# Next-Generation Cytokines for Immunotherapy: A New Frontier in Oncology

**Next-generation cytokines are transforming cancer care with improved precision, stability, and effectiveness, providing hope in immunotherapy. They regulate imbalanced immune reactions in conditions like cancer and autoimmune diseases.**

Cytokines are crucial for immune regulation, and given their extensive impact on human biology and disease, they have a long history of therapeutic use in treating cancer and other diseases. First-generation cytokines like interleukin-2 (IL-2) and interferon-alpha (IFN-α) have been limited by short half-life, high toxicity, and lack of specificity. Innovations in next-generation cytokines aim to enhance stability, reduce toxicity, and improve tumor targeting. Strategies include pegylation, fusion proteins, Immunocytokines, cytokine prodrugs, and mRNA-based therapies. Notable examples in development include NKTR-214, ALT-803, and L19-IL2, showing promise in clinical trials. Despite advances, challenges such as safety, manufacturing, combination therapies, and patient selection remain critical for future progress.

## Cytokines and Their Role in Immunotherapy

Cytokines are small proteins that play a crucial role in regulating the growth, differentiation, and activation of immune cells, making them essential for immune system functioning [1,2]. Cytokines can inhibit tumor growth by direct antiproliferative effects or by stimulating immune cells to attack tumor cells. This also reveals their great potential in cancer immunotherapy [3,4]. However, the use of natural cytokines in therapy has been limited by their short half-life, systemic toxicity, and lack of specificity [5,6].

## First-Generation Cytokines

First-generation cytokines like IL-2 and IFN-α have been pivotal in cancer immunotherapy. IL-2 was approved for advanced renal cell carcinoma and metastatic melanoma treatment, while IFN-α for hairy cell leukemia, follicular non-Hodgkin lymphoma, melanoma, and AIDS-related Kaposi's sarcoma [3]. These cytokines bolster the immune system's natural killer (NK) and T cell functions, crucial for combating tumors [5]. However, their clinical use is hindered by short *in vivo* half-life, severe toxicity at therapeutic doses, and limited efficacy in most patients [2,3]. For instance, while IL-2 can enhance T-cell proliferation and anti-tumor activity, it's accompanied by severe and frequent grade 3 and 4 adverse effects before reaching therapeutic levels [3,5,6].

## Next-Generation Cytokines

Next-generation cytokines are engineered to improve upon the properties of their predecessors. These innovations include enhanced stability, reduced toxicity, and increased specificity for tumor cells. Here are some key strategies employed in the development of next-generation cytokines:

- **Pegylation**: Pegylation refers to the covalent bonding between cytokines and polyethylene glycol (PEG) polymers, which prolongs the half-life and stability of cytokines in the bloodstream [1]. Pegylated cytokines can maintain therapeutic concentrations for longer periods, reducing the frequency of administration and associated toxicities [2,7]. Additionally, pegylation expands the hydrodynamic diameter of the cytokines, thereby reducing renal clearance and immunogenicity [2].

- **Fusion Proteins**: Fc fusion proteins covalently linked to an immunoglobulin Fc domain modify the pharmacokinetics (PK) of active molecules. By fusing cytokines with antibodies or other targeting moieties, researchers can direct cytokine activity specifically to tumor cells. The Fc domain increases the plasma half-life of the fusion protein, improving its therapeutic efficacy and slowing down renal macromolecular excretion [1]. In addition, the Fc domain binds to the Fcg receptor (FcgR) and complement, which may contribute to antibody-dependent cytotoxicity (ADCC), antibody-dependent cell phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC) [8].

- **Immunocytokines**: Antibody-cytokine fusion proteins, known as immunocytokines, including a targeting-antibody moiety, an amino acid linker, and a cytokine load. Immunocytokines may consist of cytokines fused to full-size antibodies or

antibody fragments, which provide the molecule with the ability to target tumor-associated antigens [1,8,9]. Cytokines activate and direct immune cells to tumor cells, and promote the formation of immune synapses. Activated immune cells increase cytotoxicity and reduce tumor cell proliferation[4]. The emergence of immunocytokines has enabled the localization of effector molecules in the TEM and expanded the therapeutic strategies [8].

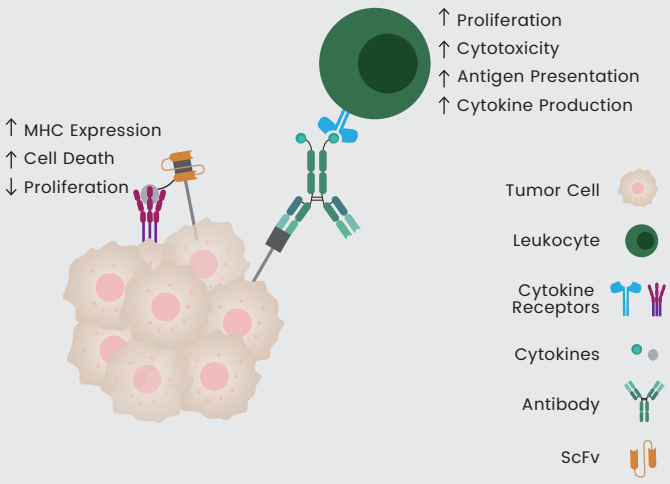


Figure 1. Mechanisms of anti-cancer action of immunocytokines [9].

