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# Chimeric Antigen Receptor-T Cell Therapy in Hematological Malignancies: Clinical Evidence and Challenges

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## Abstract

Chimeric antigen receptor-T (CAR-T) cell therapy has revolutionized the treatment of hematological malignancies, offering remarkable clinical responses in patients with refractory or relapsed disease. However, challenges such as toxicities, T-cell exhaustion, and manufacturing limitations hinder its broader application, necessitating a comprehensive review of current evidence and innovations. This review synthesizes clinical data, engineering advancements, and persistent barriers to optimize CAR-T therapy and guide future research. The review highlights key insights, including the superior efficacy of CAR-T therapy over conventional treatments in B-cell malignancies and multiple myeloma, as evidenced by high response rates (RR) and durable remissions. It explores the critical role of co-stimulatory domains in enhancing CAR-T cell persistence and the promise of dual-targeting strategies to overcome antigen escape. Clinical trials demonstrate manageable safety profiles, though cytokine release syndrome (CRS) and neurotoxicity remain significant concerns. Manufacturing advancements, such as induced pluripotent stem cell (iPSC) derived CAR-T cells, aim to improve scalability and reduce costs. The review also discusses emerging applications in T-cell neoplasms and the potential of allogeneic 'off-the-shelf' products to expand accessibility. Finally, it underscores the importance of combination therapies and personalized approaches to address tumor microenvironment immunosuppression. Future research should focus on optimizing CAR designs to reduce toxicity and enhance long-term efficacy, particularly in solid tumors and autoimmune diseases. Innovations in gene editing, logic-gated systems, and automation hold promises for democratizing CAR-T therapy globally. Collaborative efforts between academia and industry will be essential to overcome current limitations and realize the full potential of this transformative treatment modality.

**Keywords:** Allogeneic chimeric antigen receptor T, Antigen escape, B-cell malignancies, Cytokine release syndrome, Hematological malignancies, Immunotherapy, T-cell exhaustion

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## Introduction

CAR-T cell therapy has emerged as a groundbreaking treatment modality for various hematological malignancies, including B-cell lymphoma, acute lymphoblastic leukemia, and multiple myeloma [1-3]. This innovative approach harnesses the power of the immune system to target and eliminate cancer cells. Despite its remarkable success, CAR-T therapy faces several clinical challenges that need to be addressed to optimize patient outcomes. The application of CAR-T cell therapy in hematological malignancies has garnered significant clinical interest, with evidence demonstrating notable therapeutic responses [4-6]. According to Sterner and Sterner [7], CAR-T cell therapy has achieved remarkable clinical responses in certain subsets of B cell leukemia and lymphoma, highlighting its potential as a transformative treatment modality. However, despite these successes, several limitations hinder its broader efficacy, particularly in hematological malignancies and solid tumors. Challenges such as T cell exhaustion, tumor microenvironment immunosuppression, and antigen heterogeneity are prominent obstacles that need to be addressed to optimize clinical outcomes [7].

Research efforts have focused on understanding and overcoming these barriers. Gumber and Wang [8] emphasize that CAR T cell exhaustion, driven by persistent antigen stimulation and an immunosuppressive microenvironment, reduces therapeutic potency. Strategies to mitigate exhaustion include modifications to CAR receptors and targeting pathways independent of CAR signaling. Similarly, Honikel and Olejniczak [9] discussed the importance of co-stimulatory receptor signaling in enhancing CAR-T cell function, suggesting that modulation of co-stimulatory signals can improve persistence and efficacy. Innovations in CAR-T cell engineering are also aimed at improving cell phenotype and reducing adverse effects. Zhu et al. [10] demonstrated that overexpressing RUNX3 in CAR-T cells maintains a less differentiated state, reduces CRS, and enhances resistance to exhaustion, thereby potentially improving safety and durability of responses. Furthermore, understanding the

timing and process of CAR-T therapy is crucial; Zhang et al. [11] provides a systematic analysis of critical time points in multiple myeloma treatment, which could inform standardized protocols for hematological malignancies.

While most studies focus on hematological cancers, recent reviews extend the scope to other malignancies. Wang et al. [12] and He et al. [13] explore advances in CAR-T therapy for head and neck squamous cell carcinoma and breast cancer, respectively, indicating ongoing efforts to expand CAR-T applications beyond hematological settings. Additionally, the tolerability and efficacy of CAR-T therapy in older adults with hematological malignancies are promising, especially considering the risks associated with traditional treatments like allo-HCT [14]. Notably, Li et al. [15] reported on a phase I clinical study evaluating C-CAR066, a fully human anti-CD20 CAR-T therapy, for patients with relapsed/refractory (R/R) large B-cell lymphoma who had previously failed anti-CD19 CAR-T treatment. The study underscores the potential of targeting alternative antigens such as CD20 to overcome resistance and improve therapeutic outcomes in hematological cancers.

Further advancements in CAR-T technology have focused on enhancing efficacy and addressing tumor escape mechanisms. Liu et al. [16] introduced tandem CAR-T cells targeting both CD19 and CD38, which exhibited potent cytotoxicity against tumor cells expressing either antigen. This dual-targeting strategy aims to mitigate immune escape, a common challenge in CAR-T therapy, by simultaneously attacking multiple tumor-associated antigens. Similarly, Luehle et al. [17] explored cysteine-engineered CAR-T cells designed to counteract antigen escape in B cell lymphoma, highlighting innovative modifications to improve persistence and effectiveness. The safety profile of CAR-T therapies remains a critical consideration. Yuan et al. [18] compared the efficacy and safety of CD19 CAR-T cells combined with either CD22 or CD20. Their findings indicated that CD19/CD22 CAR-T therapy had a higher partial RR and a more favorable safety profile, particularly concerning immune effector cell-associated neurotoxicity syndrome, than the CD20 combination. These insights emphasize the importance of antigen selection and combination strategies to optimize clinical outcomes while minimizing adverse effects.

Manufacturing and technological innovations are also pivotal in advancing CAR-T therapy. Zong and Li [19] discussed how iPSC technology revolutionizes CAR-T production by reducing costs and manufacturing time and enabling the development of allogeneic CAR-T products suitable for multiple patients simultaneously. Such innovations could significantly expand the accessibility and scalability of CAR-T treatments in hematological malignancies.

