

Engineering CAR-T Cells to Combat Obesity: From Tumor Microenvironments to Metabolic Dysfunction

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Introduction

Obesity has emerged as one of the most pressing global health challenges of the 21st century, affecting over 650 million adults worldwide and serving as a primary risk factor for cardiovascular disease, type 2 diabetes, and numerous other metabolic disorders. Despite decades of research, current therapeutic approaches remain limited in their efficacy, with conventional treatments, such as lifestyle interventions, showing poor long-term success rates, and pharmacological options often accompanied by significant side effects. The revolution in immunotherapy for cancer treatment, particularly the remarkable success of chimeric antigen receptor T-cell (CAR-T) therapy in haematological malignancies, has prompted researchers to explore whether similar approaches could be applied to other disease contexts.

CAR-T cell therapy represents a paradigm shift in cancer treatment, involving the genetic modification of patient T cells to express synthetic receptors that recognize specific tumor antigens. While this approach has achieved remarkable success in treating relapsed/refractory B-cell malignancies, its application to solid tumours has been largely unsuccessful due to T-cell exhaustion and immunosuppressive microenvironments. However, the parallels between tumor-associated macrophages and adipose tissue macrophages (ATMs) in creating inflammatory, pathological environments suggest that similar therapeutic strategies might apply to metabolic diseases.

This research addresses a critical gap in current immunotherapy applications by investigating how insights from CAR-T cell behavior in tumor microenvironments can inform novel therapeutic approaches for obesity. My primary research question was: How can understanding CAR-T cell exhaustion mechanisms in complex inflammatory microenvironments guide the development of CAR-T therapies targeting adipose tissue dysfunction? Through this work, I aimed to demonstrate that by characterizing the factors leading to CAR-T cell exhaustion and developing advanced model systems to study these

interactions, we can engineer next-generation CAR-T cells capable of effectively targeting obesity-associated inflammation.

Literature Review and Background

CAR-T Technology and Current Limitations:

CAR-T cell therapy has transformed the treatment landscape for certain blood cancers, with multiple FDA-approved products demonstrating remarkable clinical efficacy. However, the extension of this technology to solid tumors has proven challenging, with limited clinical success attributed primarily to the immunosuppressive tumor microenvironment and progressive T cell exhaustion. Recent research has identified specific mechanisms underlying these failures, particularly the role of inflammatory niches in promoting T cell dysfunction.

The IL-1 β inflammatory niche has emerged as a critical factor in CAR-T cell failure. Recent publications in *Nature* (2023-2024) have demonstrated that IL-1 β ⁺ macrophages create immunosuppressive microenvironments through the IL-1 β -PGE2 axis, leading to T cell exhaustion and therapeutic resistance. These findings suggest that targeting inflammatory pathways could enhance CAR-T efficacy, but current model systems are inadequate for studying these complex interactions in real-time.

Obesity as an Inflammatory Disease:

Obesity is increasingly recognized not merely as an energy imbalance disorder, but as a chronic inflammatory condition characterized by the accumulation and activation of immune cells within adipose tissue. Adipose tissue macrophages play a central role in this process, shifting from an anti-inflammatory M2 phenotype in lean individuals to a pro-inflammatory M1 phenotype in obesity. These activated macrophages secrete inflammatory cytokines including IL-1 β , TNF- α , and IL-6, which contribute to insulin resistance, metabolic dysfunction, and the perpetuation of chronic inflammation.

The similarities between obese adipose tissue and tumor microenvironments are striking. Both feature chronic inflammation, immune cell infiltration, and the presence of immunosuppressive factors that could potentially interfere with CAR-T cell function. This parallel suggests that strategies developed to overcome CAR-T resistance in cancer might

be directly applicable to obesity treatment, representing an entirely novel therapeutic approach for metabolic diseases.

Research Innovation:

Current research lacks sophisticated model systems capable of recapitulating the complexity of tissue microenvironments while allowing real-time tracking of immune cell behavior. The development of Cancer Immunized Vascularized Assembloids (CIVA) and advanced lineage tracing technologies like Larry barcoding offers unprecedented opportunities to study CAR-T cell fate decisions in controlled yet physiologically relevant systems. These technological advances create the foundation for translating cancer immunotherapy insights to metabolic disease applications.

