RNA-seq, ATAC-seq, and CRISPR Screen Analysis Reveals IL-4 Regulation of CART Cell Exhaustion

Carli M. Stewart, Elizabeth L. Siegler, R. Leo Sakemura, Michelle J. Cox, Truc Huynh, Brooke Kimball, Long Mai, Ismail Can, Claudia Manriquez Roman, Kun Yun, Olivia Sirpilla, James H. Girsch, Ekene Ogbodo, Wazim Mohammed Ismail, Alexandre Gaspar-Maia, Justin Budka, Jenny Kim, Nathalie Scholler, Mike Mattie, Simone Filosto & Saad S. Kenderian

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Introduction

While Chimeric Antigen Receptor T-cell (CAR-T) therapy is a promising approach for the treatment of hematological malignancies, its effectiveness is often limited by cell exhaustion. This study investigates CAR T-cell exhaustion using a three-pronged approached: (1) a genome-wide CRISPR knockout screen, (2) RNA-seq and ATAC-seq analysis on baseline and exhausted CAR T-cells, and (3) RNA-seq and ATAC-seq analysis on preinfusion CAR T-cell products from responders and non-responders in the ZUMA-1 trial. This study successfully identified interleukin-4 (IL-4) as a key driver of CAR T-cell dysfunction, demonstrating that the targeting of IL-4 serves to improve antitumor efficacy and reduce exhaustion. Through cutting-edge **RNA-seq, ATAC-seq, and CRISPR screening** services, CD Genomics enabled comprehensive analyses that procured insights into the molecular drivers of CAR T-cell exhaustion.

Methods

RNA-sequencing

- RNA-seq was performed on healthy donor CART cells and pre-infusion axi-cel samples. RNA was isolated using the miRNeasy Micro kit (Qiagen) for healthy donor samples and from frozen pellets for axi-cel samples. Sequencing was done on the Illumina NovaSeq S4 platform..

ATAC-sequencing

- 1 × 10^5 cells were processed for ATAC-seq library preparation using the Nextera kit and sequenced on HISEQ 4000.

CRISPR Screen Sequencing

- Genomic DNA was isolated from CART cells, followed by library PCR and sequencing on the PE150 platform.

Data Analysis

ATAC-seq Data Analysis Summary:

- QC: FASTQC for quality check, Cutadapt for adapter trimming (min. 45 bp).
- Mapping: Bowtie2 for read alignment to hg38.
- Cleaning: Samtools for sorting, BAMQC for mitochondrial read removal.
- Peak Calling: MACS2 (broad peaks).
- Differential Analysis: DESeq2 in DiffBind (FDR < 0.05 for healthy, p-value < 0.05 for patient).
- Annotation: ChIPSeeker.
- Motif Analysis: MEME suite.
- Visualization: UCSC genome browser with BigWig files.

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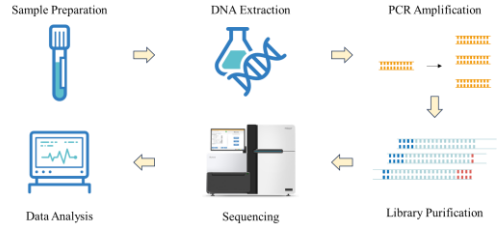
Workflow and Results

RNA-Seq Workflow in CD Genomics

CD Genomics conducts **RNA-Seq** through sample preparation, RNA library preparation, and sequencing with Illumina NovaSeq 6000 or PacBio systems. To ensure reliable results, each step includes a rigorous quality control procedure covering samples, libraries, and data. This is followed by bioinformatics analysis, including de novo assembly, gene annotation, differential expression analysis, and novel transcript discovery

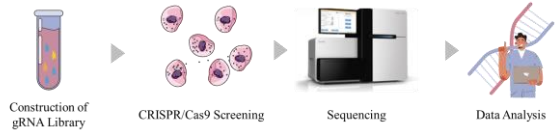
ATAC-Seq Workflow in CD Genomics

CD Genomics provides **ATAC-Seq** services for the analysis of chromatin accessibility. The workflow includes DNA fragmentation, adapter ligation, PCR amplification, sequencing, and bioinformatics analysis (such as peak calling, annotation, and motif discovery). Leveraging the Illumina sequencing platform, the ATAC-seq service supports the study of gene regulation and epigenetics, with customized analysis to meet research needs.



CRISPR Screen Sequencing Workflow in CD Genomics

The **CRISPR Screen Sequencing** workflow offered by CD Genomics consists of gRNA library construction, CRISPR/Cas9 screening, selection, sequencing, and comprehensive bioinformatics analysis. Using Illumina HiSeq platforms for paired-end sequencing, processes feature raw data quality control, reference alignment, sgRNA abundance analysis, and differential analysis. The CRISPR Screening service enables researchers to identify gene knockouts linked to specific phenotypes.