• **Cytokine Prodrugs**: Prodrug cytokines are mainly composed of cytokine, masking moiety, "half-life extension elements", and linker. Designing the prodrug constructs is an effective approach to overcoming off-target effects. In this strategy, a peptide is linked to the cytokine and renders it inactive. Proteases overexpressed at the tumor site can cleave this inactivating unit, reactivating the cytokine. Cytokine prodrug designs often pair with re-engineered cytokines, such as ProIL2, an inactive form of IL-2 [6].

• **mRNA-Based Cytokine Therapies**: In mRNA-based therapies, cytokines can be encoded and delivered via synthetic mRNA to modulate the immune system, offering a strategy to circumvent the toxicities associated with recombinant cytokine therapies [2]. Lipid nanoparticles (LNPs) efficiently deliver mRNA to host cells, allowing them to produce cytokines. These cytokines can enhance anti-tumor immunity, modify the tumor microenvironment, and inhibit tumor growth [10,11].

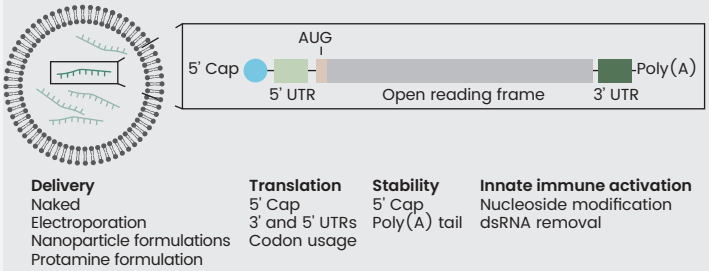


Figure 2. Delivery and Structural Elements of mRNA Therapeutics. Left: Lipid-based mRNA nanoparticle structure. Right: Synthetic mRNA structure with 5′ cap, 5′ and 3′ UTRs, start codon (AUG), and poly(A) tail. UTR: Untranslated region; ds: Double-stranded [11].

## Examples of Next-Generation Cytokines in Development

Several next-generation cytokines are currently being investigated in preclinical and clinical studies, demonstrating promising results in enhancing cancer immunotherapy.

• **ALT-803 (Nogapendekin alfa)**: An IL-15 superagonist, ALT-803 has enhanced stability and bioactivity compared to native IL-15. It promotes the proliferation and activation of NK cells and memory CD8+ T cells, providing sustained anti-tumor activity. ALT-803 is being evaluated in combination with other immunotherapies and has received FDA approval in the treatment of non-muscle invasive bladder cancer (NMIBC) [12].

• **L19-IL2 (Darleukin)**: L19-IL2 is a diabody format immunocytokine consisting of two ScFv fragments and a C-terminal-fused IL-2. This cytokine improves safety and therapeutic efficacy and can be co-treated with other therapeutics. L19-IL2 used as a single agent or in combination with rituximab and CTLA-4 blockers or L19-TNF immunocytokines can completely eradicate β-cell lymphoma xenografts [4].

• **NKTR-214 (Bempegaldesleukin)**: This engineered version of IL-2 is designed to preferentially activate CD8+ T cells and natural killer (NK) cells, key players in the anti-tumor immune response. NKTR-214 has shown reduced binding to IL-2 receptors on regulatory T cells (Tregs), which can suppress immune responses. Clinical trials have demonstrated its potential in combination with checkpoint inhibitors for treating various cancers [13].

## Challenges and Future Directions

Cytokines, powerful immune mediators, present a complex challenge in drug development. Next-generation cytokines with lower toxicity and longer half-life aim to address these issues and improve therapeutic efficacy. Despite the promising advances, the development and implementation of next-generation cytokines face several challenges:

• **Safety and Toxicity**: Even with improved designs, cytokine therapies can still induce systemic toxicity. Ensuring the safety of these treatments while maintaining their efficacy is a critical concern [4].

• **Manufacturing and Scalability**: The complex engineering and production processes for next-generation cytokines can pose manufacturing challenges. Scalability and cost-effectiveness are important factors for widespread clinical adoption [2].

• **Combination Therapies**: Integrating next-generation cytokines with other cancer therapies, such as checkpoint inhibitors, targeted therapies, and adoptive cell transfer, requires careful optimization. Understanding the synergistic effects and potential interactions is essential for maximizing therapeutic outcomes [14].

## Sino Biological's Contribution to the Research on Next-Generation Cytokines

Named "Growth Factor Supplier to Watch in 2024" by CiteAb, Sino Biological provides an extensive range of recombinant cytokines for cell culture, enhancing research in tumor immunotherapy, stem cell therapy, drug screening, and regenerative medicine. Our products undergo stringent quality control and thorough validation to ensure high purity, biological activity, stability, and low endotoxin levels. These cytokines, available from various species including human, rat, and mouse, support research on stem cells, neural cells, immune cells, and organoids. Committed to delivering high-quality reagents for drug development and clinical research, Sino Biological offers both research-use-only (RUO) and GMP-grade cytokines. Developed under a GMP quality management system, our GMP-grade cytokines provide enhanced stability and quality, comprehensively supporting cell therapy and drug development processes.


- **Vast Selection**:
  250+ molecules, 800+ cytokines (20+ GMP-grade)
  Covering growth factors, ILs, TNFs, CSFs, IFNs, and more
- **High Quality**:
  High purity, validated activity, and established stability
- **Quality Control**:
  ISO 9001/ISO 13485/GMP quality systems


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