cell undergoes apoptosis.

loncastuximab tesirine at a dose of 150 µg/kg for the first 2 cycles, and at a dose of 75 µg/kg thereafter. In terms of efficacy, the phase 2 study confirmed previous encouraging data, and loncastuximab tesirine was recently granted accelerated approval from the FDA for the treatment of r/r DLBCL after 2 or more lines of systemic therapy<sup>39</sup> (Table 1). Approval was based on achieving an objective response rate (ORR) of 48.3%, with a complete response rate of 24.1% and a median duration of response of 10.3 months.<sup>32</sup> Most responses were achieved within the first imaging assessment (after 2 cycles of therapy), with a median time to response of 41 days. The study included patients with r/r DLBCL after 2 or more multiagent systemic treatments, of whom 32% had received at least 3 prior lines of therapy. Subgroup analyses were limited by the small sample; however, similar responses were obtained also in high-risk patients, including double- and triple-hit lymphoma, primary refractory DLBCL, and transformed DLBCL from indolent disease. Further, 13 patients (9%) who relapsed after CAR T-cell therapy were treated with loncastuximab

**Table 1. Summary of phase 1 and 2 studies of loncastuximab tesirine in r/r DLBCL**

	Phase 1	Phase 2
No. of patients	139	145
Median age, y	63	66
Double/triple hit, no.	23 (17%)	15 (10%)
Transformed DLBCL	37 (27%)	29 (20%)
Median number of prior therapies, no. (range)	3 (1-10)	3 (2-4)
Prior auto-SCT, no.	22 (16%)	21 (14%)
Prior CAR T cells, no.	2 (1%)	13 (9%)
ORR	42.3%	48.3%
Complete remission	23.4%	24.1%
Median duration of response, mo	4.5 (not reached for complete responders)	10.3 (13.4 mo for complete responders)
Median PFS, mo	2.8	4.9
Median OS, mo	7.5	9.9
Time to response, d	43	41
Stopped drug from adverse event, no.	35 (19%)	34 (23%)
Grade ≥ 3 neutropenia, no.	71 (40%)	37 (26%)
Grade ≥ 3 pleural effusions, no.	7 (4%)	3 (2%)
Rash, no.	45 (24.6%)	19 (13%), only 1 case grade 3
Grade ≥ 3 elevation of GGT, no.	39 (21.3%)	24 (16%)

OS, overall survival; PFS, progression-free survival; SCT, stem cell transplant.

\*Only the DLBCL cohort was considered for patient characteristics and outcomes; safety analysis refers to all patients included in the study.

tesirine and exhibited an overall response rate of 46%. These findings support a more extended use in relapsed DLBCL and in clinical contexts in which rapid disease control is required.

Because of the paucity of immunoconjugates currently in use, there are no data regarding mechanisms of resistance to ADCs causing clinical failure. CD19 antigen loss is an important mechanism of resistance to anti-CD19 CAR T-cell therapy, occurring in up to 30% of cases.<sup>40</sup> Interestingly, available data on a small cohort of patients exposed to loncastuximab tesirine before anti-CD19 CAR T-cell therapy suggest that antigen loss may not be as common.<sup>41</sup>

### Toxicity profile

Data from safety analysis showed that all patients experienced treatment emergent adverse events, which mainly included hematologic toxicity, fatigue, nausea, and rash. Febrile neutropenia was uncommon (grade ≥ 3 = 3%). Most common grade ≥ 3 adverse events included neutropenia (26%), thrombocytopenia (18%), and elevation of γ-glutamyl transpeptidase (GGT) (16%) in the absence of other signs of liver toxicity. Grade ≥ 3 hematological and liver toxicity can be managed by holding the drug until resolution and subsequent dose reductions by 50% if a prolonged toxicity occurs (>3 weeks). Contrary to other ADCs, peripheral neuropathy was not a common adverse event. Fluid retention, including pleural effusions (all-grade = 8%, grade ≥ 3 = 2%), peripheral edema (all grade = 19%, grade ≥ 3 = 1%), and pericardial effusion (n = 1) were observed in the phase 1 trial,<sup>37</sup> and were effectively reduced by including premedication with oral dexamethasone (4 mg twice a day, from day -1 to +1) and spironolactone diuretics. Presumably, they are related to the PBD payload, although the pathogenetic mechanisms are still unclear and may be associated with direct vascular toxicity.<sup>42-45</sup> Rash was also a common adverse event and occurred mainly in sun-exposed areas (12%, only 1 grade 3 case), prompting physicians to recommend avoidance of prolonged sun exposure. Of note, no cases of tumor lysis syndrome or tumor flare occurred.

