

UNDERSTANDING IMMUNE CELL FUNCTION IN CANCER



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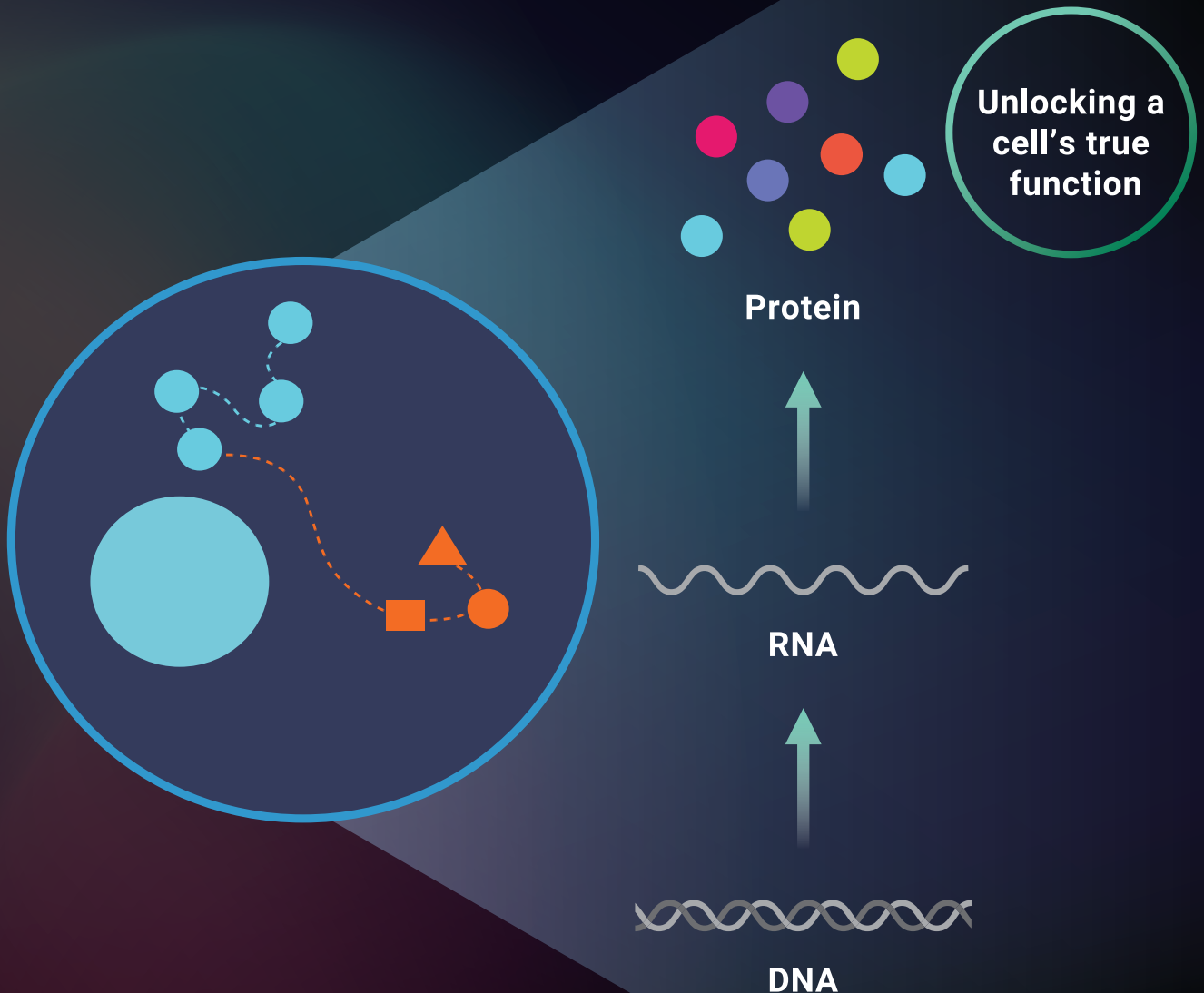
How Immune Cell
Response Relates
to Prognosis





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A LINK BETWEEN POLYFUNCTIONALITY AND PERSISTENCE

Chimeric antigen receptor (CAR) T cell therapy has shown promise against hematological cancers, leading to FDA approval of two CAR-T cell products targeting CD19 B cell malignancies. However, CAR-T cell efficacy for solid tumor cancers remains a significant challenge. To overcome this obstacle, scientists are working to identify new solid tumor targets and improve CAR engineering. In a recent study published in *Gastroenterology*,¹ Li et al. from the National Institutes of Health National Cancer Institute engineered two novel CARs targeting glypican 3 (GPC3) and used them to eliminate GPC3-positive hepatocellular carcinoma (HCC) cells in a mouse xenograft model.

