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Research Highlight

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Head-to-head: IL-21 triumphs over IL-15 in NK cell therapy for glioblastoma

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Glioblastoma (GBM) is the most aggressive primary brain tumor. Despite current treatment options, including surgery, radiotherapy, and temozolomide chemotherapy, patient outcomes remain poor, with a median survival of less than 15 months [1,2]. This dire prognosis highlights an urgent need to develop more effective therapies.

Natural Killer (NK) cells, a key component of the innate immune system, are being actively investigated as a potential treatment for GBM. NK cells continually surveil their environment for abnormal cells, including GBM stem cells (GSCs), which are central to GBM progression and recurrence [3]. While NK cells exhibit some ability to target GSCs independently, their activity can be significantly amplified by inflammatory cytokines [2]. One such cytokine, interleukin-15 (IL-15), is critical for NK cell survival and function, making it a focal point of research in GBM immunotherapy [4]. However, IL-15 is not without complications; it has been associated with toxicity, and its overexpression has been shown to induce leukemia in mouse models [5], potentially due to heightened inflammatory responses. These issues make IL-15 overexpression a less-than-ideal strategy for enhancing NK cell anti-tumor activity.

To address these limitations, Shanley and colleagues [6] recently identified interleukin-21 (IL-21) as a promising alternative to IL-15 in their study published in *Cancer Cell*. Their findings revealed that IL-21 overexpression provides prolonged NK cell activity, even under repeated exposure to GSCs, and demonstrates efficacy both *in vitro* and *in vivo*. Importantly, IL-21-expressing NK cells showed no significant toxicity when injected into mouse brains. These results suggest that IL-21 could represent a safer and more effective cytokine for boosting NK cell-mediated GBM therapy.

IL-21 NK Cells Outperform IL-15 NK Cells in Tumor Elimination and Rechallenge

The effectiveness of NK cells against GBM varies depending on the duration of their exposure to GSCs [7]. GSCs are known to suppress NK cell activity over time through mechanisms such as contact-dependent TGFβ signaling [7]. To compare the ability of IL-15 and IL-21 to enhance NK cell-mediated GBM elimination, Shanley and colleagues [6] used NK cells derived from cord blood to overexpress either cytokine. This autocrine signaling approach allowed the cytokines to stimulate the NK cells producing them. Both IL-15 and IL-21 NK cells showed greater killing activity towards GSCs than control NK cells. Using orthotopic patient-derived GBM mouse models, the authors found that a single intratumoral injection of IL-21 NK cells achieved long-term tumor eradication and significantly prolonged survival compared to IL-15 or control NK cell treatments.

Tumor recurrence driven by the persistence of GSCs remains a significant challenge in GBM treatment, as these cells often evade complete eradication [2,3]. Effective therapies must target this re-emergence. Shanley and colleagues evaluated the durability of IL-15 and IL-21 NK cell activity through repeated *in vitro* exposure to GSCs. While both NK cell populations effectively killed GSCs during the initial challenge, IL-15 NK cell activity declined rapidly with successive exposures. In contrast, IL-21 NK cells maintained robust killing efficiency. These findings highlight IL-21 NK cells as a promising alternative to IL-15 NK cells for targeting GBM recurrence.

A key factor in this enhanced efficacy is the method of IL-21 delivery. Genetically modified NK cells produce IL-21 locally and continuously within the tumor microenvironment, establishing a sustained source of IL-21 at the tumor site. This localized and stable cytokine production facilitates prolonged NK cell activation and immune modulation within the tumor. In contrast, the administration of IL-21-Fc recombinant proteins requires repeated dosing due to the short half-life of recombinant IL-21. Consistent with this, Shanley *et al.* [6] found that short-term priming of NK cells with exogenous IL-21 (3 ng/mL) for 48 h failed to control GSC growth effectively, further highlighting the advantage of sustained, localized IL-21 secretion by genetically modified NK cells over

intermittent recombinant protein administration.

IL-21 NK Cells Exhibit Reduced Toxicity Compared to IL-15 NK Cells in GBM Mouse Models

IL-15 is known to be proinflammatory, and while the exact pathways remain unclear, such cytokine activity can contribute to cancer development, with leukemia being one example of its potential oncogenic effects [5,8]. Due to these concerns, finding a cytokine with fewer off-target effects is crucial. Shanley and colleagues explored IL-21 as a safer alternative.