Despite these promising developments, challenges persist. The review by Sanomachi et al. [20] highlights ongoing efforts to translate CAR-T therapies from hematological to solid tumors, noting the unique obstacles such as impaired antigen presentation and T cell infiltration in solid tumor microenvironments. While their focus is broader, the discussion underscores the need for continued innovation to address tumor heterogeneity and immune evasion in hematological contexts as well. In summary, clinical evidence supports the efficacy of CAR-T cell therapy in hematological malignancies, with ongoing research aimed at overcoming resistance, enhancing safety, and expanding applicability through technological advancements. The integration of multi-antigen targeting, novel engineering techniques, and improved manufacturing processes are central to overcoming current challenges and realizing the full potential of CAR-T therapy in hematological cancers.

## Mechanisms of Action and CAR-T Cell Engineering

The fundamental principle of CAR-T cell therapy lies in its ability to redirect a patient's own T cells to recognize and eliminate cancer cells through synthetic CARs [21, 22]. These engineered receptors combine an extracellular antigen-binding domain, typically derived from monoclonal antibodies, with intracellular T-cell signaling domains. Upon binding to tumor-associated antigens, CARs initiate T-cell activation independent of MHC restriction, bypassing a major immune evasion mechanism employed by malignancies [23, 24]. This design enables CAR-T cells to target surface antigens with high specificity while overcoming the limitations of endogenous T-cell recognition.

The evolution of CAR design has progressed through multiple generations, each improving upon therapeutic efficacy. First-generation CARs incorporated only the CD3ζ signaling domain, demonstrating proof-of-concept but limited persistence *in vivo* [25-27]. Second-generation constructs added co-stimulatory domains (CD28 or 4-1BB), significantly enhancing T-cell proliferation, cytokine production, and persistence [28-30]. Third-generation CARs now combine multiple co-stimulatory signals (e.g., CD28 plus 4-1BB), while fourth generation 'TRUCK' (T cells redirected for universal cytokine-mediated killing) CARs include cytokine secretion capabilities to modify the tumor microenvironment [31-33]. These iterative improvements have dramatically increased the potency and durability of CAR-T cell responses in clinical settings.

Critical to CAR-T cell function is the selection of appropriate target antigens. Ideal targets are tumor-specific surface proteins with homogeneous expression across malignant cells and minimal presence on healthy tissues [34-36]. In hematological malignancies, CD19 has emerged as a near-ideal target for B-cell neoplasms due to its consistent expression and restricted tissue distribution. Other promising targets include B-cell maturation antigen for multiple myeloma and CD22 for B-cell malignancies. Recent engineering efforts have focused on overcoming antigen escape through dual-targeting CARs or logic-gated systems that require recognition of multiple antigens for activation, thereby improving tumor selectivity and reducing off-target effects [37-39].

The manufacturing process of CAR-T cells represents another crucial engineering challenge. Autologous CAR-T production requires leukapheresis, T-cell activation, viral vector-mediated gene transfer (typically using lentiviral or retroviral vectors), *ex vivo* expansion, and quality control testing - a process that can take 2 to 3 weeks [40-42]. Recent advances aim to streamline this through improved activation methods (such as nanoscale artificial antigen-presenting cells), non-viral gene delivery systems (transposons or CRISPR-based approaches), and automated closed-system bioreactors [43]. These innovations seek to reduce manufacturing failures and variability while shortening production timelines for critically ill patients.

Novel engineering strategies address key limitations of current CAR-T therapies. To mitigate toxicity, researchers are developing safety switches (e.g., inducible caspase-9) and tunable CAR systems controlled by small molecules [44, 45]. To combat immunosuppressive microenvironments, armored CARs are being engineered to secrete immunomodulatory cytokines (IL-12, IL-15) or express checkpoint inhibitors [46, 47]. Additionally, approaches to prevent T-cell exhaustion include epigenetic modifications and the incorporation of metabolic regulators. These multifaceted engineering solutions

demonstrate how synthetic biology principles are being harnessed to create more sophisticated cellular therapeutics.

Looking forward, the field is exploring transformative engineering paradigms such as allogeneic 'off-the-shelf' CAR-T products derived from healthy donors or iPSCs [48, 49]. These approaches utilize gene editing technologies (CRISPR/Cas9 or TALENs) to eliminate endogenous TCRs and MHC molecules, reducing graft-versus-host disease risks. Furthermore, the integration of synthetic biology tools enables the creation of smart CAR-T cells capable of sensing multiple inputs, executing logical computations, and responding with precision-controlled outputs [50]. These cutting-edge developments promise to expand the applicability, safety, and efficacy of CAR-T therapy across a broader range of hematologic malignancies.

## Clinical Evidence of CAR-T Cell Therapy

The efficacy of CAR-T cell therapy has been well-documented in clinical trials. For instance, studies have shown that CAR-T cells can lead to significant remission rates in patients with refractory B-cell malignancies, demonstrating a paradigm shift in treatment strategies for these conditions [51]. The introduction of CAR-T therapy has notably improved survival rates, particularly in patients who have exhausted other treatment options [52]. In the context of multiple myeloma, CAR-T cell therapy has shown promising results, with ongoing research focusing on enhancing its efficacy and safety [52]. Recent systematic reviews have highlighted the importance of understanding critical time points in CAR-T therapy for multiple myeloma, which can guide clinical practice and improve treatment regimens [11].

The clinical efficacy of CAR-T cell therapy has been demonstrated across various hematological malignancies, with particularly striking results in refractory B-cell lymphomas and leukemias. Studies have reported complete remission rates of up to 80% in patients with R/R acute lymphoblastic leukemia showcasing the transformative potential of this therapy [51]. These outcomes represent a significant advancement over traditional chemotherapy regimens, which often yield limited responses in heavily pretreated patients. The durability of these responses is further supported by long-term follow-up data, with some patients remaining disease-free for several years post-treatment. In diffuse large B-cell lymphoma, CAR-T therapy has emerged as a lifeline for patients who have failed multiple lines of therapy. Clinical trials such as ZUMA-1 and JULIET have reported objective RR exceeding 50%, with a subset of patients achieving sustained remissions [52]. These findings underscore the ability of CAR-T cells to overcome resistance mechanisms that render conventional therapies ineffective. Notably, the integration of CAR-T therapy into earlier lines of treatment is now being explored, with preliminary data suggesting improved outcomes compared to salvage chemotherapy.