Methodology

Development of Tumor Microenvironment Models:

My research began with generating Vascular Immune Organoids (VIOs) from induced pluripotent stem cells through a carefully optimized hematopoietic differentiation protocol. This process involved treating iPSCs with specific cytokine cocktails to promote the development of immune, vascular, and stromal cell populations within three-dimensional organoid structures. The resulting VIOs contained diverse cell types, including macrophages, endothelial cells, and fibroblasts, as confirmed through immunofluorescence staining for lineage-specific markers (CD45 for immune cells, CD31 for endothelial cells).

To create more complex tumour microenvironments, I co-cultured VIOs with patient-derived cancer organoids obtained from lung adenocarcinoma samples, generating CIVA assembloids. These assembloids were cultured using gravity-assisted fusion techniques, allowing the spontaneous integration of immune and tumour compartments. The resulting structures were characterized using both immunofluorescence microscopy and histological analysis to confirm proper tissue architecture and cell type distribution.

CAR-T Cell Engineering and Tracking:

For lineage tracing experiments, I employed Larry barcode technology to track individual CAR-T cell clones over time. This involved transducing primary human T cells with

lentiviral vectors carrying unique DNA barcodes alongside CAR constructs. The transduction protocol was optimized to achieve high efficiency while maintaining cell viability, with barcode diversity validated through next-generation sequencing to ensure adequate representation of individual cell lineages.

CAR constructs were designed based on obesity-relevant targets identified through analysis of published CITE-seq datasets. Following established protocols, I activated T cells using anti-CD3/CD28 beads, performed lentiviral transduction, and expanded CAR-T cells in culture media supplemented with IL-2. CAR expression was validated using flow cytometry before proceeding with functional assays.

Multi-omics Analysis Pipeline:

For comprehensive characterization of microenvironment-CAR-T interactions, I employed both single-cell RNA sequencing and spatial transcriptomics approaches. Single-cell suspensions were prepared from CIVA assembloids at multiple time points (days 3, 6, and 12) following CAR-T cell addition. Libraries were constructed using the 10x Genomics platform and sequenced to adequate depth for robust gene expression analysis.

Spatial transcriptomics was performed using the VisiumHD platform, which required careful tissue sectioning and optimization of permeabilization conditions to maintain RNA integrity while enabling efficient capture of spatial gene expression patterns. Computational analysis involved standard preprocessing pipelines, followed by cell type annotation and trajectory analysis to understand CAR-T cell fate decisions over time.

Key Findings

Characterization of IL-1 β + Inflammatory Niches:

My analysis revealed the presence of distinct IL-1 β + macrophage populations within CIVA assembloids that closely recapitulated inflammatory signatures observed in patient tumor samples. Single-cell RNA sequencing identified these macrophages as highly expressing inflammatory markers including IL1B, CCL2, and PTGES2, consistent with the pro-inflammatory M1 phenotype. Importantly, these inflammatory macrophages were specifically enriched in tumor-containing assembloids compared to VIO-only controls,

demonstrating that cancer cells actively promote the formation of immunosuppressive niches.

Spatial transcriptomics analysis using VisiumHD technology revealed the precise spatial organization of these inflammatory niches. IL-1 β ⁺ macrophages co-localised with other immunosuppressive factors, including PD-L1 (CD274) and IL-6, creating defined inflammatory zones within the assembloid architecture. Histological validation by an experienced pathologist confirmed that these structures accurately recapitulated key features of human tumor microenvironments, including the spatial relationship between immune infiltrate and malignant tissue.

To validate the clinical relevance of our findings, I analyzed public datasets from ALK-rearranged non-small cell lung cancer patients and confirmed the presence of similar IL-1 β macrophage signatures in actual patient samples. This correlation between our model system and human disease provided strong evidence for the translational potential of insights gained from CIVA assembloids.

CAR-T Cell Exhaustion Dynamics:

Flow cytometric analysis revealed progressive T cell exhaustion in CIVA co-culture systems, with significant increases in exhaustion markers LAG-3 and PD-1 expression over the experimental time course. Critically, I observed a clear hierarchy of exhaustion induction: T cells cultured alone maintained low exhaustion marker expression (LAG-3: ~20%), while co-culture with VIOs induced moderate exhaustion (LAG-3: ~30%), and co-culture with complete CIVA assembloids resulted in the highest levels of T cell dysfunction (LAG-3: ~45%).

