Engineering CAR T-Cell Therapy for Rheumatoid Arthritis: Conceptual Design and Biosensor Validation

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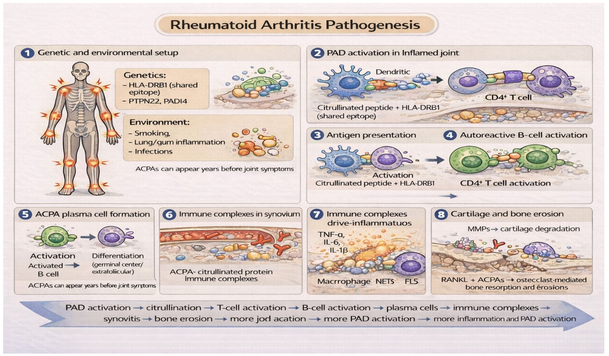
Introduction & Goals

Rheumatoid arthritis is a chronic autoimmune disease affecting millions worldwide, characterized by progressive joint destruction and disability that worsens significantly with age. The disease affects women at three times the rate of men, driven by declining immunomodulatory hormones like estrogen and progesterone during menopause, which dysregulate immune tolerance. Genetically, over 80% of RA patients carry the HLA-DRB1 surface protein, and environmental triggers like smoking accelerate disease onset by promoting protein citrullination.

At the molecular level, PAD enzymes convert arginine residues to citrulline in key structural proteins including vimentin, fibrinogen, and collagen. These citrullinated proteins are recognized as foreign by autoreactive B-cells, which produce anti-citrullinated protein antibodies called ACPAs that attack the synovial tissue lining the joints. This drives chronic inflammation, cartilage erosion, and progressive bone destruction that compounds over time, with severe cases requiring surgical joint replacement.

Current treatments including DMARDs, TNF-α inhibitors, IL-6 blockers, and B-cell depletion agents manage symptoms through broad immunosuppression but never address the underlying cause. They do not selectively eliminate the pathogenic B-cells producing ACPAs, leaving the disease mechanism intact. Patients require lifelong medication carrying compounding risks of infection and blood clots, while treatment efficacy diminishes over time requiring regular dosage increases. The lack of strong biomarkers to predict disease severity leaves treatment largely dependent on trial and error. There is a clear and urgent need for a precise, durable therapeutic approach that targets the root cause of RA rather than simply suppressing its symptoms.



Project Goals

Goal 1: CAR T-Cell Therapeutic Design (Conceptual)

We designed a CAAR T-cell engineered to selectively target autoreactive B-cells driving rheumatoid arthritis. Rather than broadly targeting CD19 like conventional CAR T-cell approaches, our CitB-CAAR T-cell displays citrullinated vimentin peptide as bait on its surface, exploiting the disease-specific BCRs of ACPA-producing B-cells against them. Only autoreactive B-cells recognizing citrullinated proteins will bind this bait, triggering activation and cytotoxic elimination of the target cell through perforin/granzyme B release. This approach selectively eliminates disease-causing B-cells while preserving healthy immune function, targeting the root cause of RA rather than broadly suppressing the immune system and providing potential for long-term remission through memory CAAR T-cells.

Goal 2: Bacterial Biosensor Validation (Experimental)

Since functional CAR T-cells require BSL-2 facilities beyond this project's scope, we developed a bacterial biosensor to validate the core principle of selective antigen recognition under BSL-1 conditions. E. coli was transformed with an IPTG-inducible GFP system to demonstrate that biological systems can achieve specific, triggered responses to molecular signals. IPTG serves as a proxy for citrullinated antigens — when present, it activates the lac operon and drives GFP expression, directly modeling how a CAAR T-cell selectively activates only upon encountering its specific target while ignoring everything else.

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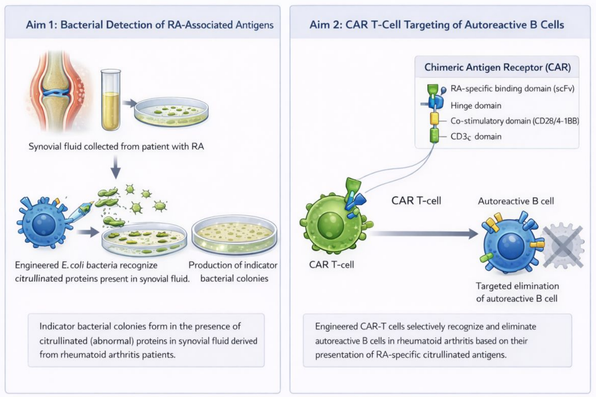


