Abstract# 536092 Targeting cfDNA and NETs with DNase I Augments CAR T-Cell Function and Antitumor Efficacy.

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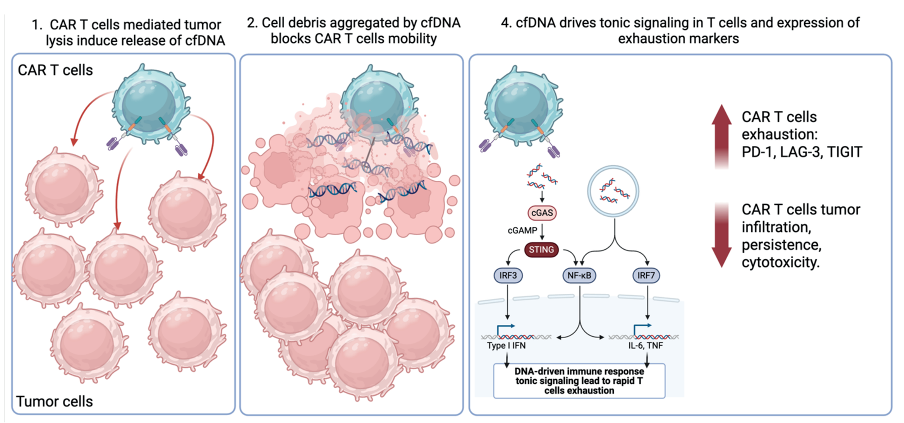
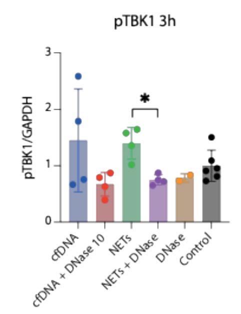
Background:
Chimeric antigen receptor (CAR) T-cell therapy induces high initial response rates in B-cell malignancies; however, limited persistence and early relapse remain major clinical challenges. Accumulation of cell-free DNA (cfDNA) and neutrophil extracellular traps (NETs) within the tumor microenvironment represents a potential mechanism of CAR T-cell dysfunction through induction of exhaustion, immunosuppression and impaired expansion. We hypothesized that enzymatic degradation of extracellular DNA using DNase I could enhance CAR T-cell persistence and antitumor efficacy.

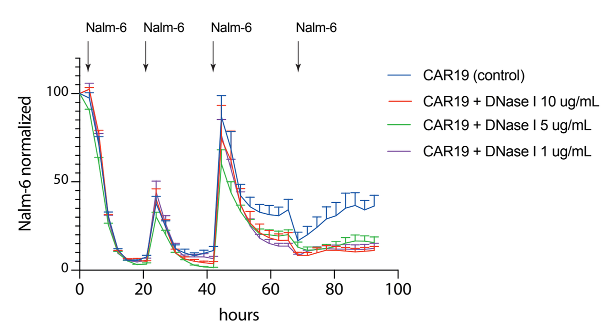
CAR-T–mediated tumor killing releases cfDNA into the tumor microenvironment. Accumulated cfDNA can form debris-like barriers, block CAR-T mobility, and trigger DNA-sensing inflammatory pathways that promote exhaustion and reduce CAR-T infiltration, persistence, and cytotoxicity.

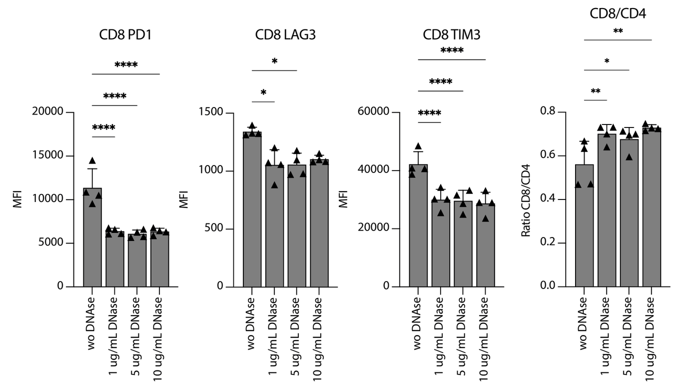


NETs and cfDNA activate DNA-sensing inflammatory signaling in CAR-T cells, reflected by increased TBK1 phosphorylation. DNase I degrades extracellular DNA and reduces this pTBK1 signal, indicating suppression of cfDNA/NET-driven cGAS–STING pathway activation.