Larry barcode tracing revealed fascinating insights into clonal dynamics during exhaustion development. While some CAR-T cell clones underwent extensive expansion before becoming exhausted, others showed limited proliferation and rapid acquisition of exhaustion markers. This heterogeneity in clonal responses suggests that intrinsic factors, in addition to microenvironmental influences, determine CAR-T cell fate decisions.

Time-course analysis demonstrated that exhaustion marker upregulation began as early as day 3 of co-culture, with progressive increases through day 12. Interestingly, the kinetics of exhaustion varied between different markers, with PD-1 showing earlier upregulation

compared to LAG-3, suggesting distinct regulatory pathways controlling different aspects of T cell dysfunction.

Mechanistic Insights into Microenvironment-Mediated Immunosuppression:

My research identified the IL-1 β -PGE2 axis as a key mechanism driving CAR-T cell exhaustion in CIVA assembloids. Single-cell analysis revealed that IL-1 β ⁺ macrophages highly expressed PTGES2, the enzyme responsible for PGE2 synthesis, creating a local inflammatory environment that suppressed T cell function. This finding aligned with recent literature demonstrating that PGE2 directly inhibits T cell activation and promotes exhaustion marker expression.

Additionally, I discovered that IL-1 β signaling induced the differentiation of inflammatory cancer-associated fibroblasts (iCAFs) within the assembloids. These iCAFs expressed high levels of inflammatory mediators and contributed to the overall immunosuppressive environment. Spatial analysis revealed that iCAFs formed a physical barrier around tumor regions, potentially limiting CAR-T cell infiltration and target access.

Surprisingly, both VIO and CIVA assembloids exhibited oncofetal gene expression programs, characterized by the expression of developmental transcription factors and embryonic signaling pathways. This finding suggests that organoid systems naturally recapitulate aspects of tissue development and repair, which may be relevant to both cancer progression and metabolic dysfunction.

Model Validation and Therapeutic Implications:

Comparative analysis between CIVA assembloids and patient tumor samples demonstrated strong correlation in inflammatory gene signatures, validating the clinical relevance of our model system. The spatial organization of immune infiltrate, the presence of exhausted T cell populations, and the expression of immunosuppressive factors all closely matched patterns observed in human tissue samples.

These findings have direct therapeutic implications, suggesting that IL-1 β blockade could serve as an effective combination therapy to enhance CAR-T efficacy. Literature support for this approach includes recent clinical trials demonstrating improved outcomes when IL-1 β inhibitors are combined with checkpoint blockade immunotherapy.

Applications to Obesity Research

Translational Framework for Obesity CAR-T Therapy:

The mechanistic insights gained from studying CAR-T cell behavior in tumor microenvironments provide a robust foundation for developing obesity-targeted immunotherapies. The identification of IL-1 β inflammatory niches as key drivers of T cell exhaustion is particularly relevant to obesity research, as adipose tissue in obese individuals is characterized by similar inflammatory signatures, including elevated IL-1 β expression by adipose tissue macrophages.

Building on published CITE-seq datasets from Hasty et al. (2022), I identified four promising surface markers specifically expressed on obesity-associated ATMs: CD9, CD36, CD163, and TREM2. These markers showed consistent upregulation in obese versus lean adipose tissue and demonstrated sufficient expression levels for potential CAR targeting. Importantly, analysis of healthy tissue expression patterns suggested minimal off-target effects, supporting the therapeutic window for ATM-directed CAR-T therapy.

CAR Design Strategy and Dual Mechanism Approach:

My proposed CAR-T strategy incorporates a dual mechanism of action: direct ATM depletion through CAR-mediated cytotoxicity, combined with anti-TGF- β payload delivery to inhibit adipocyte maturation. This approach addresses both the inflammatory component of obesity (through ATM elimination) and the metabolic component (through prevention of white adipocyte accumulation).

The anti-TGF- β component is critical because TGF- β signaling is essential for adipocyte differentiation and maturation. By delivering TGF- β inhibitors specifically to adipose tissue sites through CAR-T cells, we could potentially prevent the formation of new adipocytes while eliminating the inflammatory macrophages that perpetuate metabolic dysfunction.

Safety considerations include the potential for systemic immune activation and the need for careful dose escalation to avoid off-target effects. However, the lessons learned from

CAR-T exhaustion in tumor models suggest strategies for maintaining CAR-T persistence while limiting excessive activation.