The drug was discontinued because of treatment-emergent adverse effects in 23% of patients, whereas dose delays were generally short-lived (<1 week).<sup>32</sup> Most discontinuations were due to grade ≥ 3 elevation of GGT (10%). The rate of discontinuation, albeit not negligible, is comparable to that of other novel therapies, including tafasitamab and lenalidomide (22%)<sup>8</sup> and polatuzumab vedotin combined with chemoimmunotherapy (21%),<sup>46</sup> although considerably higher than bispecific antibodies (0%-4%).<sup>47-49</sup> Most importantly, especially when considering the demographics of DLBCL, the Loncastuximab Tesirine in Relapsed or Refractory Diffuse Large B-cell Lymphoma-2 trial did not reveal toxicity concerns for patients aged ≥ 65 years, including 14% of patients older than 75 years.<sup>32</sup>

### Clinical scenarios for future applications of loncastuximab tesirine

Loncastuximab tesirine is the most recent addition to the ADC armamentarium for NHL therapies. Its applications are potentially broad, and is, therefore, currently being tested in other clinical settings as well as in other subtypes of NHL (NCT03684694, NCT04998669, NCT04384484; www.clinicaltrials.gov). Whereas the anti-CD79b ADC polatuzumab

vedotin is a strong candidate for the treatment of transplant-ineligible *r/r* DLBCL in combination with chemoimmunotherapy, loncastuximab tesirine may address additional unmet clinical scenarios.

Among its attractive features, it displays a rapid action, and, in contrast to other novel immunotherapies and cellular therapies, does not appear to cause tumor flare or tumor lysis syndrome, allowing for a safe outpatient administration. Thus, it may be used sequentially or in combination with either lower doses or reduced cycles of chemotherapeutic agents, in elderly and/or unfit patients, to maximize treatment effectiveness. Further, a sequential strategy of ADC debulking therapy followed by chemotherapy may be appropriate for patients presenting with rapidly progressing disease.

DLBCL is a heterogeneous disease in terms of clinical features, morphology, immunohistochemistry, and genetic defects. High-grade B-cell lymphoma with rearrangements of *MYC* and *BCL2* and/or *BCL6* (HGBCL-DH/TH) is clinically aggressive, with poor prognosis.<sup>50</sup> Likewise, double and triple expressor DLBCL, as well as DLBCL transformed from indolent lymphoma or harboring a TP53 mutation/deletion are associated with poor outcomes.<sup>51-53</sup> In such subgroups, standard therapy has inferior efficacy, and it is urgent to identify effective, well-tolerated treatment alternatives. In this context, loncastuximab tesirine may be a promising addition to standard regimens; indeed, a clinical trial investigating the effects of R-CHOP and loncastuximab will soon be open to enrollment (NCT04974996; [www.clinicaltrials.gov](http://www.clinicaltrials.gov)).