cfDNA accelerates CAR-T functional exhaustion during repeated tumor challenge. DNase I preserves sustained cytotoxic activity across serial Nalm-6 rechallenges.



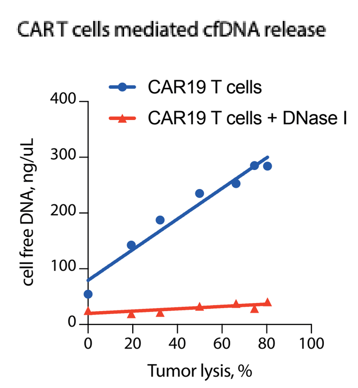


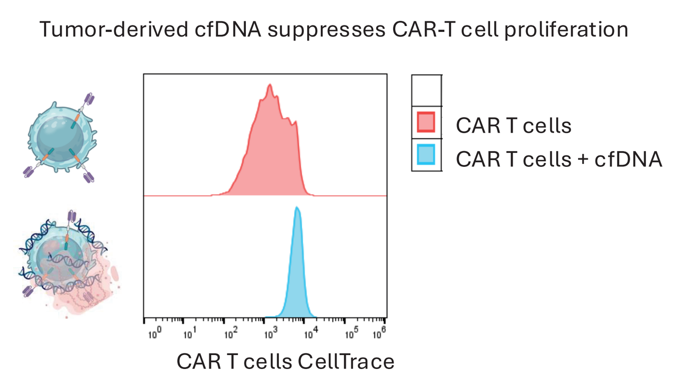
CAR-T success creates its own barrier: tumor killing releases cfDNA, and accumulated cfDNA feeds back to impair CAR-T expansion, persistence, and function. DNase I breaks this loop by degrading extracellular DNA.

More tumor killing → more cfDNA release → more CAR-T suppression.

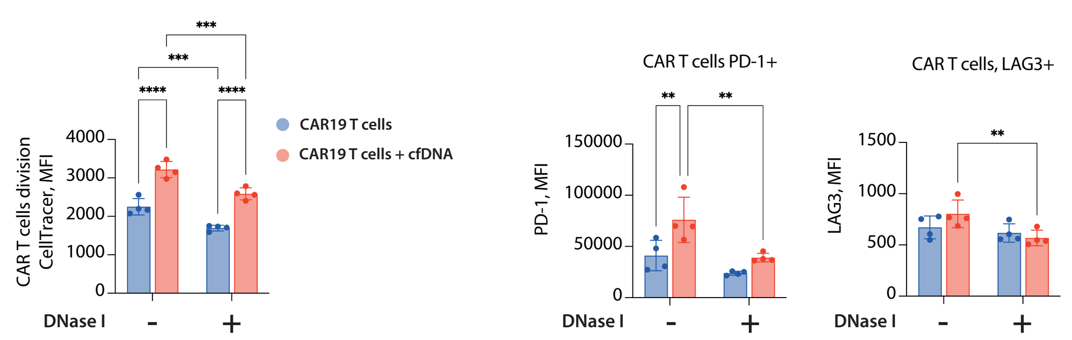
The better CAR T cells works, the more cells free DNA released in a surroundings.

DNase I eliminates cfDNA accumulation





DNase I restores CAR-T proliferation and reverses cfDNA-induced exhaustion



Tumor-derived cfDNA inhibits CAR-T cell proliferation and induces exhaustion markers PD-1 and LAG-3. DNase I degrades cfDNA, restoring CAR-T expansion and reducing exhaustion marker expression.