A meta-analysis by Aiman et al. [53], which included three randomized clinical trials (RCTs) (N = 865), evaluated the efficacy and safety of CAR-T cell therapy compared to standard therapy for large B-cell lymphoma. The results highlight significant improvements in response and survival rates with certain CAR-T cell therapies, while also detailing associated adverse effects. Based on updated results from two trials (N = 543), CAR-T cell therapy demonstrated significant effectiveness over standard therapy across several key metrics. The pooled hazard ratio (HR) for progression free survival (PFS) was 0.47 (95% confidence interval (CI) = 0.37 to 0.60, $I^2 = 0$) in favor of CAR-T cell therapy. The pooled HR for overall survival was 0.73 (95% CI = 0.56 to 0.94, $I^2 = 0$). The pooled HR for event free survival (EFS) was 0.4 (95% CI = 0.32 to 0.49, $I^2 = 0$). The pooled relative risk for complete response was 1.88 (95% CI = 1.57 to 2.25, $I^2 = 2\%$). The pooled RR for overall RR (ORR) was 1.69 (95% CI = 1.48 to 1.92, $I^2 = 0\%$). Axicabtagene ciloleucel and lisocabtagene maraleucel were found to be significantly more effective in terms of response and survival rates. Tisagenlecleucel was identified as an outlier. The Belinda RCT on tisagenlecleucel showed similar efficacy to standard care, with HR of EFS at 1.07 (95% CI = 0.82 to 1.4), RR of CR at 1.03 (0.73 to 1.47), and RR of ORR at 1.09 (95% CI = 0.85 to 1.39). The relative risk of ≥ grade 3 any adverse effects across the three RCTs was 1.02 (95% CI = 0.92 to 1.12, $I^2 = 72\%$), indicating similar rates between CAR-T cell and standard therapy. However, certain adverse effects were notably higher with CAR-T cells, neurotoxicity: The RR was 7.35 (95% CI = 0.97 to 55, $I^2 = 64\%$). Neutropenia, the relative risk was 1.41 (95% CI = 1.04 to 1.91, $I^2 = 78\%$). The pooled incidence of ≥ grade CRS was 5% (CI = 0.03 to 0.08) with CAR-T cells, compared to 0% with standard therapy. In summary, the study concludes that while CAR-T cell therapies, specifically axicabtagene ciloleucel and lisocabtagene maraleucel, offer superior response and survival rates for B-cell lymphoma compared to standard care, they are associated with a higher incidence of specific side effects like neurotoxicity, neutropenia, and CSR. Tisagenlecleucel did not show the same level of efficacy and was considered an outlier. Further large-scale trials are recommended to confirm these findings.

A meta-analysis by dos Santos et al. [54] included 15 clinical trials (9 phase I/II, 6 phase III) and 9 real-world studies (RWS), encompassing a total of 5,313 patients. The median follow-up (MFU) was longer for phase III trials (18.0 months) compared to RWS (12.9 months) and comparable to phase I/II trials (17.5 months). The most common disease entity studied was large B-cell lymphoma with 3,770 patients, followed by multiple myeloma (959 patients), mantle cell lymphoma (339 patients), and indolent lymphomas (245 patients). The CAR products included in the study were axi-cel (10 studies), tisa-cel (6 studies), ide-cel (4 studies), liso-cel (4 studies), brexu-cel (3 studies), and cilta-cel (2 studies). Over half of the studies (58%) did not report any measure of non-relapse mortality, indicating a reporting deficit. Reported non-relapse mortality rates showed considerable heterogeneity, ranging from 3.0 to 9.1%. When non-relapse mortality was estimated, patients with multiple myeloma showed a trend towards higher non-relapse mortality (9.4%), followed by mantle cell lymphoma (8.7%), large B-cell lymphoma (6.2%), and indolent lymphomas (4.1%). Non-relapse mortality was lowest with tisa-cel (3.4%) and highest with cilta-cel (14.3%). There was a numerical increase in non-relapse mortality for CD28z- vs 4-1BB harboring CAR products (8.2% vs 6.2%) and for phase III studies compared to phase I/II and RWS (11.9% vs 6.6% vs 6.2%). Out of 441 reported non-relapse deaths, nearly half (47.6%, n = 210) were caused by infections, making them the primary determinant of non-relapse mortality. CAR-T specific adverse events such as CRS, neurotoxicity, or hemophagocytic lymphohistiocytosis accounted for 11.1% of deaths. Secondary malignancies contributed to 6.1% of deaths, and hemorrhages to 3%. The distribution of CAR-specific toxicities was similar across different disease entities. In summary, the meta-analysis highlights that infections are the leading cause of non-relapse mortality after CAR-T therapy across various disease entities and CAR products, underscoring the significant immune deficits induced by this treatment. The study also points out the concerning incidence of secondary malignancies as a cause of death and the need for improved, long-term reporting of non-relapse mortality in future studies.

Multiple myeloma has also seen remarkable progress with CAR-T therapy, particularly targeting the B-cell maturation antigen. The pivotal KarMMa trial demonstrated an ORR of 73% in heavily pretreated

patients, with 33% achieving complete remission [11]. These results highlight the potential of CAR-T cells to address the high unmet need in this incurable disease. Ongoing research is focused on optimizing B-cell maturation antigen targeted therapies, including dual-targeting approaches and combination regimens, to further enhance depth and duration of responses (DOR). The role of co-stimulatory domains in CAR design has been critical to the therapy's success. Second-generation CARs incorporating CD28 or 4-1BB domains have shown improved persistence and anti-tumor activity compared to their first-generation counterparts [9]. For instance, 4-1BB-based CAR-T cells exhibit prolonged *in vivo* persistence, which correlates with durable remissions, while CD28-based constructs demonstrate rapid expansion and potent short-term cytotoxicity. These insights have informed the development of next-generation CARs tailored to specific disease contexts.