Patients who progress on or relapse after anti-CD19 CAR T-cell therapy are left with no validated treatment alternatives and often exhibit a rapidly progressing disease requiring timely treatment. The efficacy of loncastuximab tesirine in this scenario has been validated in a small cohort of patients within the Loncastuximab Tesirine in Relapsed or Refractory Diffuse Large B-cell Lymphoma-2 trial.<sup>54</sup> Patients who have CD19 persistence should still be susceptible to the direct cytotoxic effect of loncastuximab, which may also overcome immune exhaustion and possibly microenvironmental signals leading to CAR T-cell failure.<sup>40</sup> Moreover, whereas changes in antigen density are known to affect CAR-T cell activity,<sup>40</sup> the bystander effect observed with loncastuximab suggests this agent is much less dependent on high CD19 surface expression and may be effective also in cases with low/absent CD19 expression. Last, we can speculate that the CD19 antigen-binding domain FMC63, the target of chimeric T cells, is distinct from that of loncastuximab tesirine, B4. Thus, ADC and CAR T-cell activities should not be mutually exclusive. A trial specifically designed to include patients failing CAR T cells will be shortly open to accrual and will also address the issue of feasibility for patients recovering from CAR T-cell toxicities, such as peripheral cytopenias. Encouragingly, recent data have shown that the proportion of patients failing CAR T and being able to receive subsequent therapy is growing.<sup>55</sup>

Likewise, loncastuximab tesirine may be given as bridging therapy to CAR T cells. Despite being active toward CD19<sup>+</sup> malignant lymphoid cells, treatment with loncastuximab tesirine typically does not cause loss of CD19 expression and therefore malignant cells should remain susceptible to effective CAR T cell-mediated antitumor activity. Indeed, a small case series of patients with DLBCL previously treated with loncastuximab tesirine reports a high probability to respond to CD19-directed CAR T-cell therapy (ORR 50%).<sup>41</sup> However, as larger scale clinical data are lacking, an upcoming phase 2 clinical trial will address the appropriateness of its use in this scenario.

## Summary

CD19-targeting ADCs represent a novel class of immunotherapy, which is highly effective, exhibits a rapid action, and can be safely administered as monotherapy in highly pretreated patients. In the future, they can be incorporated earlier in treatment course, both as monotherapy and in combination regimens, especially for patients who cannot tolerate standard therapy or require rapid debulking. Further, they may become a treatment option for certain subtypes of NHL with unfavorable biological characteristics and for patients failing cellular therapy and novel drugs.

As the armamentarium of drugs that exhibit significant activity against *r/r* DLBCL expands, the optimal choice of therapy in such complex and heterogeneous disease is still a challenge. Numerous ongoing clinical trials will soon shed light on the therapeutic potential of CD19-targeting ADCs, as well as their optimal modality of use in the context of standard and novel treatment alternatives.