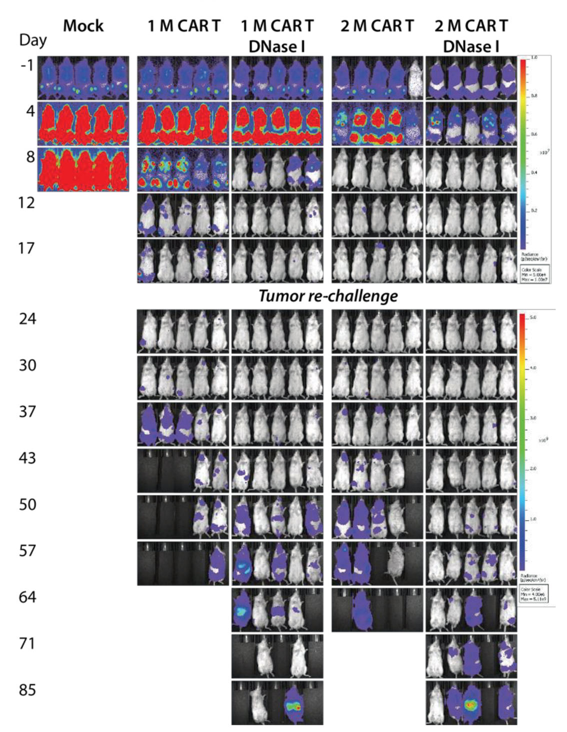
Methods:
The effects of cfDNA and NETs on human CD19 CAR T-cell function were evaluated using sequential killing assays, proliferation assays, and flow cytometry–based phenotyping. DNase I was assessed for its ability to reverse cfDNA/NET-mediated suppression and prevent exhaustion. In vivo efficacy, expansion, and persistence of CAR T cells were studied in xenogeneic B-cell acute lymphoblastic leukemia (Nalm-6) and Burkitt lymphoma (Raji) mouse models. Longitudinal in vivo tracking of CAR T-cell expansion was performed using bioluminescence imaging and serial peripheral blood analysis. Translational relevance was further explored through compassionate-use administration of DNase I in combination with CAR T-cell therapy in a pediatric patient with relapsed/refractory Burkitt lymphoma.

Results:
cfDNA and NETs suppressed CAR T-cell proliferation and cytotoxicity while promoting upregulation of exhaustion markers, including PD-1, LAG-3, and TIM-3. DNase I efficiently degraded extracellular DNA, preserved CAR T-cell effector function, improved CD8:CD4 ratios, and reduced exhaustion across multiple rounds of tumor rechallenge in vitro.
In vivo, DNase I significantly enhanced CAR T-cell expansion and persistence following infusion, as demonstrated by longitudinal tracking studies. Combination therapy resulted in improved tumor control, delayed relapse upon rechallenge, and prolonged survival in both NALM-6 and Raji xenograft models. In a single pediatric patient with highly refractory Burkitt lymphoma, DNase I co-administration was associated with marked CAR T-cell expansion and progressive reduction in tumor burden after prior CAR T-cell failure.

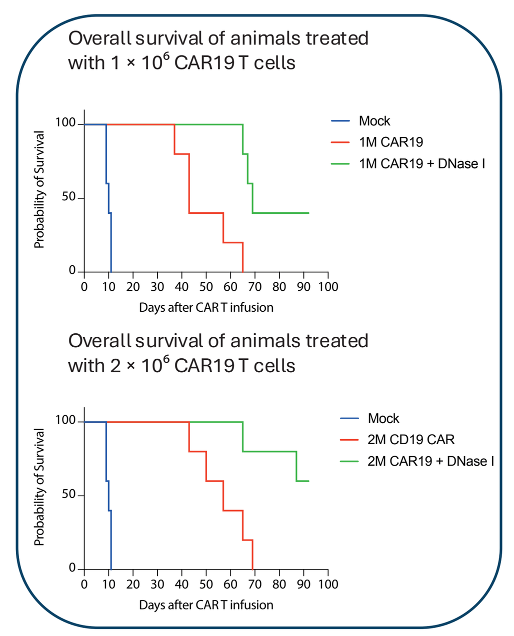
DNase I Supports CAR19 T-Cell Persistence and Delays Relapse in B-ALL