REFERENCES
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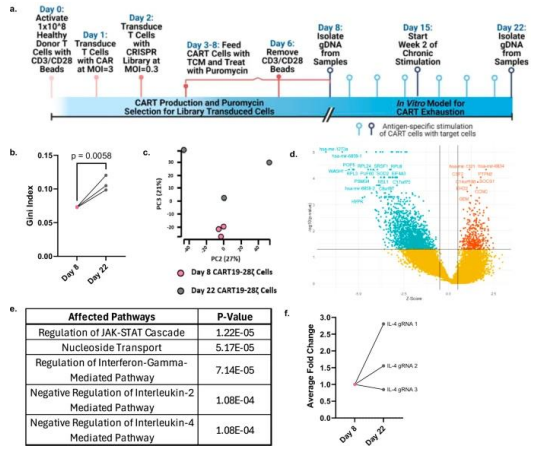
CRISPR Screen Reveals IL-4 Pathway in CART Cell Exhaustion

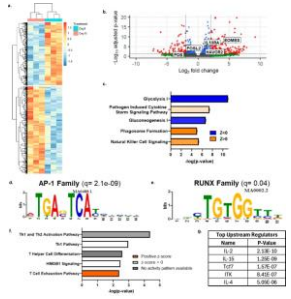
A genome-wide CRISPR knockout screen was performed on healthy donor CART19-28ζ cells to uncover genes and pathways involved in exhaustion. The screen identified several positively selected genes linked to CART dysfunction, including CSF2, SOCS1, and PTPN2, which have been previously associated with improved CART cell function upon knockout. Gene ontology analysis highlighted several cytokine signaling pathways as being central to exhaustion, particularly the IFN-γ, IL-2, and IL-4 pathways. Additionally, ingenuity pathway analysis revealed the IL-4 receptor (IL4R) as a top regulator, with enhanced representation of gRNAs targeting IL-4 in chronically stimulated samples, suggesting a critical role of the IL-4 pathway in CAR T-cell exhaustion.



Affected Pathways	P-Value
Regulation of JAK-STAT Cascade	1.22E-05
Nucleoside Transport	5.17E-05
Regulation of Interferon-Gamma-Mediated Pathway	7.14E-05
Negative Regulation of Interleukin-2 Mediated Pathway	1.08E-04
Negative Regulation of Interleukin-4 Mediated Pathway	1.08E-04

IL-4 as an Upstream Regulator of CART Cell Exhaustion: Transcriptomic and Epigenetic Insights

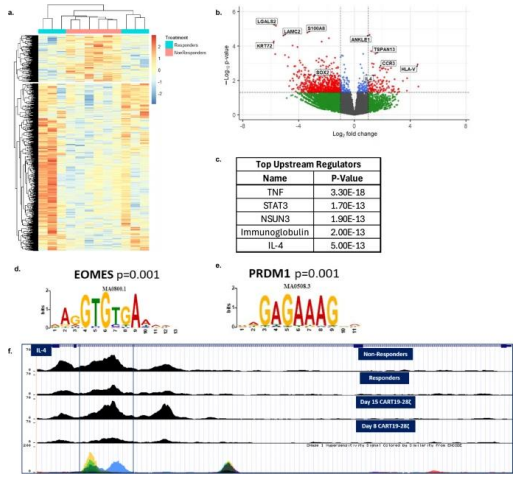
RNA sequencing and **ATAC sequencing** of baseline and chronically stimulated CART19-28ζ cells revealed transcriptomic and chromatin accessibility changes associated with exhaustion, including upregulation of exhaustion markers like EOMES and TIM-3. Pathway analysis identified cytokine signaling, particularly IL-4, as crucial for exhaustion; further analysis showed that chronic stimulation led to increased IL-4 production in CD8+ CART cells, while Th1/Th2 polarization shifted towards Th1, confirming IL-4's role as an upstream regulator of exhaustion.



We also provide other related services, such as **ChIP-Seq**, **MeDIP-Seq**, **Bacterial RNA Sequencing** and more, to support your research needs.

IL-4 Enrichment in CART Cell Products from Non-Responders

RNA sequencing and **chromatin accessibility analysis** of pre-infusion axi-cel products from responders and non-responders in the ZUMA-1 clinical trial revealed that non-responders showed increased IL-4 expression and chromatin accessibility at the IL-4 locus. Motif analysis and chromatin accessibility at exhaustion-related loci such as PDCD1 and TIM-3 mirrored the changes observed in chronically stimulated healthy donor CART cells. These findings suggest that IL-4-related epigenetic changes contribute to CART cell exhaustion and treatment failure in non-responders.



Top Upstream Regulators	
Name	P-Value
TNF	3.30E-18
STAT3	1.70E-13
NSUN3	1.90E-13
Immunoglobulin	2.00E-13
IL-4	5.00E-13

Conclusion

- This study highlights the role of IL-4 in the development of CAR T-cell exhaustion and its potential as a therapeutic target for the enhancement of CAR T-cell efficacy.
- Through CD Genomics' **RNA-seq** and **ATAC-seq** services, the research team identified IL-4 as a key regulator of CAR T-cell exhaustion, revealing that its presence induces a transcriptional and epigenetic signature of exhaustion in both preclinical and clinical models.
- Furthermore, the study demonstrates that neutralizing IL-4–either through genetic knockdown or monoclonal antibodies–significantly improves antitumor activity and reduces signs of exhaustion in CAR T-cells. These findings offer a promising approach for enhancing the therapeutic efficacy of CAR T-cell therapies.

* For research purposes only. Not intended for clinical diagnosis, treatment, or individual health assessments. .