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## Authorship

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## Footnote

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## REFERENCES

- Al-Hamadani M, Habermann TM, Cerhan JR, Macon WR, Maurer MJ, Go RS. Non-Hodgkin lymphoma subtype distribution, geodemographic patterns, and survival in the US: a longitudinal analysis of the National Cancer Data Base from 1998 to 2011. *Am J Hematol*. 2015;90(9):790-795.
- Maurer MJ, Ghesquières H, Jais JP, et al. Event-free survival at 24 months is a robust end point for disease-related outcome in diffuse large B-cell lymphoma treated with immunochemotherapy. *J Clin Oncol*. 2014; 32(10):1066-1073.
- Gisselbrecht C, Glass B, Mounier N, et al. Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. *J Clin Oncol*. 2010;28(27):4184-4190.
- Abramson JS, Palomba ML, Gordon LI, et al. Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. *Lancet*. 2020; 396(10254):839-852.
- Schuster SJ, Bishop MR, Tam CS, et al; JULIET Investigators. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. *N Engl J Med*. 2019;380(1):45-55.
- Locke FL, Ghobadi A, Jacobson CA, et al. Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1-2 trial. *Lancet Oncol*. 2019;20(1):31-42.
- Chong EA, Ruella M, Schuster SJ; Lymphoma Program Investigators at the University of Pennsylvania. Five-year outcomes for refractory B-cell lymphomas with CAR T-cell therapy. *N Engl J Med*. 2021;384(7):673-674.
- Salles G, Duell J, González Barca E, et al. Tafasitamab plus lenalidomide in relapsed or refractory diffuse large B-cell lymphoma (L-MIND): a multicentre, prospective, single-arm, phase 2 study. *Lancet Oncol*. 2020;21(7):978-988.
- Kalakonda N, Maerevoet M, Cavallo F, et al. Selinexor in patients with relapsed or refractory diffuse large B-cell lymphoma (SADAL): a single-arm, multinational, multicentre, open-label, phase 2 trial. *Lancet Haematol*. 2020;7(7):e511-e522.
- Tilly H, Morschhauser F, Bartlett NL, et al. Polatuzumab vedotin in combination with immunochemotherapy in patients with previously untreated diffuse large B-cell lymphoma: an open-label, non-randomised, phase 1b-2 study. *Lancet Oncol*. 2019;20(7): 998-1010.
- Nadler LM, Anderson KC, Marti G, et al. B4, a human B lymphocyte-associated antigen expressed on normal, mitogen-activated, and malignant B lymphocytes. *J Immunol*. 1983;131(1):244-250.
- Bradbury LE, Goldmacher VS, Tedder TF. The CD19 signal transduction complex of B lymphocytes. Deletion of the CD19 cytoplasmic domain alters signal transduction but not complex formation with TAPA-1 and Leu 13. *J Immunol*. 1993;151(6): 2915-2927.
- Bradbury LE, Kansas GS, Levy S, Evans RL, Tedder TF. The CD19/CD21 signal transducing complex of human B lymphocytes includes the target of antiproliferative antibody-1 and Leu-13 molecules. *J Immunol*. 1992;149(9):2841-2850.
- Susa KJ, Rawson S, Kruse AC, Blacklow SC. Cryo-EM structure of the B cell co-receptor CD19 bound to the tetraspanin CD81. *Science*. 2021;371(6526):300-305.
- Wang Y, Brooks SR, Li X, Anzelon AN, Rickert RC, Carter RH. The physiologic role of CD19 cytoplasmic tyrosines. *Immunity*. 2002;17(4):501-514.