In the **Nalm-6 xenograft model**, DNase I co-treatment improved the depth and durability of response to **CAR19 T cells**.

1. DNase I improve CAR T cells **efficacy**
2. DNase I-treated mice maintained **better tumor control**



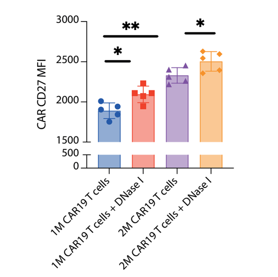
3. DNase I improved the **depth, durability, and survival benefit** of CAR19 T-cell therapy.



4. DNase I treatment was associated with a **higher frequency of CD27⁺ CAR T cells** and **reduced PD-1 expression**, consistent with improved CAR-T fitness and less exhaustion.

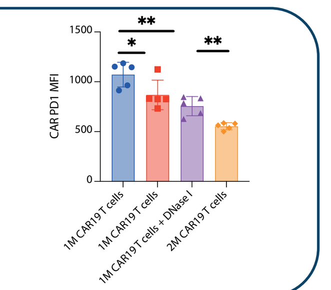
CD27 Expression on CAR T Cells

DNase I treatment increased the proportion of **CD27⁺ CAR T cells**, consistent with improved T-cell survival potential and a more durable functional state.



PD-1 Expression on CAR T Cells

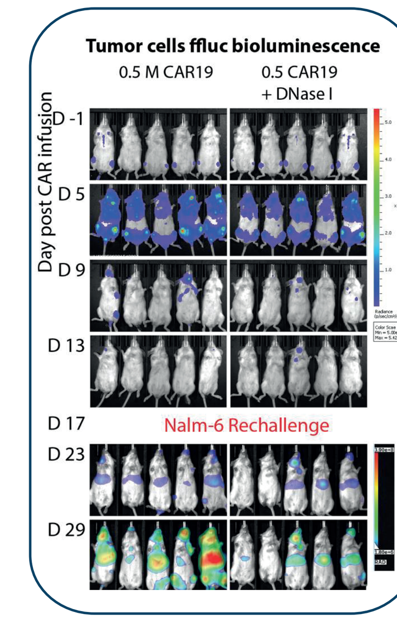
DNase I treatment reduced **PD-1 expression** on CAR T cells, supporting decreased exhaustion and improved maintenance of effector function in vivo.



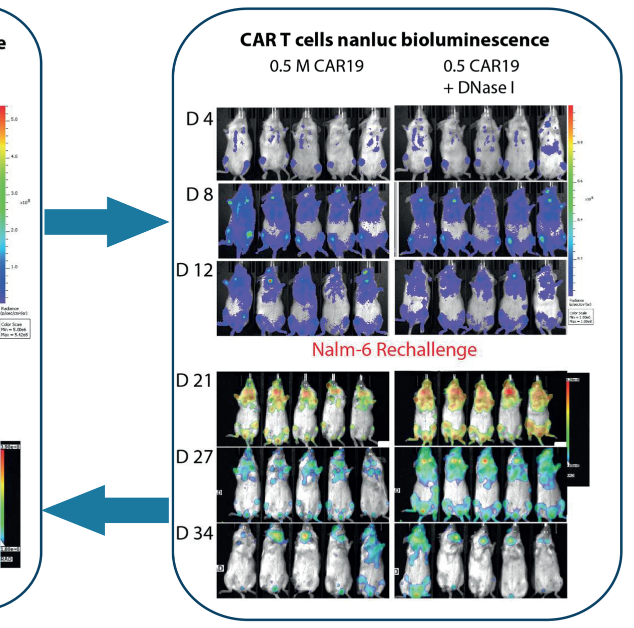
DNase I Supports CAR19 T-Cell Persistence and tumor control in B-ALL

In vivo tracking of labeled CAR T cells demonstrate that after tumor re-challenge (animal model of B-ALL relapse) CAR T cells expand better and stay longer in DNase I treated group.

