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ICOS-expressing CAR-T cells mediate durable eradication of triple-negative breast cancer and metastasis

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ABSTRACT

Triple-negative breast cancer (TNBC) remains one of the most aggressive and therapeutically challenging breast cancer subtypes. In their recent study, Cao *et al* introduced a B7H3-specific chimeric antigen receptor (CAR)-T cell with constitutive inducible co-stimulator (ICOS) expression (ICOS-B7H3-CAR-T), which demonstrated eradication of TNBC, including metastases, in preclinical models. These CAR-T cells exploit the expression of ICOS ligand on TNBC cells, enhancing antitumor cytotoxicity through ICOS signaling. Compared with conventional B7H3-CAR-T cells, the ICOS-B7H3-CAR-T cells exhibited superior antitumor efficacy, increased cytokine secretion, and prolonged survival in xenograft murine models. This study highlights ICOS as a promising co-stimulatory molecule for improving CAR-T therapy against solid tumors and underscores the critical role of ICOS signaling in enhancing therapeutic outcomes. Here, we discuss the implications of these findings for TNBC treatment, the importance of understanding and exploiting ICOS biology in immunotherapies, and future directions for optimizing ICOS CAR-T cell therapies in solid tumor immunotherapy.

THE NEED FOR IMPROVED THERAPY FOR TNBC

Triple-negative breast cancer (TNBC) is a particularly aggressive subtype of breast cancer characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression. TNBC accounts for approximately 10–15% of all breast cancer cases and is associated with poorer prognosis due to high rates of metastasis and lack of targeted therapeutic approaches. Current treatment strategies primarily rely on combinations of surgery, chemotherapy, and radiation, which frequently fail to prevent recurrence or effectively manage metastatic disease. More recently, immunotherapy has changed the paradigm of cancer treatment, with strategies such as immune checkpoint therapy and chimeric antigen receptor (CAR)-T cell therapy demonstrating potential in overcoming the immunosuppressive tumor microenvironment and eliminating disease. However, the clinical application of these therapies is often hindered by challenges such as tumor heterogeneity, immune evasion, and treatment resistance, necessitating further research and innovation.

CAR-T cell therapy has emerged as a promising modality for hematologic malignancies. However, its efficacy in solid tumors has been limited by immune suppression/exclusion within the tumor microenvironment, antigen-specific identification and heterogeneity, and poor CAR-T cell persistence. In the case of TNBC, there have been multiple potential tumor-associated antigens, including mesothelin, folate receptor-alpha, tumor endothelial marker 8, NKG2D ligands, epidermal growth factor receptor, and B7-H3 that have been targeted using CAR-T approaches. Further, TNBC is characterized by rich infiltration of tumor-infiltrating lymphocytes, particularly $CD8^+$ T cells. This suggests that the primary challenge to CAR-T therapy in TNBC lies in T cell fitness and persistence within the tumor. In their study, Cao *et al*[1] address this challenge directly by incorporating inducible co-stimulator (ICOS) signaling into B7H3-specific CAR-T cells that achieve impressive therapeutic efficacy in preclinical TNBC models.

THE ROLE OF ICOS IN CANCER IMMUNOLOGY

ICOS is a member of the co-stimulatory B7-1/B7-2-CD28/cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) family. ICOS has significant homology to the co-stimulatory molecule CD28 and the immune checkpoint CTLA-4. ICOS is not constitutively expressed by naive T cells but is rather rapidly induced following T cell activation via T cell receptor and CD28 engagement. ICOS signaling is initiated after binding its ligand (ICOSL), which is constitutively expressed by antigen-presenting cells, such as B cells, dendritic cells, and macrophages, as well as non-hematopoietic cells such as endothelial and epithelial cells. Class IA phosphatidylinositol 3-kinase (PI3K) is recruited following ICOS

activation, which in turn leads to the activation of AKT, a kinase that promotes proliferation and survival. Some of the known functions of ICOS signaling include the generation and maintenance of germinal centers, T follicular helper (Thf) cell differentiation via PI3K signaling, cytokine production such as interleukins (ILs) 4, 5, 6, 10, 21 and tumor necrosis factor-alpha (TNF-α), maintenance and function of regulatory T cells (Tregs),[2] and promotion of tissue-resident memory CD8 T cell differentiation in tissues.[3]

In cancer, the role of ICOS signaling is cell type dependent and varies between follicular helper T cells (Tfh), regulatory $CD4^+$ T cells (Tregs) and effector T cells ($CD8^+$ or $CD4^+$ Teff). While $ICOS^+$ Tfh cells express the transcription factor Bcl6 and $ICOS^+$ Tregs express the transcription factor FOXP3, it was previously demonstrated that tumor antigen-specific $CD4^+ICOS^+$ T cells, which occur after anti-CTLA-4 immunotherapy, express the transcription factor T-bet and produce the Th1 cytokine IFN-γ.[4–7] In addition, targeting ICOS in combination with anti-CTLA-4 improved anti-tumor responses in preclinical models as a result of increased $CD4^+$ Teff, with increased production of IFN-γ and TNF-α, and increased $CD8^+$ T cells.[8 9] In fact, these studies were the first to demonstrate a role for the ICOS/ICOSL pathway in anti-tumor responses. Interestingly, ICOS signaling in $CD8^+$ T cells in the context of cancer is less studied compared with $CD4^+$ T cells. It was previously reported that dual blockade of CTLA-4 and programmed death protein 1 (PD-1) enhances adoptive $CD8^+$ T cell therapy by regulating the expression of Eomes, IFN-γ, and perforin. In fact, $ICOS^{hi}$ $CD8^+$ T cells from human melanomas expressed higher levels of IFN-γ and Eomes compared with $ICOS^{lo}$ counterparts.[10] Together, these data highlight a role for ICOS in mediating anti-tumor responses.