GPC3 is a cell surface protein expressed in more than 70% of HCC, as well as other solid tumors including hepatoblastoma and lung squamous cell carcinoma. It promotes tumor growth by modulating Wnt/Frizzled signaling. Importantly, GPC3 is not expressed in non-malignant adult tissues, including normal hepatocytes. These facts make it an attractive target for anti-HCC therapeutic approaches. Indeed, GPC3-targeting CAR-T cells have already yielded modest results in a Phase I clinical trial.²

Researchers believe that modifying the CAR construct, especially the antibody domain, may lead to better functional efficacy. Li et al. used two antibodies, HN323 and humanized YP7 (hYP7), to engineer CAR-T cells against GPC3. HN323 and hYP7 both show high binding affinity for GPC3, but at different

locations of the protein; HN323 binds the N-lobe while hYP7 binds the C-lobe. In initial experiments, hYP7 CAR-T cells showed more lytic activity than HN323 CAR-T cells, and also produced more cytokines and chemokines than HN323 CAR-T cells in the presence of HepG2 and Hep3B liver cancer cells.

This finding led the researchers to investigate cellular polyfunctionality, which has been positively linked with beneficial long-term immune responses in certain clinical settings.³ Polyfunctionality is defined as the ability of an individual cell to produce two or more cytokines simultaneously, and is measured using the polyfunctional strength index (PSI).³ Through IsoPlexis' platform, the researchers employed a 32-plex panel probing key effector, stimulatory, chemoattractive, regulatory, and inflammatory cytokines to find that hYP7 CAR-T cells were more polyfunctional than HN323 CAR-T cells. Additionally, CD8+ hYP7 CAR-T cells were considerably more polyfunctional than CD4+ counterparts when co-cultured with liver cancer cell lines. Li et al. identified small polyfunctional CD8+ hYP7 CAR-T cell subsets producing granzyme B, INF- γ , perforin, and s4-1BB, indicating that the perforin/granzyme apoptosis pathway might be activated by hYP7 CAR-T cells.

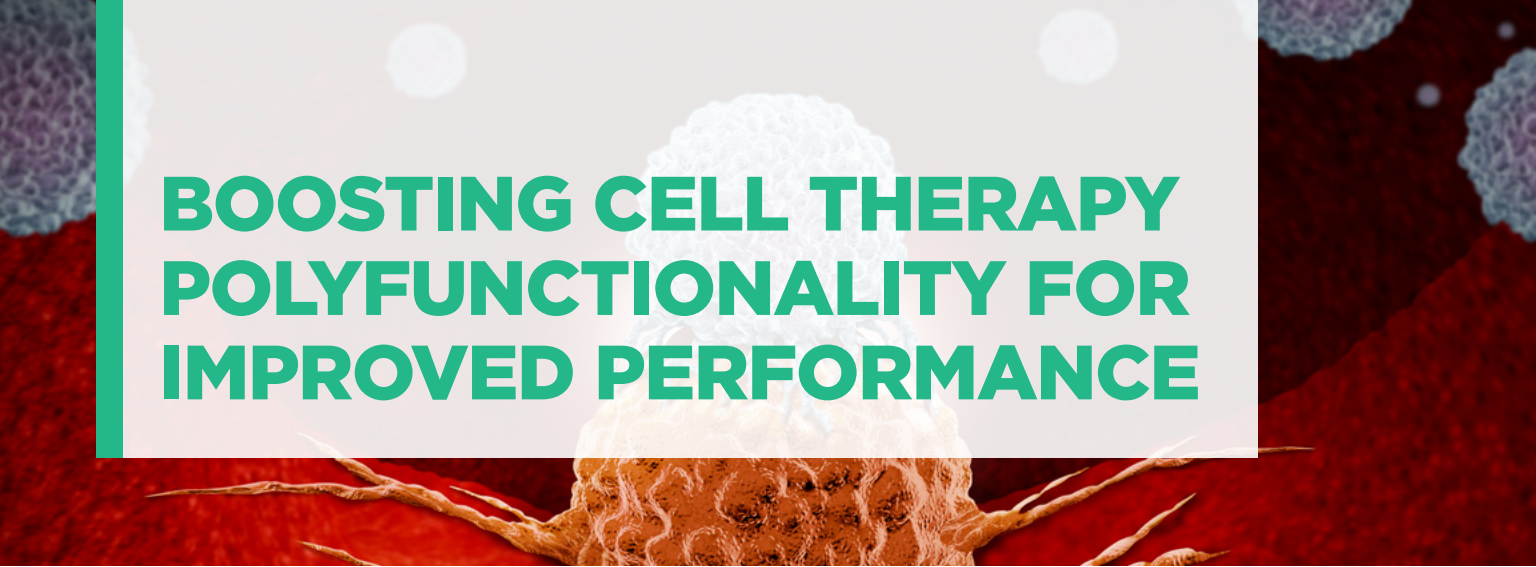
Following in vitro characterization, both hYP7 and HN323 CAR-T cells were injected into intraperitoneal and orthotopic xenograft animal models of liver cancer. Animals injected with the hYP7 CAR-T cells showed reduced tumor burden, but animals receiving HN323 CAR-T