1. DNase I-treated mice maintained better tumor control

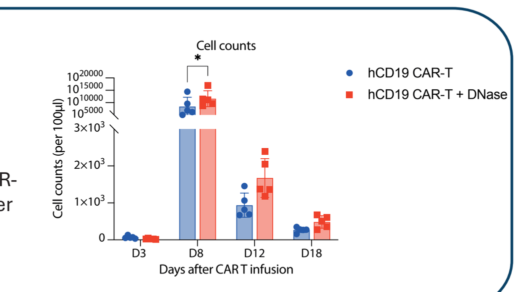


2. In vivo tracking shows enhanced CAR-T expansion and persistence after tumor re-challenge

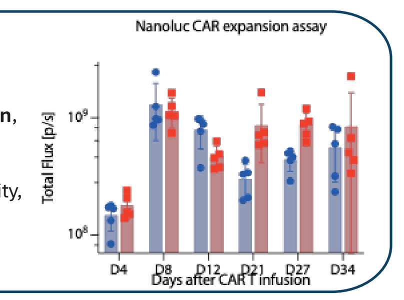


3. DNase I-treated mice showed higher circulating hCD19 CAR-T cell counts during the expansion and contraction phases.

Peripheral blood tracking showed **improved hCD19 CAR-T expansion and persistence** in DNase I-treated mice, with higher CAR-T cell counts maintained after peak expansion.



DNase I-treated mice showed improved CAR-T performance in vivo.
After tumor re-challenge, the DNase I-treated group demonstrated **renewed CAR-T expansion**, with increased signal by Day 21.
These findings indicate that DNase I supports CAR-T persistence, preserves functional capacity, and enables a stronger recall response after relapse-like re-exposure to tumor.