Beyond B-cell malignancies, CAR-T therapy is showing promise in T-cell neoplasms, a historically challenging area due to the risk of fratricide and T-cell aplasia. Innovative strategies, such as targeting CD7 or TRBC1, have yielded encouraging early-phase clinical results, with manageable toxicity profiles [55]. These advances expand the applicability of CAR-T therapy to a broader range of hematological cancers, addressing previously untreatable conditions. Real-world evidence continues to corroborate the findings from clinical trials, though with nuanced differences in outcomes. Factors such as bridging therapy, tumor burden, and patient comorbidities influence real-world efficacy and toxicity, emphasizing the need for personalized treatment approaches [56-58]. As CAR-T therapy becomes more widely accessible, ongoing data collection will be essential to refine patient selection and optimize therapeutic protocols.

Moreover, the incorporation of co-stimulatory domains in CAR-T cell design has been pivotal in enhancing their anti-tumor activity and persistence [9]. The use of co-stimulatory signals has been linked to improved response kinetics and reduced toxicity profiles, which are crucial for patient safety and treatment efficacy [9].

## Clinical Studies

CAR-T cell therapy has emerged as a transformative treatment for hematological malignancies, particularly in cases of R/R conditions. Randomized controlled trials have been pivotal in evaluating the efficacy and safety of CAR-T therapies, leading to several Food and Drug Administration (FDA) approvals (Table 1). These trials have primarily focused on hematological cancers such as B-cell lymphomas and acute lymphoblastic leukemia, demonstrating significant improvements in patient outcomes.

Abramson et al. [65] TRANSCEND NHL 001 (NCT02631044; NCT03435796) study evaluated the efficacy and safety of lisocabtagene maraleucel (liso-cel) as a treatment for R/R large B-cell lymphoma, with a 2-year follow-up period. The study included 270 liso-cel-treated patients with a median age of 63 years, ranging from 18 to 86 years. Patients received a median of 3 prior lines of systemic therapy, with a range of 1 to 8 lines. A significant portion, 67% (181 patients), had chemotherapy-refractory large B-cell lymphoma. The MFU period for the study was 19.9 months. Among 257 efficacy-evaluable patients, the objective RR was 73%. The complete RR observed was 53%. The median DOR was 23.1 months (95% CI: 8.6 to not reached), PFS was 6.8 months (95% CI: 3.3 to 12.7), and overall survival was 27.3 months (95% CI: 16.2 to 45.6). The estimated 2-year rates were 49.5% for DOR, 40.6% for progression-free survival, and 50.5% for overall survival. Grade 3 to 4 CSR occurred in 2% of patients during the 90-day treatment-emergent period. Grade 3 to 4 neurological events were observed in 10% of patients within the same 90-day period. Common grade ≥3 adverse events, neutropenia was the most common occurring in 60% during the treatment-emergent period and 7% post-treatment-emergent period. Anemia was also common, reported in 37% during the treatment-emergent period and 6% post-treatment-emergent period. Liso-cel demonstrated a manageable safety profile with no new safety signals identified during the 2-year follow-up. In summary, liso-cel showed significant and durable responses with a manageable safety profile in patients with R/R large B-cell lymphoma over a 2-year follow-up period.

Abramson et al. [61] TRANSFORM (NCT03575351) study, this international phase 3 study evaluated liso-cel against standard second-line treatment in patients with primary refractory or early relapsed (≤12 months) large B-cell lymphoma (Figure 1). Eligible adults (N = 184) who were candidates for autologous stem cell transplantation were randomized 1:1 to receive either liso-cel (100 x $10^6$ CAR-positive T cells) or standard therapy (platinum-based immunochemotherapy followed by high-dose chemotherapy and autologous stem cell transplantation for responders). With a MFU of 17.5 months, liso-cel demonstrated superior efficacy outcomes. The median event-free survival was not reached in the liso-cel arm vs 2.4 months for standard therapy ($p < 0.0001$). Patients receiving liso-cel achieved significantly higher complete RR (74% vs 43%; $p < 0.0001$) and longer median PFS (not reached vs 6.2 months; HR = 0.400; $p < 0.0001$). While median overall survival was not reached in either group, the 18-month adjusted survival rates (accounting for crossover treatment) favored liso-cel (73% vs 54%; HR = 0.415). The safety profile of liso-cel remained favorable, with grade 3 CSR and neurological events occurring in only

**Table 1:** Comparative efficacy of FDA-approved CAR-T therapies for hematological malignancies.

| Product (Target) | Indication | ORR (%) | CR (%) | Median PFS (months) | Grade ≥3 CRS (%) | Grade ≥3 neurotoxicity (%) | Unique features |
|---|---|---|---|---|---|---|---|
| Tisagenlecleucel (CD19) [59] | R/R B-cell ALL | 81 | 60 | 11.1 | 22 | 12 | First FDA-approved CAR-T |
| Axicabtagene ciloleucel (CD19) [60] | R/R LBCL | 83 | 58 | 5.9 | 13 | 28 | CD28 co-stimulatory domain |
| Lisocabtagene maraleucel (CD19) [61] | R/R LBCL | 73 | 53 | 6.8 | 2 | 10 | Defined CD4+/CD8+ ratio |
| Brexucabtagene autoleucel (CD19) [62] | R/R MCL | 93 | 67 | 25.8 | 15 | 31 | High efficacy in mantle cell lymphoma |
| Idecabtagene vicleucel (BCMA) [63] | R/R multiple myeloma | 73 | 33 | 8.8 | 5 | 3 | First BCMA-targeted CAR-T |
| Ciltacabtagene autoleucel (BCMA) [64] | R/R multiple myeloma | 97 | 67 | NR (77% at 12 mo) | 4 | 9 | Dual-epitope BCMA targeting |

