Metabolic Autonomy of Cytotoxic Programs Distinguishes Responder from Non-Responder CAR-T Effectors in DLBCL: A Single-Cell Co-Expression Network Analysis

E. KALEMOGLU, MD, PhD[1] ; **A. TURUNC, MD**[2]; **A. SEKHRI, MD**[1]

1. RUTGERS – Jersey City Medical Center, Jersey City, NJ, USA
2. Cukurova University, Turkey

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INTRODUCTION

Chimeric antigen receptor (CAR) T-cell therapy has transformed treatment of relapsed/refractory diffuse large B-cell lymphoma (DLBCL), yet approximately half of patients fail to achieve durable responses.

Whether transcriptionally similar effector populations in responder (R) and non-responder (NR) infusion products differ in their internal metabolic–cytotoxic coupling architecture **remains unknown**.

AIM

To determine whether R and NR CAR-T effectors differ in metabolic–cytotoxic gene co-expression wiring, **independent of cluster identity**, using single-cell RNA-seq.

20
DLBCL patients · 12 responders / 8 non-responders

81,143
CAR-T cells profiled · CD3D/CD3E co-expression

GSE197268
Public single-cell cohort · 18 axi-cel / 2 tisa-cel

METHODS

- Single-cell RNA-seq of CAR-T **infusion products** from 20 DLBCL patients (12 R, 8 NR; 18 axicabtagene ciloleucel, 2 tisagenlecleucel), GSE197268.
- T cells (81,143) identified by CD3D/CD3E co-expression; processed with **Seurat v5**.
- Spearman correlations between glycolytic enzymes (GAPDH, PKM, ENO1, ALDOA, LDHA) and cytotoxicity genes (GZMB, GZMA, NKG7, PRF1) computed **separately for R and NR**; nine enzyme–gene pairs were carried forward for permutation testing.
- Differences assessed by permutation testing (10,000 shuffles, Benjamini–Hochberg correction), bootstrap resampling and Fisher r-to-z transformation.
- Mitochondrial OXPHOS coupling and a composite patient-level biomarker also evaluated.

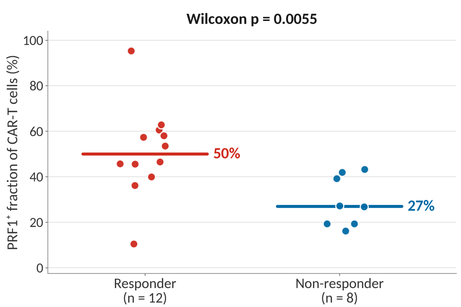
CONCLUSIONS

Responder CAR-T effectors in DLBCL achieve **metabolic autonomy**, maintaining cytotoxic machinery independent of metabolic state, while non-responder effectors exhibit **dual metabolic dependency** rendering them vulnerable to nutrient-depleted tumor microenvironments.

This co-expression wiring framework provides a phenotype-independent biomarker dimension for infusion product quality and points toward metabolic conditioning during manufacturing as a potential strategy to improve CAR-T outcomes in aggressive B-cell lymphoma.

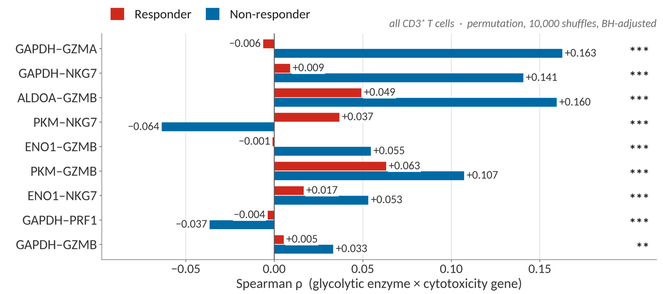
RESULTS

A. PRF1⁺ effector fraction



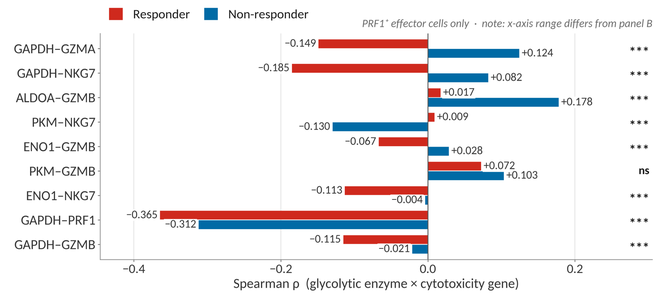
A. PRF1⁺ fraction of each infusion product, one point per patient. Median 50% in responders vs 27% in non-responders (p = 0.0055).

B. Glycolysis × cytotoxicity coupling — all T cells



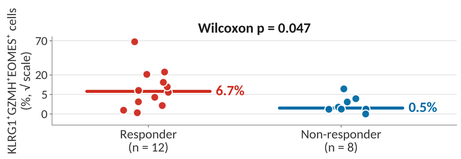
B. Spearman ρ computed separately in R and NR across all CD3⁺ T cells. All nine pairs differed significantly (permutation, 10,000 shuffles, BH-adjusted; all adj. p < 0.005), including GAPDH–GZMA (−0.006 vs +0.163) and ALDOA–GZMB (+0.049 vs +0.160). Cluster identity is not used. *** adj. p < 0.001; ** adj. p < 0.005.

C. Same nine pairs within PRF1⁺ effectors



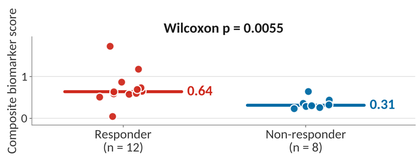
C. Restricting to PRF1⁺ effectors amplifies the difference: 8 of 9 pairs reach adj. p < 0.001 and responder ρ shifts downward in 8 of 9 pairs, turning negative for all six GAPDH and ENO1 pairs — cytotoxic output is uncoupled from glycolytic flux. *** adj. p < 0.001; ns = not significant.

D. Terminally differentiated effectors



D. KLRG1⁺GZMH⁺EOMES⁺ effectors are enriched in responder products: median 6.7% vs 0.5% (p = 0.047).

E. Composite biomarker



E. PRF1⁺ fraction combined with coupling separates R from NR (p = 0.0055).

KEY FINDINGS

- **PRF1⁺ fraction** is higher in responder infusion products (median 50% vs 27%, p = 0.0055).
- **All nine glycolysis–cytotoxicity pairs** differ between R and NR (adj. p < 0.005), computed without using cluster identity.
- **Responder coupling shifts negative** within PRF1⁺ effectors (8/9 pairs adj. p < 0.001; ρ negative for all six GAPDH and ENO1 pairs) — active metabolic–cytotoxic decoupling.
- **Dual dependency in non-responders:** within the terminally differentiated effector cluster, OXPHOS transcripts also track killing genes only in NR (MT-ATP8–GZMB +0.027 vs +0.217; MT-ND6–NKG7 −0.094 vs +0.100).
- **Composite biomarker** integrating PRF1⁺ fraction and coupling predicts response (p = 0.0055).

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CONTACT INFORMATION

Ecem Kalemoglu, MD, PhD
RUTGERS – Jersey City Medical Center
Jersey City, NJ, USA
ecem.kalemoglu@rutgers.edu

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