- Deaglio S, Vaisitti T, Billington R, et al. CD38/CD19: a lipid raft-dependent signaling complex in human B cells. *Blood*. 2007;109(12):5390-5398.
- Cherukuri A, Cheng PC, Sohn HW, Pierce SK. The CD19/CD21 complex functions to prolong B cell antigen receptor signaling from lipid rafts. *Immunity*. 2001;14(2): 169-179.
- Buhl AM, Nemazee D, Cambier JC, Rickert R, Hertz M. B-cell antigen receptor competence regulates B-lymphocyte selection and survival. *Immunity*. 2000;17(1): 141-153.
- Shivtiel S, Leider N, Sadeh O, Kraiem Z, Melamed D. Impaired light chain allelic exclusion and lack of positive selection in immature B cells expressing incompetent receptor deficient of CD19. *J Immunol*. 2002;168(11):5596-5604.
- Engel P, Zhou LJ, Ord DC, Sato S, Koller B, Tedder TF. Abnormal B lymphocyte development, activation, and differentiation in mice that lack or overexpress the CD19 signal transduction molecule. *Immunity*. 1995;3(1):39-50.
- Chung EY, Psathas JN, Yu D, Li Y, Weiss MJ, Thomas-Tikhonenko A. CD19 is a major B cell receptor-independent activator of MYC-driven B-lymphomagenesis. *J Clin Invest*. 2012;122(6):2257-2266.
- Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N Engl J Med*. 2018;378(5):439-448.
- Chau CH, Steeg PS, Figg WD. Antibody-drug conjugates for cancer. *Lancet*. 2019; 394(10200):793-804.
- Chalouni C, Doll S. Fate of antibody-drug conjugates in cancer cells. *J Exp Clin Cancer Res*. 2018;37(1):20.
- Frigerio M, Kyle AF. The chemical design and synthesis of linkers used in antibody drug conjugates. *Curr Top Med Chem*. 2017;17(32):3393-3424.
- Dean AQ, Luo S, Twomey JD, Zhang B. Targeting cancer with antibody-drug conjugates: promises and challenges [published correction appears in *MAbs*. 2021;13(1):1966993]. *MAbs*. 2021;13(1): 1951427.
- Younes A, Bartlett NL, Leonard JP, et al. Brentuximab vedotin (SGN-35) for relapsed CD30-positive lymphomas. *N Engl J Med*. 2010;363(19):1812-1821.
- Castaing S, Pautas C, Terré C, et al; Acute Leukemia French Association. Effect of gemtuzumab ozogamicin on survival of adult patients with de-novo acute myeloid leukaemia (ALFA-0701): a randomised, open-label, phase 3 study. *Lancet*. 2012;379(9825): 1508-1516.
- Kantarjian HM, DeAngelo DJ, Stelljes M, et al. Inotuzumab ozogamicin versus standard therapy for acute lymphoblastic leukemia. *N Engl J Med*. 2016;375(8): 740-753.
- Kreitman RJ, Dearden C, Zinzani PL, et al. Moxetumomab pasudotox in relapsed/refractory hairy cell leukemia. *Leukemia*. 2018;32(8):1768-1777.
- Lonlal S, Lee HC, Badros A, et al. Belantamab mafodotin for relapsed or refractory multiple myeloma (DREAMM-2): a two-arm, randomised, open-label, phase 2 study. *Lancet Oncol*. 2020;21(2):207-221.
- Caimi PF, Ai W, Alderuccio JP, et al. Loncastuximab tesirine in relapsed or refractory diffuse large B-cell lymphoma (LOTIS-2): a multicentre, open-label, single-arm, phase 2 trial. *Lancet Oncol*. 2021;22(6): 790-800.
- Hafeez U, Parakh S, Gan HK, Scott AM. Antibody-drug conjugates for cancer therapy. *Molecules*. 2020;25(20):E4764.