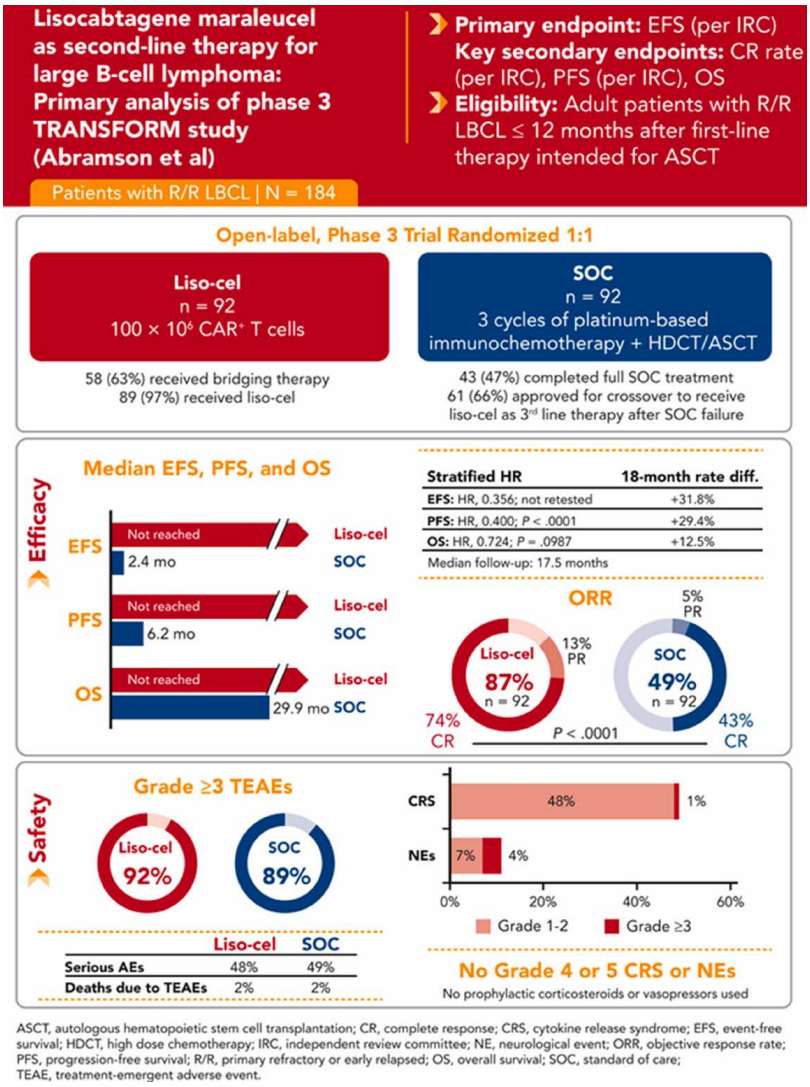


**Figure 1:** Overview of TRANSFORM study [61].

1% and 4% of patients respectively, and no grade 4/5 events reported. These results establish liso-cel as a potentially transformative second-line treatment option for this high-risk patient population, showing substantial improvements in multiple efficacy endpoints compared to conventional therapy.

Berdeja et al. [64] CARTITUDE-1 (NCT03548207) study, investigated ciltacabtagene autoleucel (cilta-cel) in heavily pretreated patients with R/R multiple myeloma, yielded significant results at approximately two years of MFU. The findings highlight the therapy's efficacy and manageable safety profile. This study evaluated cilta-cel in 113 enrolled patients between July 2018 and October 2019, with 97 patients (29 in phase 1b and 68 in phase 2) ultimately receiving the recommended phase 2 dose of 0.75 x $10^6$ CAR-positive viable T cells per kg. At the September 2020 data cutoff, with an MFU of 12.4 months (IQR 10.6 to 15.2), the trial population had received a median of six prior therapies. The therapy demonstrated remarkable efficacy, with an ORR of 97% (95% CI 91.2 to 99.4), including 67% of patients achieving stringent complete response. Responses appeared rapidly (median time to first response: 1 month) and continued to deepen over time. Both median DOR and PFS were not reached, with 77% of patients remaining progression-free in 12 months (95% CI 66.0 to 84.3) and an OSR of 89% (80.2 to 93.5). Hematologic toxicities were prevalent, with grade 3 to 4 events including neutropenia (95%), anemia (68%), leukopenia (61%), thrombocytopenia (60%), and lymphopenia (50%). CRS occurred in 95% of patients (4% grade 3 to 4), typically emerging at a median of 7 days post-infusion and lasting 4 days. Neurotoxicity affected 21% of patients (9% grade 3 to 4). Among 14 reported deaths, 6 were treatment-related (including one fatal CRS with hemophagocytic lymphohistiocytosis), 5 resulted from disease progression, and three were unrelated to treatment. These findings demonstrate cilta-cel's potent activity in heavily pretreated multiple myeloma patients, with high RR and durable remissions, albeit with expected CAR-T cell-associated toxicities that were generally manageable. The results support cilta-cel as a promising therapeutic option for this difficult-to-treat population.

Neelapu et al. [60] ZUMA-1 (NCT02348216) trial is a pivotal study that evaluates the safety and efficacy of axicabtagene ciloleucel (axi-cel), a CD19-directed CAR-T cell therapy, in patients with refractory aggressive non-Hodgkin lymphomas. The median age of enrolled patients was 58 years (range, 23 to 76), with 67% male, 85% having stage

III-IV disease, 47% with IPI 3-4, 77% refractory to at least a second line of therapy, and 21% relapsing within 12 months of autologous stem cell transplant. The primary analysis, based on 92 patients with at least 6 months of follow-up, yielded significant findings. The study met its primary endpoint with an ORR of 82% ($p < 0.0001$) in the primary analysis set (n = 92). In the modified intent-to-treat analysis set of 101 patients, the ORR was also 82%, comprising a 54% complete RR and a 28% partial RR. This consistency was observed across various patient characteristics, including disease subtype, refractory status, stage, and IPI score. The complete RR of 54% was noted to be seven-fold higher compared to historical controls. At a MFU of 8.7 months, 44% of patients were still in response, with 39% in complete response. The median DOR was 8.2 months overall, and it had not been reached for patients who achieved a complete response. Median OS was not reached, and 80% of patients remained alive at 6 months. The most frequently observed grade ≥3 adverse events included neutropenia (66%), leukopenia (44%), anemia (43%), febrile neutropenia (31%), and encephalopathy (21%). Grade ≥3 CRS occurred in 13% of patients, and grade ≥3 neurological events occurred in 28% of patients. All reported CRS and neurological events resolved, with the exception of one grade 1 memory impairment. There were 3 grade 5 adverse events (fatal adverse events) reported. Axi-cel was successfully manufactured for 110 out of 111 enrolled patients (99%), with an average turnaround time from apheresis to clinical site of 17 days. In conclusion, axi-cel demonstrated a significant improvement in objective RR and complete RR in patients with refractory aggressive non-Hodgkin lymphoma, coupled with a manageable safety profile, offering a promising option for patients with limited curative treatments.

