RESEARCH HIGHLIGHT

A precision counterstrike on central nervous system autoimmunity

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Autoantibodies targeting the N-methyl-D-aspartate receptor (NMDAR) induce debilitating neuropsychiatric symptoms and potentially fatal complications due to brain swelling and autonomic instability, requiring aggressive treatments to eliminate the autoreactive B-cells and autoantibodies causing disease. In a recent *Cell* paper, Reincke et al. genetically engineer T-cells to express an autoantigen-based chimeric autoantibody receptor (CAAR) to direct T-cell cytotoxicity specifically against anti-NMDAR B-cells, establishing the basis for a novel precision cellular immunotherapy for NMDAR encephalitis.

N-methyl-D-aspartate receptor (NMDAR) encephalitis is the most common of the autoimmune encephalitides, often presenting in children and young adults with dyskinesia, seizures, agitation, hallucinations, or catatonia. Autoantibodies target the NMDAR GluN1 subunit, leading to decreased synaptic NMDAR density. Therapy aims to suppress or eliminate B-cells or autoantibodies with corticosteroids, intravenous immunoglobulin, plasmapheresis, rituximab, and/or cyclophosphamide. B-cell depletion with rituximab decreases rates of disease relapse and improves symptoms, although repeated infusions are needed to maintain disease control and can lead to serious infections, highlighting the need for better and safer therapies.

Toward this end, Reincke and co-authors designed chimeric autoantibody receptors (CAARs) with ectodomains comprising the main immunogenic region of the NMDAR GluN1 subunit (Fig. 1a), either as the GluN1 amino-terminal domain (ATD) alone ($N1_{ATD}$), or all GluN1 extracellular domains, or $N1_{ATD}$ in tandem with GluN2B-ATD (Fig. 1b).[1] The NMDAR-CAAR ectodomain targets the anti-NMDAR B cell receptor (BCR), a membrane-bound autoantibody that marks the autoreactive B-cell population in NMDAR encephalitis. Upon target BCR engagement, the CAAR 4-1BB costimulatory and CD3ζ cytoplasmic domains direct NMDAR-CAAR T-cell (NMDAR-CAART) proliferation and cytolysis of NMDAR-specific B-cells, sparing healthy B-cells that do not express anti-NMDAR BCRs (Fig. 1c).

To better understand the autoreactive B-cells targeted by NMDAR-CAART, the authors first characterized epitopes recognized by serum IgG and recombinant mAbs cloned from patients with NMDAR encephalitis. Patient serum IgG and mAb binding to $N1_{ATD}N2B_{ATD}$-CAAR is greater than to $N1_{ATD}$-CAAR alone, suggesting that NMDAR autoantibodies bind a quaternary epitope at the GluN1–GluN2B interface or $N1_{ATD}$ epitopes stabilized by $N2B_{ATD}$. Accordingly, $N1_{ATD}N2B_{ATD}$ NMDAR-CAART killed a broader range of anti-NMDAR B-cells including low-affinity clones, even in the presence of physiologic levels of soluble anti-NMDAR antibodies that might have inhibited NMDAR-CAART cytolysis. Bystander killing of primary human B-cells was not observed, establishing the specificity of NMDAR-CAART cytolytic activity. A mouse passive transfer model using a monoclonal high-affinity Nalm6 target B-cell, with or without soluble anti-NMDAR antibody secretion, demonstrated evidence of NMDAR-CAART cytolytic activity in vivo. Although the 003-102 mAb is known to be pathogenic, NMDAR-specific brain pathology was not observed after passive transfer of 003-102 antibody-secreting Nalm6 cells, reflecting a relatively low level of anti-NMDAR antibodies in this model. NMDAR-CAART function depended on the quality of CAART manufacturing, as CAARTs expanded for 9 days controlled target cell outgrowth in the absence of soluble autoantibodies, whereas those expanded for 13 days allowed target cell escape. Off-target cytotoxic interactions of NMDAR-CAART against brain or other organs were not detected.

How likely is NMDAR-CAART to successfully address the root cause of disease? The necessity and sufficiency of anti-NMDAR antibodies for disease pathology has been well-established through passive transfer models in mice and humans.[2,3] Complement fixation is not observed in patient brain tissue despite IgG1/IgG3 autoantibody predominance[4]; similarly, complement-mediated cytotoxicity against NMDAR-CAART in circulation may not occur if NMDAR-CAAR cell-surface density is low enough to avoid complement deposition. B-cells and plasma cells are abundant in the brain of NMDAR encephalitis patients, and autoantibody levels in cerebrospinal fluid are more sensitive than those in serum for disease diagnosis and prediction of relapse.[5] Therefore, effective therapy would depend on absence of long-lived autoreactive plasma cells, which do not express surface BCRs and are not targetable by NMDAR-CAART, and may require CAART trafficking to brain or intrathecal administration, which could risk neurologic side effects from inflammatory cytokine secretion. The authors observed engineered T-cells in the leptomeninges, a tissue with direct access to the brain, and further demonstrated that the tyrosine kinase inhibitor dasatinib inhibits NMDAR-CAART cytotoxicity as a safety switch in case of unexpected adverse events.