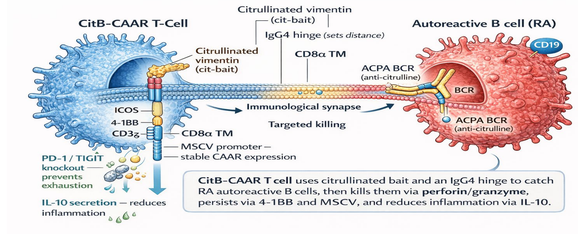
Materials and Methods

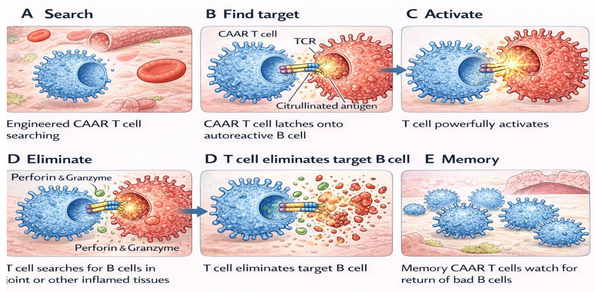
Conceptual Model: CitB-CAAR T-Cell Design
Our CitB-CAAR T-cell represents a significant advancement beyond conventional CD19-targeted CAR T-cell approaches. Rather than broadly targeting all CD19-positive B-cells, our construct exploits the disease-specific B-cell receptors of autoreactive B-cells against them through a precision bait and catch mechanism.

The Bait-The extracellular domain displays a citrullinated vimentin peptide, citVim60-71, as molecular bait on the T-cell surface. Only ACPA-producing autoreactive B-cells carry BCRs shaped by the disease itself to recognize this specific citrullinated epitope — healthy B-cells have no affinity for it and are completely ignored. A short IgG4 hinge of 12 amino acids positions this bait at the optimal 15 nanometer distance from the T-cell surface, enabling the tight immunological synapse necessary for strong activation. The CD8α transmembrane domain anchors the construct stably within the membrane, facilitating receptor clustering that amplifies downstream signaling.

The Catch-Once an autoreactive B-cell binds the bait, three intracellular domains trigger elimination in concert. ICOS drives rapid T-cell proliferation to sustain the response. 4-1BB converts activated T-cells into long-lived stem cell memory T-cells capable of persisting 2 to 5 years. CD3ζ serves as the kill switch — triggering perforin and granzyme B release that destroys the target cell within 30 minutes, with FasL providing a secondary apoptosis signal as backup. The MSCV promoter maintains stable high-density CAAR expression over time by favoring Tscm differentiation and resisting epigenetic silencing.

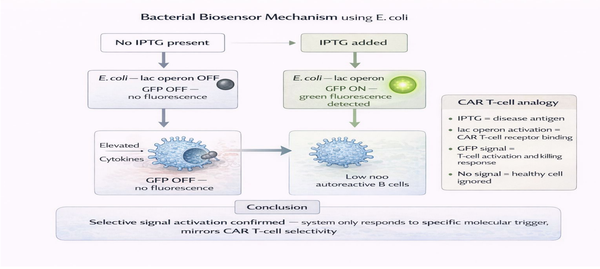
Safety and Durability-A synNotch AND gate requires confirmation of both citrulline recognition and CD19 presence before full activation — a double lock that virtually eliminates off-target killing. Armored IL-10 secretion dampens post-killing inflammation and promotes synovial tissue tolerance, preventing cytokine storm. CRISPR knockout of PD1 and TIGIT removes inhibitory checkpoints that the inflamed RA environment would otherwise exploit to exhaust the CAAR T-cells, ensuring sustained cytotoxic function throughout treatment.





Experimental Model: Bacterial Biosensor
Experiment Protocol:

1. To start the experiment you must first have enough E.coli, so make sure to grow enough colonies
2. Then once you have enough colonies you then extract it from the plate, and put them into 2 tubes. One tube will contain Calcium chloride, and will be labelled DNA- while the other that contains nothing will be labelled DNA+.
3. You then resuspend the bacterial cells in the DNA- tube and transfer around 250 microliters into the DNA+ tube.
4. Next, add 10 microliters of the GFP protein to the DNA+ tube and then incubate both tubes for 10 minutes.
5. Afterwards you quickly place the transformation tubes in 40 degrees celsius water to heat shock, and then you incubate for 2 minutes.
6. You then put 250 microliters of recovery broth into both tubes and incubate for 10 minutes in a 37 degree celsius water bath. While you wait, label 4 plates DNA- with ampicillin and DNA- without it, and do the same for DNA+.
7. You then streak the bacteria according to this and incubate overnight for around 16-18 hours in a 37 degree celsius oven.