34. Gerratana B. Biosynthesis, synthesis, and biological activities of pyrrolbenzodiazepines. *Med Res Rev.* 2012;32(2):254-293.
35. Hartley JA, Flynn MJ, Bingham JP, et al. Pre-clinical pharmacology and mechanism of action of SG3199, the pyrrolbenzodiazepine (PBD) dimer warhead component of antibody-drug conjugate (ADC) payload tesirine. *Sci Rep.* 2018;8(1):10479.
36. Zammarchi F, Corbett S, Adams L, et al. ADCT-402, a PBD dimer-containing antibody drug conjugate targeting CD19-expressing malignancies. *Blood.* 2018;131(10):1094-1105.
37. Kahl BS, Hamadani M, Radford J, et al. A phase I study of ADCT-402 (loncastuximab tesirine), a novel pyrrolbenzodiazepine-based antibody-drug conjugate, in relapsed/refractory B-cell non-Hodgkin lymphoma. *Clin Cancer Res.* 2019;25(23):6986-6994.
38. Hamadani M, Radford J, Carlo-Stella C, et al. Final results of a phase 1 study of loncastuximab tesirine in relapsed/refractory B-cell non-Hodgkin lymphoma. *Blood.* 2021; 137(19):2634-2645.
39. US Food and Drug Administration. FDA grants accelerated approval to loncastuximab tesirine-lpyl for large B-cell lymphoma. Available at: <https://www.fda.gov/drugs/resources-information-approved-drugs/fda-grants-accelerated-approval-loncastuximab-tesirine-lpyl-large-b-cell-lymphoma>. Accessed 6 June 2022.
40. Shah NN, Fry TJ. Mechanisms of resistance to CAR T cell therapy. *Nat Rev Clin Oncol.* 2019;16(6):372-385.
41. Thapa B, Caimi PF, Ardeshtna KM, et al. CD19 antibody-drug conjugate therapy in DLBCL does not preclude subsequent responses to CD19-directed CAR T-cell therapy [published correction appears in *Blood Adv.* 2020;4(19):4606]. *Blood Adv.* 2020; 4(16):3850-3852.
42. Rudin CM, Pietanza MC, Bauer TM, et al; SCRX16-001 investigators. Rovalpituzumab tesirine, a DLL3-targeted antibody-drug conjugate, in recurrent small-cell lung cancer: a first-in-human, first-in-class, open-label, phase 1 study. *Lancet Oncol.* 2017;18(1):42-51.
43. Puzanov I, Lee W, Chen AP, et al. Phase I pharmacokinetic and pharmacodynamic study of SJG-136, a novel DNA sequence selective minor groove cross-linking agent, in advanced solid tumors. *Clin Cancer Res.* 2011;17(11):3794-3802.
44. Janjigian YY, Lee W, Kris MG, et al. A phase I trial of SJG-136 (NSC#694501) in advanced solid tumors. *Cancer Chemother Pharmacol.* 2010;65(5):833-838.
45. Stein EM, Walter RB, Erba HP, et al. A phase 1 trial of vadastuximab talirine as monotherapy in patients with CD33-positive acute myeloid leukemia. *Blood.* 2018;131(4):387-396.
46. Sehn LH, Herrera AF, Flowers CR, et al. Polatumab vedotin in relapsed or refractory diffuse large B-cell lymphoma. *J Clin Oncol.* 2020;38(2):155-165.
47. Hutchings M, Morschhauser F, Iacoboni G, et al. Glofitamab, a novel, bivalent CD20-targeting T-cell-engaging bispecific antibody, induces durable complete remissions in relapsed or refractory B-cell lymphoma: a phase I trial. *J Clin Oncol.* 2021;39(18):1959-1970.
48. Budde LE, Assouline S, Sehn LH, et al. Single-agent mosunetuzumab shows durable complete responses in patients with relapsed or refractory B-cell lymphomas: phase I dose-escalation study. *J Clin Oncol.* 2022;40(5):481-491.
49. Hutchings M, Mous R, Clausen MR, et al. Dose escalation of subcutaneous epcoritamab in patients with relapsed or refractory B-cell non-Hodgkin lymphoma: an open-label, phase 1/2 study. *Lancet.* 2021; 398(10306):1157-1169.
50. Rosenthal A, Younes A. High grade B-cell lymphoma with rearrangements of MYC and BCL2 and/or BCL6: Double hit and triple hit lymphomas and double expressing lymphoma. *Blood Rev.* 2017;31(2):37-42.
51. Chapuy B, Stewart C, Dunford AJ, et al. Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes [published correction appears in *Nat Med.* 2018;24(8):1292]. *Nat Med.* 2018;24(5):679-690.
52. Hu S, Xu-Monette ZY, Tzankov A, et al. MYC/BCL2 protein coexpression contributes to the inferior survival of activated B-cell subtype of diffuse large B-cell lymphoma and demonstrates high-risk gene expression signatures: a report from The International DLBCL Rituximab-CHOP Consortium Program. *Blood.* 2013;121(20):4021-4031, quiz 4250.
53. Perry AM, Alvarado-Bernal Y, Laurini JA, et al. MYC and BCL2 protein expression predicts survival in patients with diffuse large B-cell lymphoma treated with rituximab. *Br J Haematol.* 2014;165(3):382-391.
54. Caimi PF, Ardeshtna KM, Reid E, et al. The antiCD19 antibody drug immunoconjugate loncastuximab achieves responses in DLBCL relapsing after antiCD19 CAR-T cell therapy. *Clin Lymphoma Myeloma Leuk.* 2022;22(5):e335-e339.
55. Alarcon Tomas A, Fein JA, Fried S, et al. Novel agents may be preferable to chemotherapy for large B-cell lymphoma progressing after CD19-CAR-T: a multicenter observational study. *Blood.* 2021;138 (suppl 1):883-883.

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