From a technical perspective, incomplete killing of target cells after a 24-h killing assay suggest that NMDAR-CAART cytotoxic potency is inferior to anti-CD19 chimeric antigen receptor T-cell (CART)-mediated cytolysis, which induces complete killing even at low effector-to-target cell ratios. Ideally, sensitive bioluminescence

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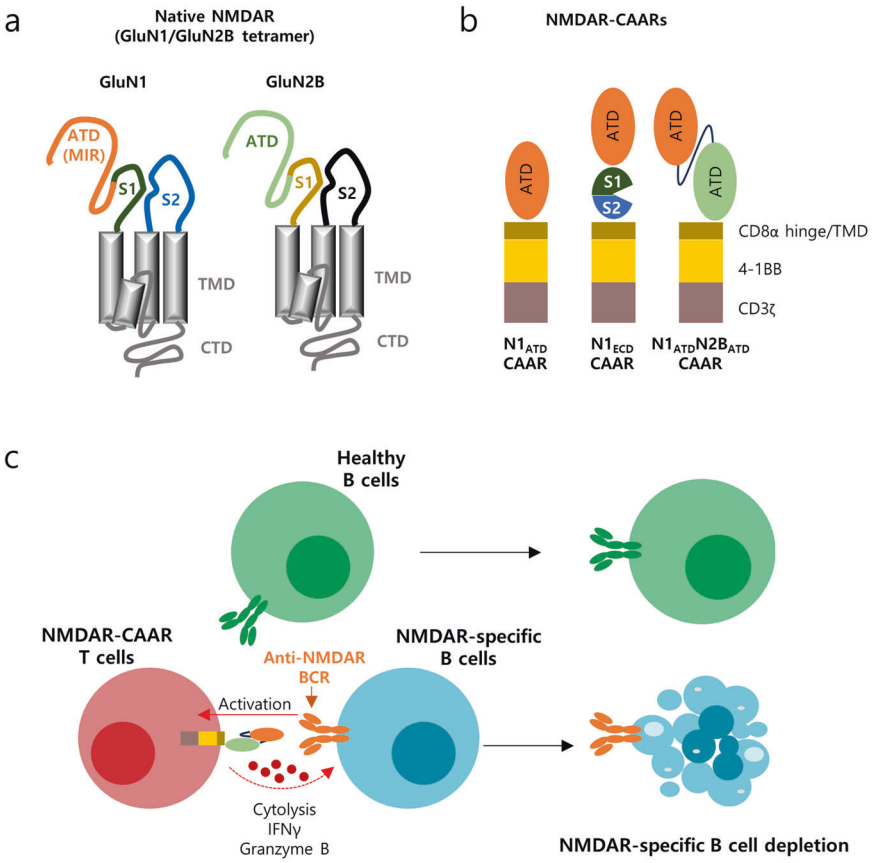

Fig. 1 NMDAR-CAAR T cells for NMDAR-specific B-cell depletion. a Native NMDAR is a heterotetramer consisting of two GluN1 and two GluN2B subunits, each with an extracellular ATD and S1-S2 domains comprising a ligand binding domain, linked through transmembrane domains (TMDs) to a C-terminal domain (CTD). The GluN1 ATD contains the main immunogenic region (MIR) for NMDAR autoantibodies. **b** NMDAR-CAARs incorporate the GluN1-ATD, the GluN1-ATD-S1-S2 extracellular domains ($N1_{ECD}$), or the GluN1-ATD linked to the GluN2B-ATD, fused to CD8α hinge and TMD, 4-1BB co-stimulatory domain, and CD3ζ activation domain. **c** NMDAR-CAART engages anti-NMDAR BCRs on autoreactive B-cells, leading to CAART activation, secretion of IFNγ and granzyme B, and specific cytolysis of anti-NMDAR B-cells.

analysis of in vivo NMDAR-CAART cytotoxic activity should be investigated against a broader variety of target cells including those expressing low-affinity anti-NMDAR BCRs. NMDAR-CAART was enriched for CAAR-expressing cells, which introduces manufacturing complexity and is typically not required for efficacy in preclinical models. Nevertheless, NMDAR-CAART therapy resulted in reduction of target cell burden and soluble anti-NMDAR antibodies in vivo, establishing preliminary proof of concept for NMDAR-CAART therapy of NMDAR encephalitis.

In regard to clinical application, phase 1 trials of CAART technology in antigen-specific subtypes of pemphigus vulgaris[6] (NCT04422912) and myasthenia gravis[7] (NCT05451212) are currently ongoing. Emerging clinical data indicate CAART persistence up to 3 months after infusion in the absence of preconditioning and a tolerable safety profile with no dose-limiting toxicities. Clinical improvements are observed in some patients, without a consistent pattern of effect on serum autoantibodies.[8] Recently, CD19- or BCMA-directed CARTs have demonstrated therapeutic efficacy without permanent target cell aplasia in systemic lupus erythematosus, myositis, systemic sclerosis, myasthenia gravis, and neuromyelitis optica,[9–13] with several patients achieving clinical and serologic remission off systemic immunosuppressives. NMDAR-CAART adds a potential precision targeted approach to the growing armamentarium of options for patients suffering with autoimmunity worldwide.

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COMPETING INTERESTS

A.S.P. reports equity, consulting, research funding, patent licensing (Cabaletta Bio); consulting (Janssen). S.O. reports patent licensing (Cabaletta Bio).

ADDITIONAL INFORMATION

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