Application Brief

Immunotherapy

Agilent

Trusted Answers

Improved CAR-NK Metabolic Fitness Leads to More Persistent Killing and is Predictive of Superior Clinical Outcomes

Author

Hannah Simmonds, PhD
Reema Vazirani, PhD
Agilent Technologies, Inc.

Introduction

The development of cell-mediated immunotherapies is saving patients' lives and has revolutionized both cancer research and the study of the immune system. One of the most promising types of cell therapy involves the genetic engineering of immune cells to express novel chimeric antigen receptors (CARs), to target and destroy cancer cells. While T cells are often used, recent studies have highlighted the potential benefits of using engineered natural killer (NK) cells and other cell types for cancer treatments.[1,2]

Metabolism has emerged as a key driver of immune cell fate and function. NK cells require both glycolysis and oxidative phosphorylation for NK cell activation, proliferation, and effector functions. Metabolic fitness is, therefore, an important determinant of effective antitumor NK cell responses.[3] Understanding immune cell metabolism and how to modulate immunometabolism is consequently critical to optimizing immune cell therapies.

Here we review a 2024 publication highlighting a clinical trial of CAR19/IL-15 NK cells by David Marin and colleagues at the University of Texas MD Anderson Cancer Center in Houston, Texas, USA.[4] During the trial, they evaluated the functional metabolism and serial killing capabilities of CAR-NK cells generated from umbilical cord blood units (CBUs). Their studies allowed them to identify CBU characteristics that can be used to select the donor cord samples most likely to induce a clinical response. Their findings uncover new features of CAR-NK cell biology, and underscore the importance of metabolism and functional phenotyping in the optimizing development of CAR-immunotherapies.

Results and discussion

In the phase 1/2 clinical trial, 37 subjects with CD19+ B cell malignancies received an infusion of cord blood unit (CBU)-derived NK cells expressing anti-CD19 CAR, interleukin 15 (IL-15), and inducible caspase-9. Their primary objective was to assess the safety and efficacy of CAR19/IL-15 NK cells.[4] They observed overall response rates at both day 30 and day 100 of 48.6%. These responses were also durable, with nine of the ten individuals who had achieved a complete response by day +30 remaining in remission on day +180.[4] What is more, they observed no notable toxicities such as cytokine release syndrome, neurotoxicity, or graft-versus-host disease. Further analyses were performed to identify CBU characteristics associated with favorable outcomes, and to define optimal cords (Opt-Cs) versus suboptimal cords (Sub-Cs).

Analysis of mitochondrial metabolism and glycolytic activity using the Agilent Seahorse XF Pro analyzer showed higher basal-oxidative phosphorylation and maximal respiration rates in CAR19/IL-15 NK cells derived from Opt-Cs, compared to those from Sub-Cs with no difference in their glycolytic capacity (Figure 1). These results were replicated in CAR19/IL-15 NK cells produced from an independent cohort of 12 further CBUs.[4] Together, these findings indicate that CAR19/IL-15 NK cells from Opt-Cs have superior mitochondrial fitness relative to Sub-Cs.

In in vivo mouse studies, Marin et. al. observed increased persistence and enhanced antitumor activity from CAR19/IL-15 NK from Opt-Cs, compared to those from Sub-Cs (see Figure 9 in the cited publication[4]). This is consistent with previous studies where CAR T cell-enhanced mitochondrial fitness has often been associated with increased in vitro persistence.[5,6] Conversely, impaired mitochondrial respiration has previously been observed to be a driver of exhaustion in T cells, further demonstrating the importance of cellular metabolism in immune cell function.[7]