Model System Adaptation:

The CIVA organoid platform could be readily adapted for obesity research by incorporating adipocyte organoids with VIOs to create metabolically relevant assembloids. These "adipose immunized vascularized assembloids" would enable real-time study of CAR-T behavior in inflammatory adipose environments, providing a platform for optimizing CAR design and combination therapies before advancing to animal studies.

Patient-specific organoid testing represents a particularly exciting application, potentially allowing personalized assessment of CAR-T efficacy before clinical administration. This approach could help identify patients most likely to benefit from therapy while minimizing exposure to ineffective treatments.

Discussion and Future Directions

Significance and Innovation:

This research represents the first systematic application of cancer immunotherapy insights to metabolic disease, opening an entirely new field of investigation. The development of CIVA assembloids provides a broadly applicable platform technology for studying immune-microenvironment interactions across diverse disease contexts. The demonstration that IL-1 β inflammatory niches drive CAR-T exhaustion has immediate therapeutic implications, suggesting combination approaches that could enhance immunotherapy efficacy in both cancer and metabolic diseases.

The precision medicine implications are particularly significant. Patient-specific organoid testing could revolutionize treatment selection by providing functional readouts of therapeutic efficacy before clinical administration. This approach could dramatically improve success rates while reducing healthcare costs and patient morbidity.

Limitations and Future Research:

Several limitations must be addressed in future studies. In vitro model systems, while sophisticated, cannot fully recapitulate the complexity of in vivo microenvironments,

including systemic immune responses and tissue-specific factors. The relatively short timeframe of organoid experiments limits our ability to assess long-term CAR-T persistence and memory formation, which are critical for sustained therapeutic efficacy.

Species differences between mouse-derived data and human biology represent another challenge, particularly given that many obesity-related pathways show species-specific regulation. Careful validation in humanized mouse models will be essential before advancing to clinical testing.

Future research priorities include in vivo validation studies using diet-induced obesity mouse models, development of manufacturing protocols for clinical-grade CAR-T production, and biomarker identification for predicting treatment response. The potential expansion to other metabolic diseases, including type 2 diabetes and non-alcoholic fatty liver disease, represents an exciting avenue for broader therapeutic impact.

Broader Implications:

This work positions immunotherapy as a potentially transformative approach for metabolic diseases, expanding the field beyond its traditional cancer focus. The identification of shared inflammatory pathways between cancer and obesity suggests that many immunotherapeutic strategies could have dual applications, accelerating development timelines and reducing costs.

The implications for fundamental immunology are equally important. Understanding how tissue microenvironments shape immune cell fate decisions provides insights into both pathological processes and normal immune homeostasis. These findings could inform therapeutic approaches for autoimmune diseases, aging-related immune dysfunction, and vaccine development.

Conclusion

This research demonstrates the power of cross-disciplinary approaches in advancing therapeutic innovation, showing how insights from cancer immunotherapy can be systematically applied to address other major health challenges. Through the development

of sophisticated model systems and advanced cell tracking technologies, I have not only advanced our understanding of CAR-T cell biology but also opened new avenues for treating metabolic diseases.

The identification of IL-1 β inflammatory niches as key determinants of immune cell fate provides actionable targets for combination therapies, while the CIVA organoid platform offers a versatile tool for future immunotherapy development. The successful demonstration that cancer-derived insights can inform obesity therapeutic strategies validates the broader principle that mechanistic understanding in one disease context can accelerate progress in others.

My experience conducting this research has been transformative, providing hands-on experience with cutting-edge technologies while fostering interdisciplinary collaboration skills. Working at the interface of cancer biology, immunology, and metabolism has broadened my scientific perspective and reinforced my commitment to translational research approaches.

Looking forward, the potential global health impact of successful obesity CAR-T therapy could be enormous, given the worldwide prevalence of metabolic diseases and the limited efficacy of current treatments. This work establishes the scientific foundation for advancing this novel therapeutic approach toward clinical testing, while the CIVA platform provides a valuable tool for the broader immunotherapy research community.

The vision of precision medicine approaches using patient-specific organoid testing to optimize therapeutic outcomes is now within reach, promising to transform how we develop and deploy immunotherapies across diverse disease contexts. As we continue to expand immunotherapy applications beyond traditional oncology contexts, the principles and platforms developed through this research will serve as valuable guides for future innovation.