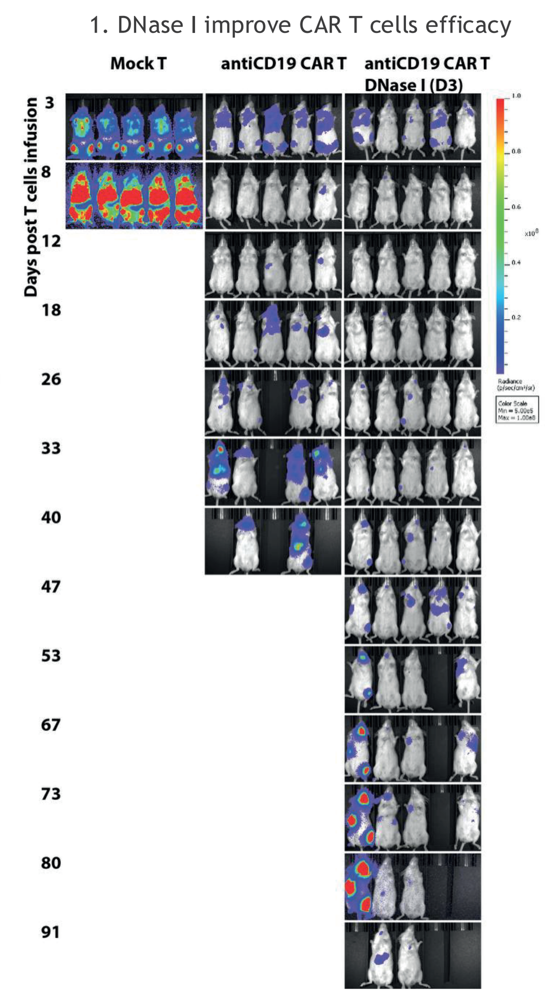


DNase I - CAR T combination for management of refractory B-Cell Lymphoma

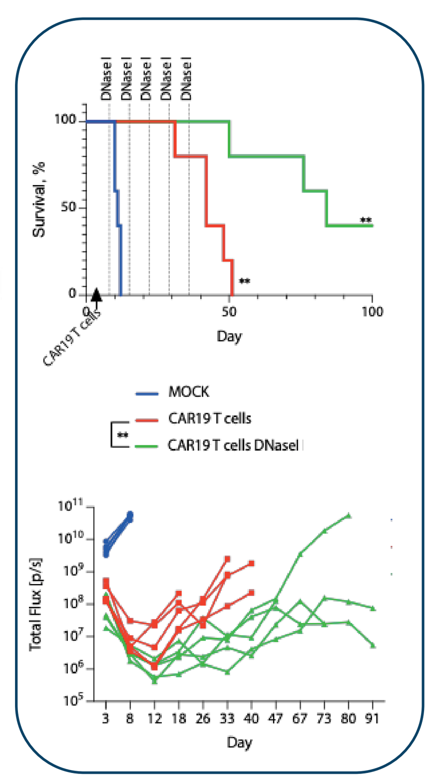
DNase I improves in vivo efficacy of CAR19 T cells in the Burkitt lymphoma model, supporting extracellular DNA as a targetable barrier to durable CAR-T activity.

1. DNase I injections **improve response and survival** in Raji Burkitt lymphoma xenograft
2. Mice treated by CAR19 T cells in combination with weekly dose of DNase I demonstrate **better tumor control and extended survival**
3. DNase I treatment was associated with **greater CAR T-cell expansion and persistence in vivo** translating into improved survival

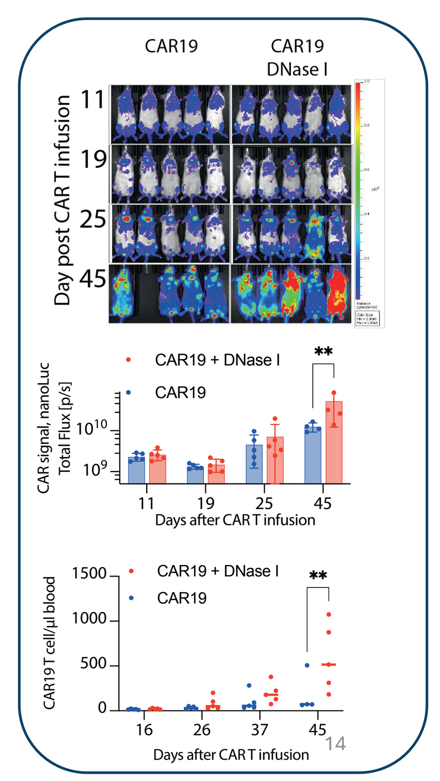
1. DNase I improve CAR T cells efficacy



2. DNase I improve survival



3. DNase I support CAR T cells expansion



Phase 1, single-center, open-label study to evaluate the safety of CLS-015 (DNase I) in combination with anti-CD19 CAR-T therapy in patients with large B-cell lymphoma. The goal is to improve clinical response by reversing the negative effects of NETs on immune function and CAR-T cells.

Recruiting

Investigator Initiated Study to Assess the Safety of Combination of CLS-015 With Anti-CD-19 CAR-T Cells in Patients With Stable/Progressive Large B Cell Lymphoma at Lymphodepletion.

ClinicalTrials.gov ID NCT07361224
Sponsor Tel-Aviv Sourasky Medical Center
Information provided by Tel-Aviv Sourasky Medical Center (Responsible Party)
Last Update Posted 2026-05-01

ClinicalTrials.gov

Contact: Ron Ram, Prof.
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Conclusions:
Extracellular DNA and NETs constitute a critical barrier to durable CAR T-cell efficacy. DNase I enhances CAR T-cell persistence and antitumor activity by eliminating cfDNA/NET-driven immunosuppression, supporting further clinical evaluation of DNase I as a combinatorial strategy to improve CAR T-cell therapy outcomes.