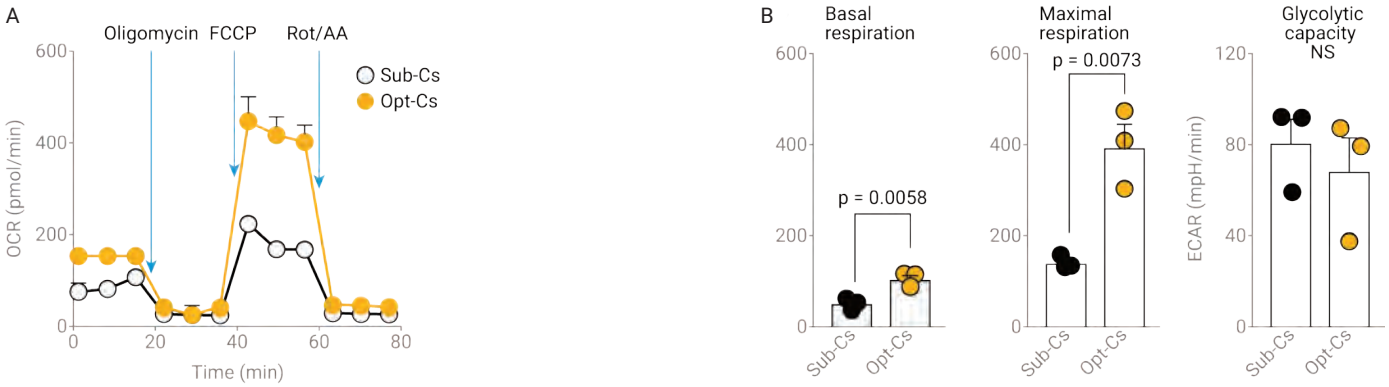

Figure 1. CAR19/IL-15 CBU NK cells from Opt-Cs have superior metabolic fitness compared to those from Sub-Cs. (A) Agilent Seahorse XF Mito Stress Test of CAR19/IL-15 NK cells from Opt-Cs versus Sub-Cs, bar graphs of basal respiration (middle) and maximal respiration (right). (B) Bar graphs of basal respiration (left) and maximal respiration (center), and glycolytic capacity measured by Agilent Seahorse XF Glycolysis Stress Test (right) of CAR19/IL-15 NK cells from Opt-Cs versus Sub-Cs.

Figure reproduced from Marin, D., Li, Y., Basar, R. et al. Safety, efficacy, and determinants of response of allogeneic CD19-specific CAR-NK cells in CD19+ B cell tumors: a phase 1/2 trial. Nat Med 30, 772–784 (2024). Figure used under the Creative Commons Attribution 4.0 International License. *Note: Future NK cell metabolism studies can take advantage of the Agilent Seahorse XF NK Cell Metabolic Profiling assay, which delivers a comprehensive set of mitochondrial and glycolytic measurements within the same experiment.

They also showed that CAR19/IL-15 NK cells from Opt-Cs were able to avoid exhaustion and demonstrate greater long-term cytotoxicity using in vitro tumor rechallenge assays. While CAR19/IL-15 NK cells from both Opt-Cs and Sub-Cs were both effective at killing Raji tumor cells during the first tumor challenge, cells from Sub-Cs could not control subsequent tumor growth after rechallenges (Figure 2).[4]

Moreover, individuals who received CAR19/IL-15 NK cells from Opt-Cs also had a significantly superior probability of one-year progression-free survival at 69%, compared with 5% for subjects who received cells from Sub Cs (Figure 3).[4] This drastic improvement was also observed for one-year overall survival, with 94% overall survival observed for individuals who received CAR19/IL-15 NK cells from Opt-Cs, compared to 48% for those who received cells derived from Sub-Cs (Figure 3).[4,8] This approximately two-fold enhancement in overall survival further demonstrates the greater clinical activity of CAR-NK cells generated from Opt-Cs and the importance of identifying optimal donor material characteristics for cell therapy product development.

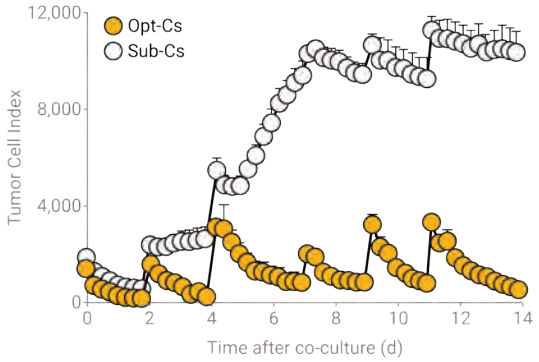

Figure 2. Tumor rechallenge assay with CAR19/IL-15 NK cells from either Sub-Cs or Opt-Cs (effector-to-target ratio of 5:1). Raji tumor cells (100,000 cells) were added every 2 to 3 days; target killing was measured by mCherry detection.

Figure reproduced from Marin, D., Li, Y., Basar, R. et al. Safety, efficacy, and determinants of response of allogeneic CD19-specific CAR-NK cells in CD19+ B cell tumors: a phase ½ trial. Nat Med 30, 772–784 (2024). Figure used under the Creative Commons Attribution 4.0 International License.

