InVivoKines[™], A New Generation of Cytokines for *In vivo* Research

Optimized long-lasting and high activity Fc-Cytokines

he choice of the best protein reagents for *in vivo* research is crucial to ensure good reproducibility and results. Optimized proteins are often developed at pharma or biotech companies for their own purposes. They are not accessible to the research community.

To this end, AdipoGen Life Sciences recently launched a new generation of recombinant fusion Fc-cytokines, called InVivoKines. They are produced under GMP-like conditions for immunological, immunotherapeutic, and preclinical *in vivo* research.

Cytokines are small proteins that facilitate communication among immune cells and orchestrate the response to infections and tumors as well as overall immune homeostasis, making them attractive for preclinical and clinical research for a variety of immune-related disorders.

However, for *in vivo* experiments, use of recombinant cytokines is limited by their short half-lives in serum, meaning higher doses need to reach the expected effect followed by dose-induced toxicities. To overcome this drawback and use lower amounts of cytokines, half-life needs to be extended while preserving the cytokines native engagement of their respective receptors.

One widely used strategy to increase the cytokine half-lives *in vivo* is to fuse the biologically active protein to the constant fragment (Fc) domain of a human immunoglobulin (Ig) to allow vascular retention mechanisms mediated by neonatal Fc receptor (FcRn) recycling and, therefore, increasing the presence of the Fc-protein in the serum. A typical Fc fusion protein prolongs half-life to 1 to 3 weeks instead of a few minutes/hours for the untagged cytokine.

Many Fc-fusion cytokines are used in research or have been approved as biotherapeutics with a half-life of almost a week(s). However, Fc-fusion frequently leads to daisy-chaining and aggregation during production, severely impacting activity. In addition, using a classical Fc approach leads to dimeric cytokines, while most cytokines are highly active only as monomers.

To solve these issues, AdipoGen utilizes the Knobs-into-Holes Fc technology (KIH), already used to create bi-specific antibodies, to design new long-lasting and highly active Fc-cytokines, called InVivoKines. This Fc-KIH technology allows Fc heterodimerization to create a structure with two different arms to design naturally occurring active monomeric or heterodimeric proteins with conformational stability and FcRn binding.



Figure. Structure of classical Fc versus Fc-KIH fused proteins

Advantages of InVivoKines

InVivoKines show identical activity compared to the endogenous or untagged recombinant cytokines but exert much more activity compared to classical Fc-cytokines that form dimer or higher structures. InVivoKines demonstrated excellent performance when validation testing first began. For example, comparison of mouse IL-21 InVivoKine with the standard IL-21 (mouse):Fc in a T-cell activation assay showed that the IL-21 InVivoKine is the appropriate reagent to study T cells with 500x higher activity compared to the standard IL-21 (mouse):Fc. The monomeric structure of the IL-21 InVivoKine, as well as that of other tested InVivoKines, allows the preservation of the high endogenous activity of the cytokine together with the extended half-life. So, highly active InVivoKines, reagents will help researchers to successfully perform crucial *in vivo* experiments.

Abrogation of the Fc activity by LALA-PG mutations

To avoid any Fc activity during *in vivo* experiments, AdipoGen engineers silenced Fc-KIH domains of InVivoKines using the hlgG1-P329G LALA mutations (called LALA-PG), which completely abolishes FcγR and C1q interactions with unaffected FcRn interactions and Fc long-lasting effect. Thus, InVivoKines with the LALA-PG mutations do not elicit Fc activities such as functional cell-based assays and inflammatory cytokine responses.

In summary, AdipoGen Life Sciences released most cytokines as InVivoKines to provide researchers with the best tools that they will need for *in vivo* experiments in mouse or rat. In addition, since the activity of InVivoKines is identical to recombinant untagged cytokines, these reagents can also be used for *in vitro* assays.

For more information, visit: adipogen.com/invivokines