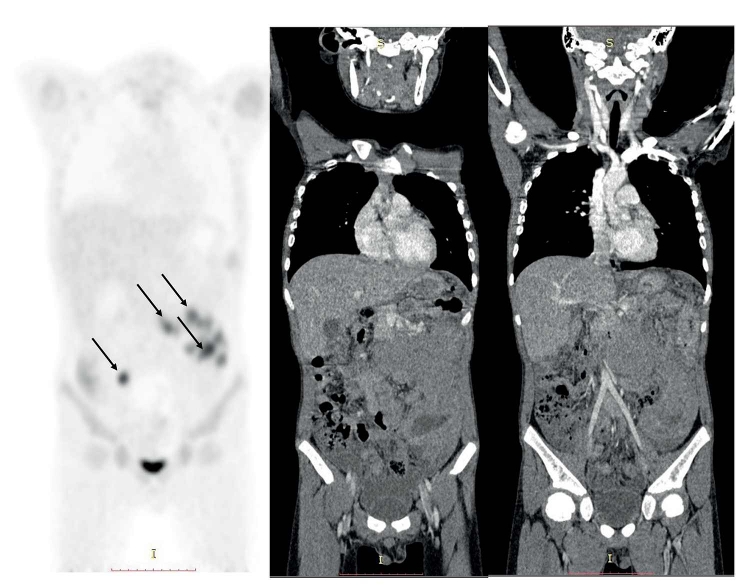
2026 ASCO ANNUAL MEETING

Session Date: June 1, 2026,
Session Time: 9:00 AM-12:00 PM CT
Poster Board Number is: 410

Clinical case report:
DNase I - CAR T combination for management of refractory Burkitt lymphoma

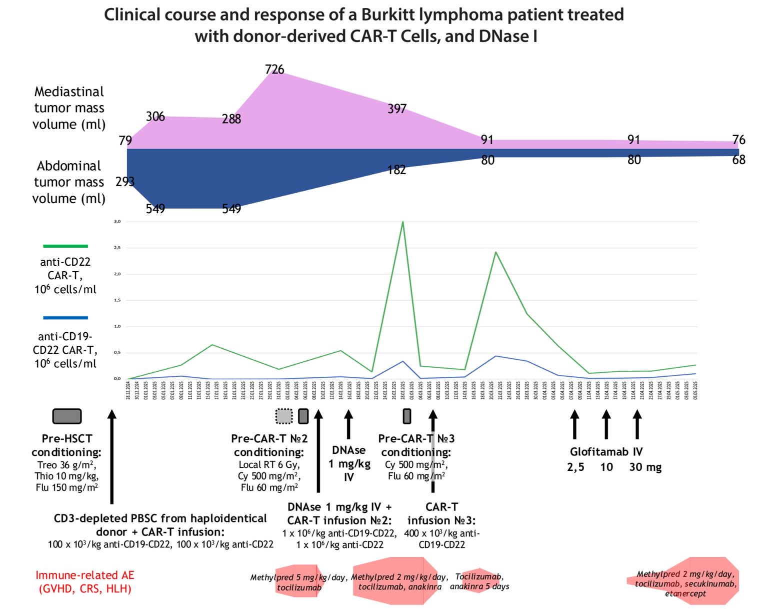
- A 10-year-old male patient presented with vomiting after food intake, weight loss, generalised itching and intraabdominal masses.
- Thoracoabdominal CT scan revealed the presence of retroperitoneal masses infiltrating mesentery, small intestines and omentum, ascites and hepatomegaly.
- The cytological exam of ascitic fluid, histochemical and molecular exam were consistent with the diagnosis of Burkitt's lymphoma with t(8;14)(q24;q32) MYC::IGH. Bone marrow and CNS were not involved. Stage III disease, risk group 4 (LDH >2N) were stated. NGS of tumor tissue revealed TP53 exon-6 mutation.
- Treatment according to B-NHL 2021 protocol: disease massively progressed during and after 6 courses of chemotherapy and 6 rituximab infusions. Old lesions enlarged and new masses appeared, including tumor in mediastinum.
- Patient had not enough CD3+ cells in peripheral blood to perform autologous CAR-T cell manufacturing, the decision to proceed to allogeneic HSCT together with CAR-T cell therapy was made.
- 1x106/kg of bi-specific anti-CD19-CD22 and 1x106/kg of anti-CD22 CAR T in combination with IV infusion of 1 mg/kg DNase I were given after cyclophosphamide/fludarabine preconditioning in combination with local tumor irradiation (6 Gy). Repeated IV infusion of 1 mg/kg DNase I was given 7 days later.
- Two infusions of DNase I (1 mg/kg IV) combined with CAR-T re-infusion was accompanied by significant expansion of both anti-CD22 and bispecific anti-CD19/CD22 CAR T cells and progressive decrease of both mediastinal and abdominal tumor mass.