cells did not. The hYP7 CAR-T cells also showed much greater in vivo persistence than HN323 counterparts, and mice treated with hYP7 CAR-T cells did not develop tumors even after additional Hep3B cells were introduced following elimination of the original tumor. Similar to in vitro results, CD8+ hYP7 CAR-T cells recovered from murine spleens exhibited significantly higher lytic activity than CD4+ counterparts in the presence of Hep3B cells, as well as increased polyfunctionality.

In this study, Li et al. demonstrated that hYP7 CAR-T cells targeting GPC3 can induce sustained HCC tumor regression in mice. They highlighted the possibilities of CAR engineering, the importance of antibody affinity, and the relationship between cellular polyfunctionality, persistence, and efficacy. Most importantly, their work offers a potential therapeutic roadmap for treating solid tumors using CAR-T cells.

References

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BOOSTING CELL THERAPY POLYFUNCTIONALITY FOR IMPROVED PERFORMANCE

Adoptive cell transfer (ACT) is a promising anti-cancer therapeutic approach. Conventionally, ACT entails transfusing tumor-infiltrating lymphocytes (TILs) or T cell receptor (TCR)-engineered blood T cells into patients, along with high doses of interleukin-2 (IL-2) to support the expansion and function of the transferred cells in the body. However, at high doses, IL-2 induces toxicity and immunosuppression, creating tolerance problems for patients and limiting the clinical use of ACT. In a study published in *Nature Communications*, Parisi et al. from the University of California, Los Angeles presented an alternative for IL-2 by demonstrating that a novel agonist, NKTR-214, outperforms IL-2 for improving ACT anti-tumor efficacy.

NKTR-214 (also known as bempegaldesleukin or BEMPEG) is a kinetically engineered IL-2 receptor agonist with the same amino acid sequence as IL-2. However, NKTR-214 is conjugated to multiple releasable polyethyleneglycol (PEG) chains. To examine how NKTR-214 performs relative to IL-2 in combination with ACT, Parisi et al. implanted murine melanoma cells into lymphodepleted mice. The researchers then injected T cells obtained from separate donor mice into these tumor-bearing mice, either alone, with IL-2, or with NKTR-214. The mice treated with NKTR-214 showed significantly less tumor growth than untreated and IL-2-treated counterparts, while exhibiting no significant immune-related adverse

events such as toxicity. Mice treated with ACT and NKTR-214 together showed greater T cell expansion and tumor infiltration than untreated and IL-2-treated ACT mice, indicating that NKTR-214 administration reduced tumor growth by promoting T cell activation and persistence in the tumor.

The researchers next examined the phenotypes of T cells involved in anti-tumor responses by examining intracellular proteins with immunohistochemistry, surface proteins with mass cytometry, and extracellular protein expression with single-cell functional proteomics. They found three main T cell subpopulations within murine tumors: CD8⁺ T cells, CD4⁺ T cells, and regulatory T cells (T_{regs}). Mice treated with ACT and IL-2 preferentially activated T_{regs} over CD8⁺ T cells in the spleen and tumor, whereas mice treated with ACT and NKTR-214 showed preferential activation of CD8⁺ T cells instead. Additionally, elevated CD4⁺ T cell proportions appeared in the tumors of IL-2 treated mice, but not in NKTR-214 treated counterparts. T cells from mice treated with ACT and NKTR-214 showed increased polyfunctionality compared to the traditional therapy.

