A Double Strike Against Tumor Resistance: IL-24 Enhances CAR-T Therapy by Disarming Cancer Stem Cells

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Despite the transformative success of chimeric antigen receptor (CAR)-T cell therapy in hematologic malignancies, its efficacy against solid tumors remains disappointing. One increasingly recognized obstacle is the persistence of cancer stem cells (CSCs), which are resistant to conventional therapies and capable of repopulating tumors.[1] A 2024 study published in the British Journal of Cancer by Zhang et al., addresses this challenge by engineering CAR-T cells to co-express interleukin-24 (IL-24), a cytokine with known tumor-suppressive functions.[2] This strategy not only augments T cell activation and persistence but also directly suppresses cancer stem cells (CSCs) through apoptosis and stemness inhibition. This dual-targeting approach reframes our understanding of CAR-T limitations and introduces a promising new direction for overcoming therapeutic resistance in solid tumors.

Numerous factors contribute to the failure of CAR-T cells in solid tumors—including poor infiltration, immunosuppressive microenvironments, antigen heterogeneity, and T cell exhaustion.[3,4] A recurring theme in recent literature was the crucial but underappreciated role of CSCs in therapy resistance and tumor relapse. These rare, self-renewing tumor-initiating cells exhibit enhanced plasticity, resist immune-mediated killing, and regenerate tumor bulk following treatment.

Zhang et al. introduce CAR-T cells engineered to express IL-24, a cytokine previously characterized for its tumor-suppressive and immunomodulatory roles. By pairing antigen targeting with IL-24 secretion, the authors demonstrate improved tumor control through both T cell enhancement and direct CSC eradication.

The study opens by confirming the limited effectiveness of standard CAR-T cells in killing CSCs. Using NKG2D and Her2-targeting CARs in lung and esophageal cancer models, they observed that while CAR-T cells effectively lysed bulk tumor cells, a population of residual cells remained. These surviving cells exhibited expression of CSC markers (e.g., CD133, SOX2, PROM1) and characteristics (e.g., greater sphere-forming capacity and increased tumor-initiating potential in vivo). Importantly, antigen loss was ruled out as a cause of escape, confirming that CSCs resist CAR-T killing despite maintaining antigen expression.

This finding echoes the insights from prior work including Prager et al., which highlight CSCs as central architects of tumor heterogeneity and resilience and reinforces the notion that therapies failing to eliminate CSCs merely prune the tumor mass, allowing regrowth from the root.[5]

IL-24, a member of the IL-10 cytokine family, has been studied for its ability to selectively induce apoptosis in tumor cells without harming normal tissue. Beyond its direct cytotoxicity, IL-24 is known to modulate the tumor immune microenvironment and sensitize tumors to other therapies.[2,6] Zhang et al. build on this legacy by exploring IL-24's effects in the CAR-T context.

First, they show that exogenous IL-24 suppresses CSC viability, reduces sphere formation, and downregulates stemness-associated genes. These effects are partially mediated through inhibition of the Wnt/β-catenin pathway, a key regulator of CSC maintenance. Western blot data revealed that IL-24 treatment decreased active β-catenin and increased cleaved caspase-3, indicating apoptosis validating previous reports that IL-24 suppresses tumor growth by targeting CSCs.[7]

Second, Zhang et al. demonstrate that IL-24 boosts T cell function. In vitro, IL-24-treated T cells showed elevated expression of activation markers (CD69, CD28), increased central memory differentiation (CCR7+ CD45RO+), and enhanced proliferation. Interestingly, IL-24 did not increase apoptosis of tumor cells directly, suggesting that its effects are mediated by T cells rather than them being directly toxic to tumor cells.

To integrate these dual benefits, the authors generated CAR-T cells that co-express IL-24 using a lentiviral construct. These CAR.IL-24-T cells exhibited superior cytotoxicity against tumor cells compared to conventional CAR-Ts and produced more IL-2 and IFN-γ in co-culture. In sphere-formation assays, residual tumor cells from IL-24-CAR-T co-cultures had dramatically reduced stemness, suggesting effective CSC elimination.

In mouse xenograft models of lung and esophageal cancer, CAR.IL-24-T cells

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demonstrated improved tumor control, higher T cell infiltration, reduced PD-1 expression (indicative of lower exhaustion), and longer survival. Strikingly, in late-stage tumors, only CAR.IL-24-T cells prevented relapse and conferred durable responses. These in vivo results strongly support the idea that CSCs are central to recurrence and that targeting them is key to long-term remission.