Thoracoabdominal CT in Burkitt Lymphoma patient.

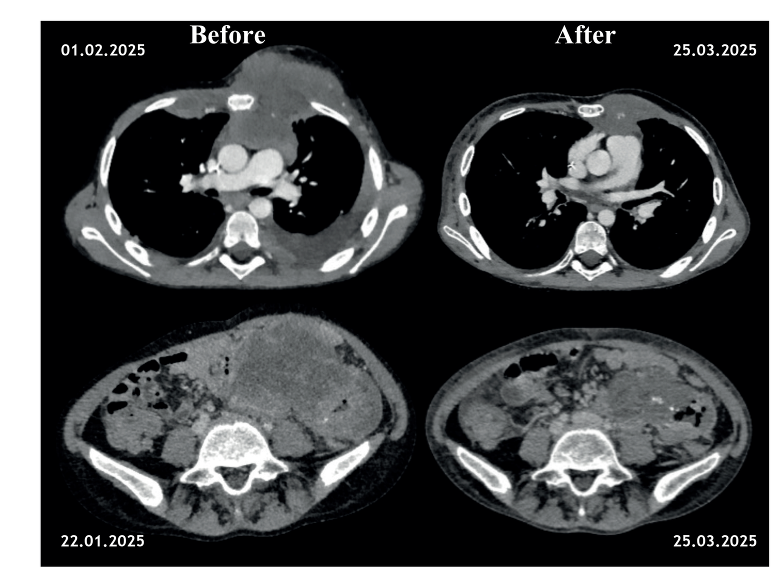


Contrast-enhanced thoracoabdominal CT demonstrating large retroperitoneal masses infiltrating the mesentery, small intestines, and omentum. Associated findings include ascites and hepatomegaly, consistent with advanced-stage Burkitt lymphoma.

Clinical course and response of a Burkitt lymphoma patient treated with donor-derived CAR-T Cells, and DNase I



Progressive reduction in mediastinal and abdominal tumor burden following combination therapy



Timeline and clinical outcomes from a 10-year-old male with relapsed/refractory Burkitt lymphoma who received simultaneous haploidentical HSCT and donor-derived CAR-T cell therapy. Initial infusion of bispecific anti-CD19/CD22 CAR-T cells and anti-CD22 CAR-T cells following treosulfan/thiotepa/fludarabine conditioning resulted in limited CAR-T cell expansion and no objective tumor response. A subsequent CAR-T cell re-infusion, combined with DNase I. Two additional DNase I infusions administered alongside CAR-T cells were associated with marked CAR-T cell expansion and progressive reduction of mediastinal and abdominal tumor burden.

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