Parisi et al. next sought to better characterize T cell subsets on an individual-cell level based on both marker expression profiles and function. Using IsoPlexis' highly multiplexed functional phenotyping, they found greater proportions of polyfunctional T cells in the tumors and spleens of NKTR-214 treated mice, which aligned with the superior persistence and anti-tumor response

previously observed. In fact, the proportion of polyfunctional cells in the tumors of NKTR-214-treated mice was ten-fold higher than that found in the tumors of IL-2-treated mice. Moreover, the functionally activated cells found in NKTR-214-treated mice were more polyfunctional, with a significant number capable of secreting four or more cytokines.

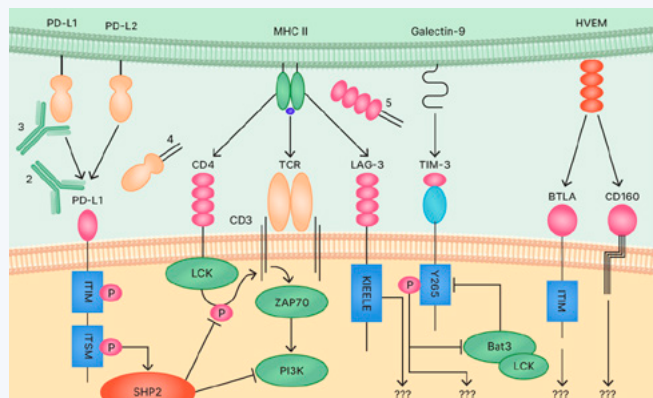
This study showed that NKTR-214 is potentially superior to IL-2 for limiting tumor growth, stimulating T cell expansion, and promoting a more robust and persistent immune response. Cellular analysis indicated a link between polyfunctionality and T cell efficacy. NKTR-214 may serve as a clinical alternative to IL-2, and is undergoing evaluation in several clinical trials, either as a monotherapy or in conjunction with PD-1 antagonists or Toll-like receptor agonists.

Reference

G. Parisi et al., "Persistence of adoptively transferred T cells with a kinetically engineered IL-2 receptor agonist," *Nat Commun*, 11:660, 2020.

Cellular Function in Cancer

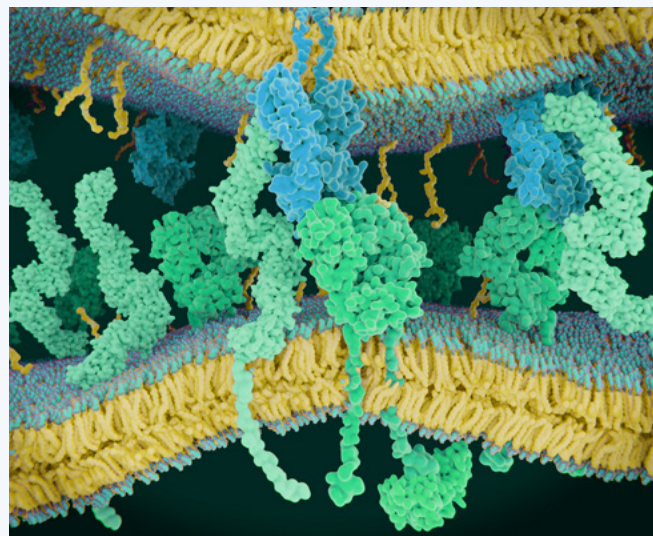
The immune response is heavily involved in endogenous anti-cancer responses. Naturally, many anti-cancer therapeutic approaches seek to harness and augment existing mechanisms to combat tumor growth. Decades of research, including genomic and proteomic analyses, show that immune-cancer interactions are highly diverse and deeply complex. The emphasis now is on function. How does cellular functional phenotype influence therapeutic efficacy or cancer progression?



Basic Research

Charting Roadmaps

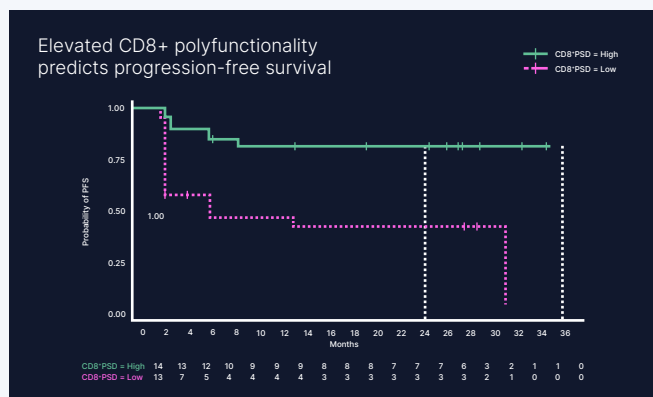
Because cells are heterogeneous, the ability to conduct functional proteomics at the single-cell level is crucial. Characterizing the function of cells involved in processes that eliminate cancerous cells and those that enable tumor formation is critical to identifying new therapeutic targets.