Results

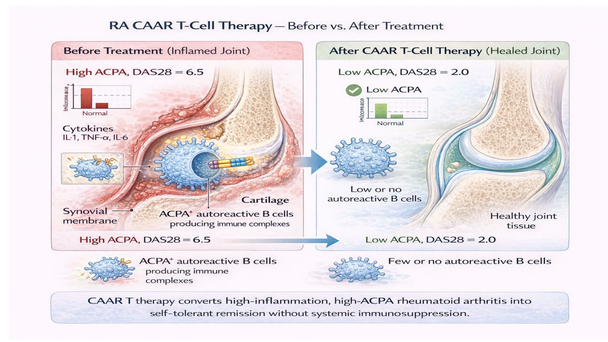
Expected CitB-CAAR T-Cell Outcomes
Based on our conceptual model and emerging clinical trial data from CAR T-cell therapies in other autoimmune diseases, we predict the following outcomes across four key areas.

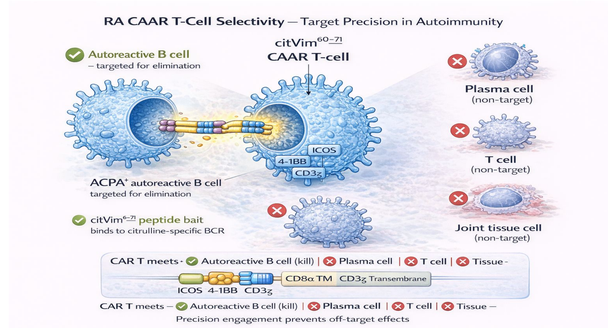
Cellular Response
Following infusion, CAR T-cells traffic to lymphoid tissues and sites of inflammation, where they recognize and bind autoreactive B-cells displaying BCRs specific to citrullinated proteins. Upon target engagement, CAR T-cells activate, proliferate, and eliminate pathogenic B-cells. Within 1 to 2 weeks post-infusion, autoreactive B-cells are expected to become undetectable in circulation.

Disease Markers
As ACPA-producing B-cells are eliminated, autoantibody titers decline over a 2 to 4 week half-life. Downstream inflammatory markers including CRP, ESR, IL-6, and TNF-α decrease progressively as the autoimmune response is disrupted at its source rather than merely suppressed at the surface.

Clinical Improvement
As inflammation resolves and synovial tissue begins healing, patients are expected to experience measurable improvements in joint pain, morning stiffness, swollen joint counts, and functional assessments over a 4 to 12 week window following infusion.

Long-Term Durability
SInce pathogenic B-cell clones are eliminated rather than suppressed, the reconstituted B-cell repertoire that emerges over 6 to 12 months lacks autoreactive populations entirely. This offers the potential for durable, drug-free remission without the need for continuous immunosuppression addressing the core limitation of every existing RA treatment.





Feature	Current Drugs	Rituximab	CAR T
Mechanism	Broad suppression of the immune system by using immunosuppressants.	Anti-CD20 surface protein antibodies that deplete these faulty B-cells that cause inflammation	Engineered T-cells induce apoptosis to faulty b-cells by targeting them using an engineered biosensor.
Duration	Continuous(dosage increases as RA progresses)	6 months for 80% of people, but cells may repopulate	1 hour for injection of t-cells but 4 weeks of close monitoring at home.
Cost	$30-60K/year	$50K/year	$400K once
Selectivity	Low	Moderate	High

Discussion & Conclusion

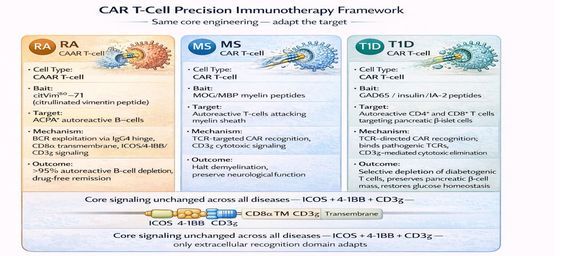
Discussion

Our bacterial biosensor validates the fundamental principle underlying CAAR T-cell therapy — that biological systems can be engineered to achieve specific, dose-dependent responses to defined molecular signals, directly modeling the discriminatory capability required to distinguish diseased from healthy targets.

Early clinical trials of CD19 CAR T-cells in severe lupus showing drug-free remission further validate that this recognition-and-eliminate approach has real world credibility beyond our model, demonstrating that antigen-specific B-cell depletion can achieve what decades of immunosuppressive therapy could not.