In their study [6], mice with human GBM tumors received a single intratumoral (i.t.) injection of either IL-15- or IL-21-expressing NK cells. IL-15 NK cells caused significant toxicity, including pronounced weight loss, extensive NK cell infiltration, microglial activation, and notable astrocytosis. Even when the dose of IL-15 NK cells was lowered, similar levels of toxicity were observed. Interestingly, when IL-15 NK cells were administered intravenously (i.v.), they did not induce the same toxic effects, likely due to minimal NK cell infiltration into the brain. In contrast, mice treated with IL-21 NK cells showed no evidence of toxicity or weight loss. Further testing in a rechallenge assay showed that IL-21 NK cells produced fewer cytokines associated with neurotoxicity compared to IL-15 NK cells. This supports the notion that IL-21 is a safer alternative to IL-15 for GBM treatment.

The IL-21-IL-21R-STAT1/3-CEBPD Axis Drives the Persistent and Effective Anti-tumor Activity of IL-21 NK Cells in GBM

To explore the pathways that give IL-21 NK cells a distinct advantage over IL-15 NK cells in targeting GBM, Shanley and colleagues [6] employed single-cell ATAC-seq and RNA-seq techniques. Their analysis revealed that the CEB/P family, particularly the CEBPD transcription factor, was upregulated in IL-21 NK cells. When *CEBPD* was knocked out, IL-21 NK cells exhibited reduced efficacy in eliminating GSCs *in vitro* and GBM in mouse models, as well as diminished proliferation. On the other hand, NK cells overexpressing CEBPD exhibited enhanced anti-tumor activity comparable to IL-21 NK cells in targeting GSCs and GBM. Shanley and colleagues proposed a model in which IL-21 upregulates CEBPD transcription via STAT1 and STAT3, with these transcription factors binding to the *CEBPD* promoter, as confirmed by ChIP results. CEBPD, in turn, activates the expression of genes such as *KLF2* and *BNIP3L*, which promote NK cell survival and metabolic fitness (Figure 1). This IL-21-induced signaling cascade drives the enhanced persistence and cytotoxicity of NK cells against GSCs and GBM.

The IL-21 receptor (IL-21R), a type I cytokine receptor, plays a pivotal role in this process. Its expression on NK cells is dynamically regulated by cytokine stimulation and cellular activation. In this study, IL-21-expressing NK cells may establish an autocrine loop, sustaining IL-21R expression and enhancing NK cell responsiveness. The authors demonstrated that deletion of IL-21R significantly reduced the cytotoxicity of IL-21 NK cells against GSCs [6], underscoring the critical role of autocrine IL-21 secretion and IL-21R signaling in NK cell activation.

While CEBPD was identified as a key regulator of the IL-21-mediated NK cell response, other transcriptional regulators found in the study may also contribute to the observed phenotype. Further research is needed to fully understand their roles and refine the molecular mechanisms underlying the effect of IL-21 in NK cells. Moreover, since IL-21-expressing NK cells have demonstrated efficacy against other cancers, this research could open up new clinical applications beyond GBM. Overall, this study [6] underscores the importance of CEBPD as a novel regulator in NK cell responses to

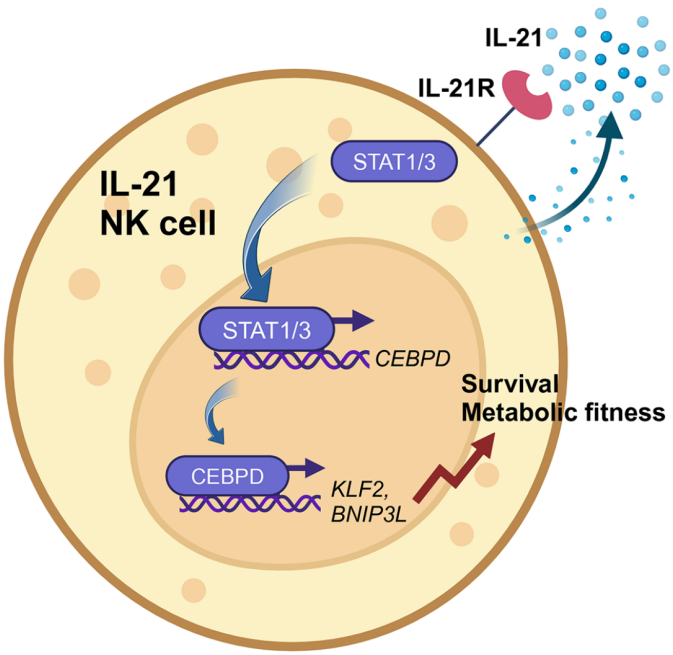

Figure 1. Schematic overview of the signal pathway in IL-21 NK cells IL-21 binds to the IL-21 receptor (IL-21R), activating STAT1 and STAT3, which then bind to the *CEBPD* promoter to drive its expression. CEBPD subsequently activates target genes such as *KLF2* and *BNIP3L*, supporting NK cell survival and enhancing metabolic fitness.

GBM and supports IL-21-armored NK cells as a promising and clinically valuable immunotherapeutic approach for GBM.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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