The study by Cao *et al* builds on prior evidence that ICOS signaling enhances T cell activation, survival, and cytokine production. Here, the authors engineered ICOS-B7H3-CAR-expressing $CD8^+$ T cells that combine induced expression of ICOS with the third-generation CAR construct targeting B7H3, a tumor-associated antigen highly expressed in TNBC. Unlike previous CAR-T strategies that have incorporated only the intracellular signaling domain of ICOS into the construct of $CD4^+$ CAR-Ts in preclinical models,[11] the ICOS-B7H3-CAR-T cells constitutively express the full-length ICOS protein in addition to an anti-B7H3 single-chain variable fragment fused to the endodomains of CD28, CD137, and CD3ζ—requiring interaction with exogenous ICOS ligand for full effect.

The authors first showed that elevated ICOSL in tumor cells is associated with poor clinical outcomes in patients with TNBC—thus motivating the development of ICOS-enhanced B7-H3 third-generation CAR-T cells which mediated efficient eradication of primary breast cancer tumors and metastases in preclinical models. The ICOS-B7H3-CAR-T cells demonstrated significantly greater cytotoxic activity against TNBC cell lines compared with conventional B7H3-CAR-T cells. This effect was attributed to increased production of effector cytokines, IFN-γ and TNF-α. Interestingly, ICOS-B7H3-CAR-T cells also had prolonged antitumor activity in vivo and contributed to superior tumor regression, prolonged survival, and durable metastasis eradication when compared with the B7H3-CAR-T cells. These effects were accompanied by enhanced T cell infiltration into tumor tissues.

This study also highlights the critical role of ICOSL expression on TNBC cells and the surrounding tumor microenvironment in enhancing CAR-T efficacy. The efficacy of ICOS-B7H3-CAR-T cells was enhanced when ICOSL was overexpressed in cancer cells, while the antitumor effect was abrogated when ICOSL was genetically silenced in cancer cells. It is important to note that this study used xenograft preclinical mouse models (NSG) that are immunodeficient. It will be interesting to see whether these findings are recapitulated in immunocompetent mice, especially since ICOSL expression is also high in antigen-presenting cells. Altogether, Cao *et al* highlight a promising potential clinical application strategy to enhance CAR-T cell persistence and efficacy using ICOS signaling.

CLINICAL IMPLICATIONS OF ICOS-B7H3-CAR-T

As previously mentioned, TNBC could be an ideal target for CAR-T cell therapy. The findings of Cao *et al* underscore the potential of ICOS as a co-stimulatory molecule to overcome some of the existing barriers limiting CAR-T therapy in solid tumors. One of the most striking observations of the study is the ability of ICOS-B7H3-CAR-T cells to eliminate metastatic lesions throughout various organs, which represents a major advancement in addressing TNBC's lethal progression. Further, this potential therapy comes with a natural biomarker (ICOSL) to identify patients who are more likely to respond. Given the dependence of ICOS-B7H3-CAR-T efficacy on ICOSL expression, patient stratification based on ICOSL levels may be used to optimize therapeutic outcomes. These findings also shed light on the development of novel combination strategies to target TNBC.

CHALLENGES AND FUTURE DIRECTIONS

While the results are exciting, several challenges remain before ICOS-B7H3-CAR-T cells can be translated into clinical practice. First, variability in ICOSL levels across TNBC tumors may limit the general applicability of ICOS-B7H3-CAR-T therapy. Future studies should explore strategies to upregulate ICOSL expression in the tumor microenvironment, either on tumor cells or on potentially antigen-presenting cells. While not explicitly addressed here, it would be interesting to evaluate the ICOS-B7H3-CAR-T cells against B7H3-CAR-T cells containing the intracellular signaling domain of ICOS—thus reducing the reliance on ICOSL expression. Although preclinical studies did not report significant off-target effects, the safety of

ICOS-B7H3-CAR-T cells in humans remains to be evaluated. In an immunocompetent setting, other cell populations may probably express high levels of ICOSL and/or B7-H3, which could unintentionally affect therapeutic outcomes and safety. In addition, sustained antitumor activity requires durable CAR-T cell persistence; therefore, understanding how constitutive ICOS signaling impacts T cell memory and function should be explored.

CONCLUSIONS

The study by Cao *et al* represents a significant milestone in the development of CAR-T therapies for TNBC. By simultaneously leveraging ICOS signaling and targeting B7H3, the authors demonstrate a promising strategy for enhancing CAR-T function and persistence to eliminate metastatic disease. These findings highlight the role of ICOS in CAR-T cell function in TNBC and have the potential to improve CAR-T design for additional solid tumor types.

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