Current RA treatments manage inflammation without ever addressing the immunological root cause, requiring lifelong administration with compounding risks. Our CitB-CAAR T-cell design overcomes this by exploiting the disease-specific BCRs of ACPA-producing autoreactive B-cells against them selectively eliminating pathogenic cells while leaving healthy B-cells and protective immunity completely intact, offering potential for durable drug-free remission from a single treatment course.

The modular architecture of our construct means this framework is not limited to rheumatoid arthritis. By simply modifying the antigen recognition domain while keeping intracellular signaling machinery constant, the same engineering logic adapts to multiple sclerosis and type 1 diabetes. The core never changes, only the bait does.



Conclusions

Goal 1-We successfully designed a comprehensive CitB-CAAR T-cell framework targeting autoreactive B-cells in rheumatoid arthritis. Moving beyond conventional broad CD19 depletion, our construct incorporates a precision bait and catch mechanism using citrullinated vimentin as a disease-specific molecular target, combined with a multi-domain signaling architecture optimized for selective elimination, long-term persistence, and clinical safety.

Goal 2-Our bacterial biosensor experiments validated the core principle underlying CAAR T-cell function — that biological systems can be engineered to produce specific, dose-dependent responses to defined molecular signals. Using an IPTG-inducible GFP system under BSL-1 conditions, we demonstrated selective antigen recognition and titratable reporter output, providing an accessible and reproducible proof-of-concept model for the recognition principles that govern CAR T-cell targeting.

Future Directions-Clinical translation of our CitB-CAAR construct would require molecular cloning into lentiviral vectors, optimization in mammalian cell lines, primary T-cell isolation and transduction, in vitro cytotoxicity assessment against autoreactive B-cells, preclinical evaluation in animal models, and ultimately first-in-human clinical trial design targeting refractory RA patients who have failed existing therapies.

Significance-This project demonstrates both a conceptual framework for precision immunotherapy and experimental validation of its underlying recognition principles. The modular architecture of our CAAR design — where the antigen recognition domain can be swapped while core signaling machinery remains constant — establishes a reproducible platform adaptable to multiple autoimmune diseases beyond RA. This represents a fundamental shift in how autoimmune disease can be approached: moving away from chronic symptom management and broad immunosuppression toward a curative immune reset that offers the potential for drug-free remission and a lasting improvement in patient quality of life.

Risk	Severity	Management	Benefits opposed to Risks
CRS	Mod-Severe	Tocilizumab, steroids, ICU monitoring	Potential Cure to the disease
B-cell aplasia	Moderate	Temporary, IgG replacement, 6-12mo recovery	One time treatment without constant hospital visits
Infection	Moderate	Antiblotics, monitoring during aplasia	Preserved normal immunity, leading to no changes in quality of life.
Off-target	Low	CD19 is B-cell specific	Cost breaks even at 8-13 years of remission.

Resources

1. Muller, I. et al. CAR T-cell therapy in Rheumatoid Arthritis. *PubMed* (2025). https://pubmed.ncbi.nlm.nih.gov/41258618/
2. Review: CAR-T cells in autoimmune diseases. *PubMed* (2024). https://pubmed.ncbi.nlm.nih.gov/41027249/
3. B-cell abnormalities in RA — CD86, FcγRIIb expression. *PubMed* (2010). https://pubmed.ncbi.nlm.nih.gov/20398308/
4. Wegner, N. et al. The immunopathogenesis of seropositive rheumatoid arthritis: from triggering to targeting. *PubMed* (2016). https://pubmed.ncbi.nlm.nih.gov/27916980/
5. Ellebrecht, C.T. et al. Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease. *Science* (2016). https://pubmed.ncbi.nlm.nih.gov/27365313/
6. Advances of CAR-T cell therapy in autoimmune diseases — technological iteration to clinical breakthrough. *PubMed* (2025). https://pubmed.ncbi.nlm.nih.gov/41657283/
7. Reincke, S.M. et al. Chimeric autoantibody receptor T cells deplete NMDA receptor-specific B cells. *Cell* (2023). https://pubmed.ncbi.nlm.nih.gov/37918394/
8. CAAR T cells for targeted B cell depletion in multiple sclerosis — in vitro study. *PubMed* (2023). https://pubmed.ncbi.nlm.nih.gov/37809446/
9. Chimeric autoantibody receptor T cells specifically eliminate Graves' Disease autoreactive B cells. *PubMed* (2025). https://pubmed.ncbi.nlm.nih.gov/40264777/
10. CAR T-cell therapy in autoimmune diseases — a promising frontier. *PubMed* (2025). https://pubmed.ncbi.nlm.nih.gov/40873568/