Zhang et al.'s work aligns with a growing appreciation that immune therapies must address not only the bulk tumor and microenvironment but also the intrinsic plasticity of tumor cells. CSCs represent a resilient population capable of adapting to selective pressure and reigniting disease. As seen in this study, IL-24 is a promising agent that simultaneously disarms CSCs and fortifies CAR-T cells (**Figure 1)**.

This dual-function strategy may prove particularly useful in combination with other next-generation CAR-T innovations, such as checkpoint inhibition, chemokine receptor engineering,[8] or metabolic modulation.[9] Moreover, IL-24's synergy with existing chemotherapeutics suggests that it may serve as a broadly useful adjuvant, both within and beyond CAR-T platforms.[10]

However, several questions remain. The precise mechanisms by which IL-24 regulates T cell memory and exhaustion warrant further study. Additionally, the lack of a patient-derived xenograft (PDX) model limits its direct clinical translation. Finally, while IL-24 appears safe in preclinical models, its effects on human immune homeostasis in vivo remain to be tested. A Phase I clinical trial involving IL-24 CAR-Ts is reportedly underway, which may address these concerns.

Zhang et al. (2024) have delivered a pivotal contribution to the field of cancer immunotherapy. By incorporating IL-24 into CAR-T cells, they achieve a rare and valuable synergy: improving T cell fitness while directly targeting the elusive CSC population. This study reframes how we think about cytokine engineering—not just as immune adjuvants, but as molecular tools that reshape tumor biology at its core. As the field moves forward, such multi-targeted designs may become the new blueprint for overcoming therapeutic resistance in solid tumors.

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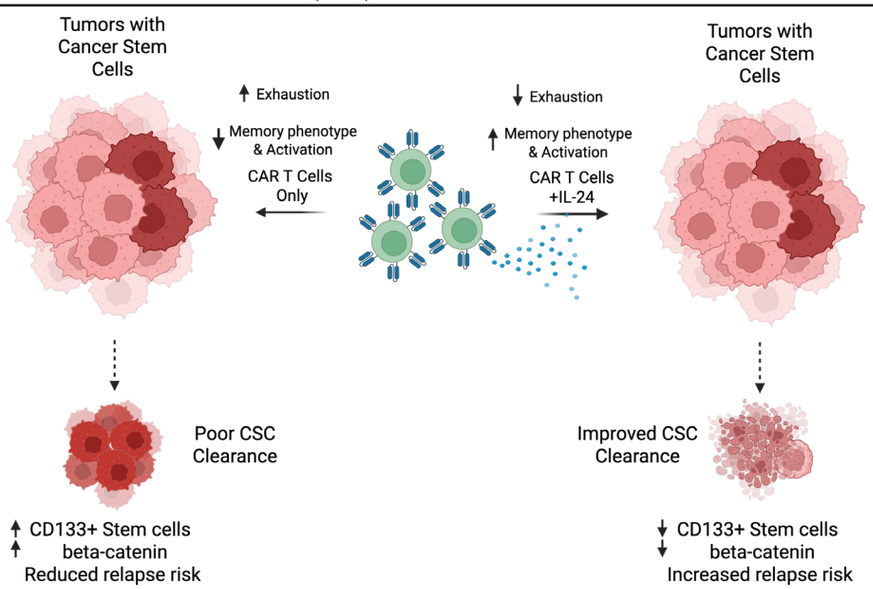

Figure 1 | IL-24-armed CAR-T cells overcome cancer stem cell (CSC)-mediated resistance in solid tumors. (Left) Conventional CAR-T cells (lacking IL-24) effectively target bulk tumor cells but fail to eliminate CD133+ CSCs due to persistent Wnt/β-catenin signaling, which maintains stemness and survival. Residual CSCs drive tumor relapse. CAR-T cells exhibit limited activation (low CD69+/CD28+) and increased exhaustion. (Right) IL-24-expressing CAR-T cells dual-target bulk tumor cells and CSCs. IL-24 disrupts CSC resistance by: (i) suppressing Wnt/β-catenin activity, reducing CD133+ stemness; (ii) inducing apoptosis; and (iii) enhancing CAR-T cell fitness (elevated activation markers, memory differentiation, and reduced exhaustion). This combined action improves tumor clearance and reduces relapse risk.