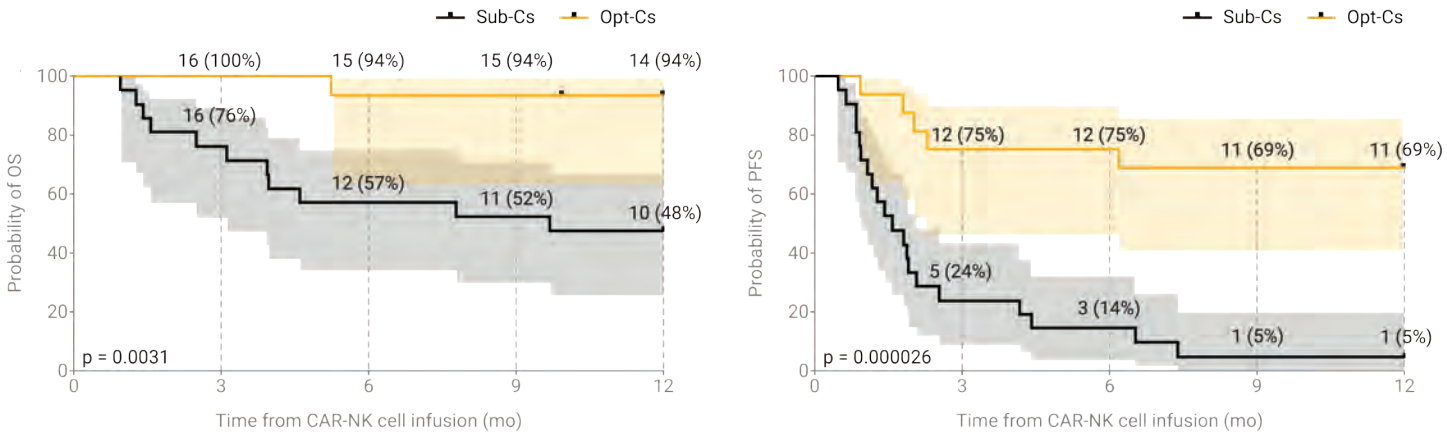

Figure 3. Kaplan–Meier curves showing the overall survival and the progression-free survival of subjects who received CAR19/IL-15 NK cell therapy derived from Opt-Cs versus Sub-Cs. The shaded areas represent 95% CI of survival probability.

Figure reproduced from Marin, D., Li, Y., Basar, R. et al. Safety, efficacy, and determinants of response of allogeneic CD19-specific CAR-NK cells in CD19+ B cell tumors: a phase 1/2 trial. Nat Med 30, 772–784 (2024). Figure used under the Creative Commons Attribution 4.0 International License.

Conclusion

The study by Marin et. al. was one of the first to demonstrate the close correlation between metabolic fitness and in vivo clinical results. It shows that immune cell product quality is a key predictor of clinical outcome. Immune cell metabolic phenotype and serial killing ability are key factors to be considered in the selection of the best quality immune cell products, which can avoid exhaustion and maintain effector function.[4] The critical importance of delineating these functional capabilities is highlighted by the dramatic clinical differences between individuals treated with Opt-C and those treated with Sub-C-derived CAR-NK cells.

This work substantiates a large body of research indicating the importance of cellular metabolism in immune cell fitness and function. Agilent Seahorse XF and Agilent xCELLigence RTCA technologies are recognized as leading tools for the study of immune cell metabolism and killing potency, providing opportunities to optimize cell therapy products and achieve meaningful outcomes.

David Marin, the University of Texas MD Anderson Cancer Center, and the other authors of this study are not affiliated or associated with Agilent.

References

1. Moscarelli, J.; Zahavi, D.; Maynard, R.; Weiner, L.M. The Next Generation of Cellular Immunotherapy: Chimeric Antigen Receptor-Natural Killer Cells. *Transpl. Cell. Ther.* **2022**, *28*(10), 650-656.
2. Wang, W.; Liu, Y.; He, Z.; Li, L.; Liu, S.; Jiang, M.; Zhao, B.; Deng, M.; Wang, W.; Mi, X.; et al. Breakthrough of Solid Tumor Treatment: CAR-NK Immunotherapy. *Cell Death Discov.* **2024**, *10*(1), 40.
3. Choi, C.; Finlay, D.K. Optimising NK Cell Metabolism to Increase the Efficacy of Cancer Immunotherapy. *Stem Cell Res. Ther.* **2021**, *12*(1), 320.
4. Marin, D.; Li, Y.; Basar, R.; Rafei, H.; Daher, M.; Dou, J.; Mohanty, V.; Dede, M.; Nieto, Y.; Uprety, N.; et al. Safety, Efficacy and Determinants of Response of Allogeneic CD19-Specific CAR-NK Cells in CD19+ B Cell Tumors: A Phase 1/2 Trial. *Nat. Med.* **2024**, *30*(3), 772-784. Figures reproduced from Marin, D. et al. Under the Creative Commons Attribution 4.0 International License http://creativecommons.org/licenses/by/4.0/.
5. Kawalekar, O. U.; O'Connor, R. S.; Fraietta, J. A.; Guo, L.; McGettigan, S. E.; Posey Jr. A. D.; Patel, P.R.; Guedan, S.; Scholler, J.; Keith, B.; et al. Distinct Signaling of Coreceptors Regulates Specific Metabolism Pathways and Impacts Memory Development in CAR T Cells. *Immunity.* **2016**, *44*(2), 380-390.
6. López-Cantillo, G.; Urueña, C.; Camacho, B. A.; Ramírez-Segura, C. CAR-T Cell Performance: How to Improve Their Persistence? *Front. Immunol.* **2022**, *13*, 878209.
7. Wu, H.; Zhao, X.; Hochrein, S. M.; Eckstein, M.; Gubert, G. F.; Knoepper, K.; Mansilla, A.M.; Oener, A.; Doucet-Ladeveze, R.; Schmitz, W.; et al. Mitochondrial Dysfunction Promotes the Transition of Precursor to Terminally Exhausted T Cells Through HIF-1α-Mediated Glycolytic Reprogramming. *Nat. Commun.* **2023**, *14*(1), 6858.
8. Romero, D. Promising Early Results with CAR NK Cells. *Nat. Rev. Clin. Oncol.* **2024**, *21*(3), 168-168.

www.agilent.com/lifesciences/cellanalysis

Published in the USA, August 30, 2024
5994-7661EN

Agilent
Trusted Answers