Munshi et al. [63] KarMMa trial (NCT03361748) is a pivotal study investigating the efficacy and safety of idecabtagene vicleucel (ide-cel), a B-cell maturation antigen-directed CAR T-cell therapy, in patients with R/R multiple myeloma who have been exposed to three major classes of treatment: immunomodulatory agents, proteasome inhibitors, and anti-CD38 antibodies. In this clinical study involving 140 enrolled patients, 128 received treatment with ide-cel. After a MFU period of 13.3 months, the therapy demonstrated substantial clinical benefit, with 73% of treated patients (94/128) achieving an objective response. Notably, 33% of patients (42/128) attained a complete response or better, indicating deep tumor regression. Among these complete responders, 79% (33/42) achieved minimal residual disease-negative status, defined as fewer than $10^{-5}$ nucleated cells, representing 26% of all treated patients. The median PFS was 8.8 months (95% CI: 5.6 to 11.6), suggesting meaningful disease control in this heavily pretreated population. The safety analysis revealed expected but manageable toxicities characteristic of CAR-T cell therapies. Hematologic adverse events were nearly universal, with neutropenia occurring in 91% of patients (117/128), followed by anemia (70%; 89/128) and thrombocytopenia (63%; 81/128). CRS developed in 84% of cases (107/128), though only 5% (7/128) experienced severe (grade ≥ 3) events. Neurotoxicity was observed in 18% of patients (23/128), with just 3% (4/128) reporting grade 3 events and no higher-grade neurological complications. Cellular persistence data showed detectable CAR-positive T cells in 59% of evaluated patients (29/49) at 6 months post-infusion, declining to 36% (4/11) by 12 months. These findings demonstrate that ide-cel can induce clinically meaningful responses, including deep minimal residual disease-negative remissions, in a majority of heavily pretreated multiple myeloma patients. While the treatment was associated with significant but manageable toxicities, primarily hematologic effects and CRS, the favorable RR and durability of effect support its therapeutic potential for this refractory patient population. The persistence of CAR-T cells in a substantial proportion of patients at 6 months may contribute to the observed clinical benefit, though longer follow-up is needed to fully characterize the durability of responses.

Larson et al. [66] clinical trial (NCT04007029) evaluated autologous naive and memory T (TN/MEM) cells engineered to express a bispecific anti-CD19/CD20 CAR (CART19/20) for patients with R/R non-Hodgkin lymphoma. The primary endpoint of the study was safety. Ten patients were treated in the study. The dosage administered ranged from 36 to $165 \times 10^6$ CART19/20 cells. Neurotoxicity, no patient experienced neurotoxicity of any grade. No patient experienced CRS beyond grade 1. One case of dose-limiting toxicity was observed, which was persistent cytopenia. Nine out of ten patients achieved an objective response, resulting in a 90% objective RR. Seven patients achieved complete remission, indicating a 70% complete remission rate. One patient relapsed after 18 months of complete remission but returned to complete remission after receiving a second dose of CART19/20 cells. With a MFU of 17 months, the median PFS and OS were not reached. In conclusion, the study found that CART19/20 TN/MEM cells are safe and effective for patients with R/R non-Hodgkin lymphoma, demonstrating durable responses even at low dosage levels.

Kersten et al. [67] phase 1 Euplagia-1 (CTIS: 2022-501686-47-00) trial evaluated the feasibility, safety, and efficacy of GLPG5201, a point-of-care manufactured CD19 CAR T-cell therapy, in patients with R/R chronic lymphocytic leukemia and small lymphocytic lymphoma, including those with Richter's transformation. As of April 26, 2023, 12 patients were enrolled in the phase 1 study at either dose level 1 ($35 \times 10^6$ CAR+ T cells, n = 6) or dose level ($100 \times 10^6$ CAR+ T cells, n = 6). All patients had R/R chronic lymphocytic leukemia, with 7 out of 12 also having concurrent Richter's Transformation. The median age was 66 years (range 58 to 71), and 8 of the 12 patients were male. Patients had received a median of 4 prior lines of therapy (range 2 to 10). Most had received a BTK inhibitor (10/12) and venetoclax (9/12), and one patient had undergone an allogeneic stem-cell transplant. Six patients had a TP53 mutation, one had a 17p deletion, 11 had an unmutated IGHV status, and two had a complex karyotype. GLPG5201 was successfully manufactured for all enrolled patients. The median vein-to-vein time (from apheresis to infusion) was 7 days, with a range of 7 to 14 days. This demonstrates the feasibility of rapid treatment delivery. The final product maintained a preserved early memory phenotype for both CD4+ and CD8+ CAR T cells compared to the apheresis starting material. Most treatment-emergent adverse events were grade 1-2. The majority of grade ≥3 events were hematological. Six patients (50%) experienced grade 1-2 CRS, but no grade ≥3 CRS was reported. No neurotoxicity was reported. One patient experienced a dose-limiting toxicity of grade 4 neutropenia at DL2, which was manageable. No unexpected GLPG5201-related toxicities were observed, and no deaths occurred while patients were on study. Eleven out of 12 patients responded to treatment, resulting in a best objective RR of 92%. Nine patients achieved a complete response, leading to a complete RR of 75%. At DL1, ORR was 83% and complete RR was 67%. At DL2, ORR was 100% and complete RR was 83%. All but one patient with Richter's transformation responded (ORR 86%), and 5 out of 7 patients with RT achieved complete response (complete RR 71%). One Richter's Transformation patient was refractory due to CD19-negative disease. At the time of analysis, 9 out of 11 (82%) responding patients had ongoing responses, with duration up to 9 months post-infusion. Two patients progressed after an initial response, one with CD19-negative disease. Robust CAR T-cell expansion was observed in all patients by

qPCR, with a median maximum expansion (Cmax) of 4.4 x $10^5$ copies/µg DNA. Time to Max Expansion (Tmax): Median Tmax was 14 days (range 9 to 20 days). GLPG5201 could be detected in peripheral blood for up to 9 months post-infusion, demonstrating durable persistence. In conclusion, the phase 1 Euplagia-1 study demonstrated that point-of-care manufacturing of GLPG5201 with a short vein-to-vein time is feasible. The therapy showed an encouraging safety profile with no unexpected toxicities and no high-grade CRS or neurotoxicity. Efficacy results were promising, with high overall response and complete RR, including in patients with Richter's Transformation, supported by robust CAR T-cell expansion and persistence.