Translational Research

Plotting a Course

Characterizing how cancer affects immune cell function allows researchers to identify potential therapeutic targets and devise novel avenues of attack. They can gain insights into the cells that orchestrate the immune response; they can create agents to kill cancer cells directly or mark them for lysis; and they can engineer customized receptors to improve immune cell activity and efficacy. Understanding cancer drivers also helps scientists create disease models that better recapitulate the properties and dynamics of cancers in human patients.



Adapted from Diab et al., *J Clin Oncol*, 2021, discussed on page 6.

Clinical Research

Towards the Goal

In addition to primary outcomes, analyzing cellular function during clinical trials gives scientists a way to chart the specific mechanisms activated during a treatment strategy. This information helps identify predictive biomarkers for treatment efficacy, areas for potential adjustments or improvements, and patient profiles for which the treatment is particularly effective or ineffective.

HOW IMMUNE CELL RESPONSE RELATES TO PROGNOSIS

In a Phase II clinical trial, researchers further examined NKTR-214 (bempegaldesleukin; BEMPEG) in the treatment of metastatic melanoma. In particular, they investigated whether combining NKTR-214 with an immune checkpoint inhibitor (ICI) might improve patient responses. Recently, Diab et al.² looked at the toleration and effectiveness of NKTR-214 in tandem with the ICI nivolumab (NIVO) as a first-line treatment against metastatic melanoma. In a recent paper published in the *Journal for Clinical Oncology*, they highlight how this approach was tolerated and demonstrated encouraging anti-tumor activity. Importantly, they also found CD8+ T cell polyfunctionality to be an early prognostic biomarker for treatment efficacy.

Interleukin-2 (IL-2) can enhance the survival of CD4+ and CD8+ T cells, as well as natural killer (NK) cells, and is often used in tandem with ICIs. However, IL-2 has a short half-life, necessitating high doses that result in significant toxicities. In contrast, NKTR-214 is an IL-2 pathway agonist that increases CD8+ T cell and NK cell proliferation and infiltration into the tumor microenvironment (TME) without inducing compensatory regulatory T cell (T_{reg}) expansion.^{3,4} Interestingly, NKTR-214 has also been linked with elevated PD-1 expression on TME lymphocytes and PD-L1 expression on tumor cells.^{3,4} This led to the initiation of PIVOT-02, an international, multicenter, phase I/II clinical trial designed to exam-

ine NKTR-214 plus NIVO in patients with advanced solid tumors.

When evaluating responses to NKTR-214 plus NIVO in 38 patients with unresectable or metastatic melanoma, Diab et al. found that tumor burden decreased in 53% of patients, with 34% achieving a complete response. The median change in lesion size was -78.5%. Responses appeared to deepen with time, as eight of the 13 patients achieving a complete response did so on or after the third post-baseline scan. Furthermore, at the data cutoff point, 80% of remaining enrolled patients had an ongoing measurable response. Encouragingly, only 17.1% of patients experienced grade 3 or 4 treatment-related AEs. There were no treatment-related deaths.

Diab et al. assessed blood-based biomarkers in order to identify potentially predictive markers of treatment response. Using IsoPlexis-powered single-cell cytokine analysis, the researchers found that circulating polyfunctional CD8+ T cell abundance was robustly upregulated in patients showing positive response to treatment. Importantly, greater increases in CD8+ T cell polyfunctionality were associated with higher response rates and longer progression-free survival than smaller increases. Furthermore, this increase in polyfunctionality can be detected as early as day 8 following treatment, which offers prognostic information for patient response and potential outcome far earlier than other methods.

In this study, Diab et al. demonstrated that NKTR-214 plus NIVO delivers deep and durable positive responses against metastatic melanoma without inducing severe toxicity. They further identified CD8+ T cell polyfunctionality as both a key mechanistic element driving responses to treatment and an easily measured biomarker that may be used to predict treatment response.

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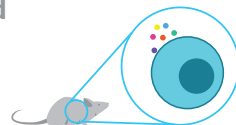
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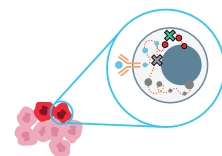
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