## Challenges in CAR-T Cell Therapy

Despite its remarkable success, CAR-T cell therapy is associated with significant clinical challenges (Table 2). One of the most pressing issues is the risk of severe toxicities, including CRS and immune effector cell-associated neurotoxicity syndrome [68, 69]. CRS, characterized by high fever, hypotension, and multi-organ dysfunction, occurs due to excessive immune activation following CAR-T cell infusion [70]. Immune effector cell-associated neurotoxicity syndrome, on the other hand, can lead to confusion, seizures, and even coma. Although management strategies, such as tocilizumab and corticosteroids, have improved outcomes, these adverse events remain a major concern, particularly in high-risk patients [7]. Another critical challenge is CAR-T cell exhaustion, which limits long-term therapeutic efficacy [71, 72]. Persistent antigen exposure and an immunosuppressive tumor microenvironment can drive T cells into a dysfunctional state, reducing their ability to sustain anti-tumor activity [73, 74]. Research has identified inhibitory receptors such as PD-1 and TIM-3 as key contributors to exhaustion. Strategies to counteract this phenomenon, including checkpoint blockade and genetic modifications to enhance T cell fitness, are under investigation [8]. Additionally, antigen escape-where tumors downregulate target antigens-poses a major obstacle, necessitating the development of multi-targeted CAR-T approaches.

Manufacturing complexities and high costs further hinder the widespread adoption of CAR-T therapy. The autologous nature of current treatments requires personalized production, leading to lengthy turnaround times and logistical challenges [75]. Moreover, the expense of CAR-T therapy places a significant financial burden on healthcare systems, limiting accessibility for many patients. Efforts to develop 'off-the-shelf' allogeneic CAR-T products, derived from healthy donors or iPSCs, aim to address these limitations by reducing costs and production time [76]. Finally, infections following CAR-T therapy represent a substantial risk, particularly during the period of B-cell aplasia and prolonged cytopenias. Studies report infection rates of 23 to 42%, with bacterial, viral, and fungal pathogens posing serious threats to immunocompromised patients. Prophylactic antimicrobials and close monitoring are essential, but optimizing immune recovery without compromising CAR-T cell efficacy remains an ongoing challenge. Future directions include engineered CAR-T cells with enhanced safety profiles and adjunct therapies to bolster immune reconstitution [77]. Addressing these challenges will be crucial to maximizing the potential of CAR-T therapy in hematological malignancies.

## Future Directions

The future of CAR-T cell therapy in hematological malignancies looks promising, with ongoing research focused on enhancing the safety and efficacy of this treatment [78, 79]. Innovations in CAR design, such as the incorporation of additional co-stimulatory signals and the use of iPSC-derived CAR-T cells, are being explored to improve patient outcomes [19]. Moreover, the potential of CAR-natural killer cell therapy is gaining traction as a safer alternative to CAR-T cells, particularly for patients at high risk of severe adverse effects [55, 80]. The development of standardized manufacturing techniques for CAR-natural killer cells could further streamline the therapeutic process and improve accessibility for patients.

One promising avenue is the development of next-generation CAR designs incorporating novel co-stimulatory domains, safety switches, and logic-gated systems to enhance precision [81, 82]. For instance, 'armored' CAR-T cells engineered to secrete cytokines like IL-15 or IL-18 show improved persistence and resistance to exhaustion in preclinical models [83]. Similarly, inducible suicide switches (e.g., caspase-based systems) offer better control over toxicity, allowing for rapid elimination of CAR-T cells if adverse events occur [84]. These innovations aim to boost efficacy while mitigating safety concerns that currently restrict broader use. Another key focus is the expansion of CAR-T therapy beyond CD19 and B-cell maturation antigen targets [85]. Researchers are exploring alternative antigens such as CD22, CD38, and GPRC5D to address antigen escape and broaden applicability across hematological malignancies. Dual-targeting CARs, which engage two tumor-associated antigens simultaneously, have shown promise in early trials by reducing relapse rates [86]. Additionally, efforts are underway to adapt CAR-T technology for T-cell malignancies, where fratricide and T-cell aplasia pose unique challenges [87]. Targeting markers like CD7 or TRBC1 with fratricide-resistant CAR designs could open new treatment avenues for these aggressive cancers.

**Table 2:** Strategies to overcome key challenges in CAR-T therapy.

| Challenge | Mechanism | Solutions |
|---|---|---|
| CRS | IL-6/IFN-γ hyperproduction | • Tocilizumab prophylaxis<br>• Kinase inhibitors (ruxolitinib)<br>• Anakinra for CRS/neurotoxicity |
| Antigen escape | Target downregulation | • Tandem CARs (CD19/CD22)<br>• Sequential targeting<br>• CAR-natural killer combination |
| T-cell exhaustion | Chronic antigen exposure | • PD-1 knockout<br>• IL-7/IL-15 armoring<br>• Epigenetic modulators |
| Manufacturing barriers | Autologous production delays | • Allogeneic platforms<br>• Point-of-care systems (7-day V2V)<br>• iPSC-derived CAR-T |
| Solid tumor penetration | Physical/immune barriers | • Stroma-degrading enzymes<br>• Hypoxia-resistant CARs<br>• Local delivery (intracavitary) |

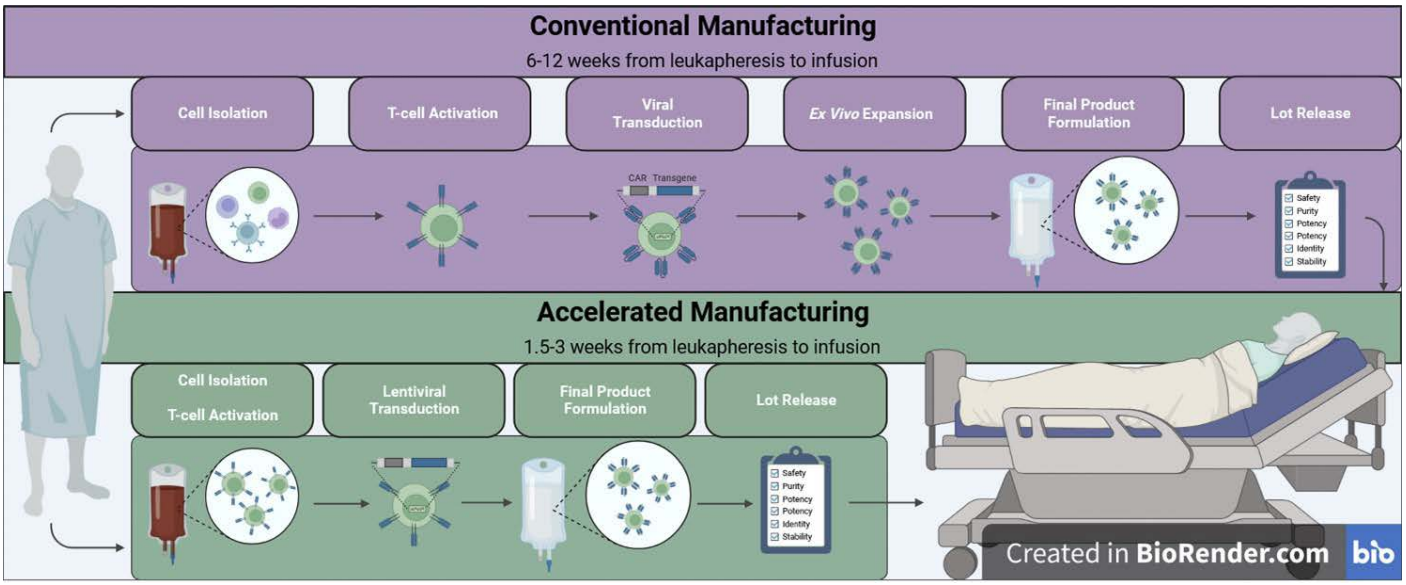


**Figure 2:** Representation of autologous CAR-T cell manufacturing processes [94].

The shift toward allogeneic, off-the-shelf CAR-T products represents a major paradigm shift in the field. Universal CAR-T cells derived from healthy donors or iPSCs could dramatically reduce manufacturing time and costs while improving accessibility [88]. Early clinical trials of allogeneic CAR-T therapies have demonstrated feasibility, though challenges such as graft-versus-host disease and host immune rejection remain. Advances in gene editing (e.g., CRISPR-mediated TCR knockout) and immune cloaking technologies may help overcome these barriers, paving the way for scalable, standardized CAR-T products [89]. Combination therapies are also being explored to enhance CAR-T cell efficacy. Preclinical data suggest that pairing CAR-T cells with immune checkpoint inhibitors, bispecific antibodies, or small-molecule drugs can counteract immunosuppressive tumor microenvironments and prolong responses. For example, PD-1 blockade has been shown to reinvigorate exhausted CAR-T cells in some settings [90]. Similarly, combining CAR-T therapy with targeted agents like BTK inhibitors or IMiDs (in multiple myeloma) may synergize to improve outcomes [91]. These strategies could help address resistance mechanisms and expand the durability of responses.

Beyond hematologic malignancies, efforts are underway to adapt CAR-T technology for solid tumors and autoimmune diseases. While challenges like poor T-cell infiltration and antigen heterogeneity persist, innovations such as local delivery, hypoxia-resistant CAR-T cells, and stromal-targeting approaches are being tested [92]. In parallel, CAR-T cells engineered to target autoimmune drivers (e.g., B cells in lupus or rheumatoid arthritis) are entering early clinical trials, offering potential cures for chronic inflammatory conditions [93]. These applications could redefine the therapeutic landscape in the coming decade. Finally, advancements in manufacturing and automation hold the key to democratizing CAR-T therapy globally (Figure 2) [94]. Closed-system bioreactors, artificial intelligence-driven quality control, and decentralized production facilities could streamline processes and reduce costs [95]. Regulatory agencies are also working to harmonize guidelines, facilitating faster approvals and broader implementation. As these technologies mature, CAR-T therapy may transition from a last-resort option to a frontline treatment, fulfilling its promise as a transformative modality in modern medicine [96]. Continued collaboration between academia, industry, and clinicians will be essential to realize this vision.

In summary, while CAR-T cell therapy has revolutionized the treatment landscape for hematological malignancies, addressing the associated challenges is crucial for maximizing its clinical benefits. Continued research and innovation will be essential in overcoming these hurdles and improving the overall efficacy and safety of CAR-T cell therapy.

## Conclusion

CAR-T cell therapy has undeniably transformed the treatment landscape for hematological malignancies, demonstrating unprecedented efficacy in patients with refractory or relapsed disease. Clinical trials have consistently shown high RR and durable remissions, particularly in B-cell malignancies and multiple myeloma, establishing CAR-T therapy as a cornerstone of modern oncology. However, challenges such as toxicities, antigen escape, and manufacturing complexities persist, necessitating continued innovation in CAR design, targeting strategies, and production processes. The integration of co-stimulatory domains, dual-targeting approaches, and safety switches has already improved outcomes, while advancements in allogeneic and iPSC-derived products promise to enhance accessibility. As the field evolves, addressing these limitations will be critical to maximizing the therapeutic potential of CAR-T cells and expanding their applicability to a broader patient population.

Looking ahead, the future of CAR-T therapy lies in overcoming current barriers through multidisciplinary collaboration and cutting-edge research. Efforts to optimize CAR-T cells for solid tumors, reduce immune-related toxicities, and streamline manufacturing will be pivotal in shaping the next generation of cellular therapies. Additionally, the exploration of combination regimen, logic-gated systems, and novel targets holds promise for further improving efficacy and safety. By leveraging advances in synthetic biology, gene editing, and automation, CAR-T therapy may transition from a last-resort option to a frontline treatment, offering hope for patients with

otherwise untreatable cancers. Ultimately, sustained innovation and clinical translation will be essential to fully realize the transformative potential of this groundbreaking therapeutic modality.

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## Conflict of Interest

None.

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