Utility and Application of the *VeriKine*™ Human IFN-α Multi-Subtype ELISA Kit

A pbl interferonsource White Paper

Abstract

Generation of type I interferons (IFNs) occurs downstream of a multitude of immunologic stimuli. The production of these cytokines can be either a positive prognostic, as in defensive immunity against a pathogenic insult, or a negative prognostic, as in promotion of autoimmunity. In humans, the designation "IFN–α" is used to represent a family of proteins comprised of at least 12 highly homologous protein subtypes that exhibit diverse biologic activities. IFN–α can be used as a therapeutic agent in disease treatment regimens, as a downstream readout of the effectiveness of several drug therapies currently in the pipeline, and as a marker of pathology in human clinical and preclinical studies. The necessity of having a simple, cost effective, and highly reproducible assay for detecting IFN–α is unquestionable. However, the requirements of such an assay are complicated by the need to simultaneously detect not just one, but numerous subtypes of IFN–α proteins. Of the various medium- to high-throughput assays available that may be useful in such efforts, the *VeriKine*™ Human Interferon Alpha Serum enzyme linked immuno-sorbent assay (ELISA) stands out for its ease of use, sensitivity, reproducibility, and ability to simultaneously detect multiple IFN–α subtypes. The application of this assay to a variety of R&D and clinical trial settings is described below, followed by an overview of the unique methodological benefits of this assay.

Systemic lupus erythematosus (SLE) and IFN-α

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the loss of tolerance to several nuclear antigens. Patients with SLE exhibit symptoms ranging from a mild rash to central nervous system involvement and life-threatening nephritis. Greater than half of all SLE patients exhibit an excess of type I IFN production, which is associated with renal disease and production of autoantibodies[1-3]. Therefore, decreasing type I IFN production is a logical pharmacologic goal [4, 5], and careful monitoring of IFN–α levels during clinical trials may aid in drug or biologic agent dose selection. In addition, the ability to accurately quantify IFN–α expression in patient serum may help tailor clinical approaches on an individual basis. The need for novel therapeutic options for SLE treatment is underscored by the fact that it has been over 50 years since the FDA has approved a new SLE medication. While several assays are currently available for measuring type I IFN levels and downstream effects, identifying the most cost effective and simple assays for high throughput screening is critical for further drug discovery.

Virology and IFN-α

IFN–α proteins, either as single agents or in combination with other anti-viral drugs, are currently used to treat patients with chronic hepatitis B and/or hepatitis C infections[6, 7]. The response rate to IFN–α based therapies is far from optimal, and significant side effects are common; these include exacerbation of preexisting autoimmune disorders or de novo induction of autoimmunity[8, 9]. Both of these side effects are attributed to the pro-inflammatory properties of type I interferons. As a possible solution, current drug development is aimed at inducing a more natural complement and level of endogenous IFN–α proteins by using immunomodulatory molecules. Use of the *VeriKine*™ IFN–α Serum ELISA to monitor induced IFN–α levels in plasma is a rapid means by which to analyze responses to these new therapies. As opposed to other IFN–α ELISA assays, which tend to selectively detect IFN–α2 protein, PBL's Serum ELISA is particularly important for virology as it detects multiple native human IFN–α protein subtypes.

The *VeriKine*™ IFN–α Serum ELISA kit is also a powerful tool for basic virology research. It can be used to evaluate and compare differences in immune responses, allowing the user to detect changes in the kinetics or strength of interferon production following infection with unique viral variants. For some viruses, including those for which highly sensitive detection proves difficult, the *VeriKine*™ IFN–α Serum ELISA kit may be used to quantify increases in host IFN–α production as an early indicator of viral load. Numerous mechanistic questions can be studied with a highly sensitive assay that sees far more than just the classical IFN–α2 protein.

IFN-α and pharmacokinetics (PK)

Currently there are several IFN–α therapeutics on the market used in the treatment of a wide range of diseases. These include recombinant IFN–α2a and IFN–α2b proteins, as well as their respective pegylated forms. The introduction of pegylated modifications for IFN–α proteins has led to a longer protein half-life in serum[10]. These second generation molecules have allowed patients to reduce their injections from three to one per week thereby enabling better patient compliance. Several new formulations of long-acting or oral IFN–α products are in development that may eventually compete with the pegylated interferons. In addition, numerous pharmacokinetic studies (www.clinicaltrials.gov) involving IFN–α are underway to examine new IFN–α formulations and dosing regimens.

Combinatorial therapies using IFN–α plus other drugs, such as anti-cancer agents or a second anti-viral, are being explored in a variety of clinical trials[10-12]. In these studies, the efficacy of a given drug combination may depend on novel dosing regimens and the resulting kinetic profiles of the different agents being administered. As IFN–α is a common drug of choice in combinatorial therapies for viral diseases and oncology, verification of the intended IFN pharmacokinetic profile is often essential for designing and optimizing dosing regimens. These studies require a means for accurate detection of IFN–α-based therapeutic lead molecules.

IFN-α and pharmacodynamics (PD)

Some of the most exciting new drugs currently in the pipeline are a class of small molecule agonists and antagonists of the toll-like receptor (TLR) family of molecules. TLRs are highly conserved receptors of the innate immune system that recognize pathogen–associated molecular patterns (PAMPs). These short recognition sequences are found in bacterial and viral components ranging from bacterial lipopolysaccharide (LPS) and flagellin to double-stranded RNA and unmethylated CpG DNA motifs. Ligation of TLR receptors that bind dsRNA, LPS, ssRNA , or CpG DNA (TLRs 3, 4, 7, 8, and 9, respectively) initiates signaling cascades that culminate in the production of proinflammatory cytokines, IFNs, and chemokines[13, 14]. One of the best-known FDA-approved TLR agonists is *Aldara™* (imiquimod), which targets TLR-7 and is used for the treatment of several skin conditions including actinic keratosis and superficial basal cell carcinoma. Current studies are focused on the use of TLR agonists as adjuvants in vaccines for cancer and infectious diseases, and TLR antagonists for treatment of SLE. Many TLR agonists and antagonists have progressed to Phase I and II clinical trials evaluating safety and efficacy (www.clinicaltrials.gov).

Because IFN–α production is a major downstream readout of TLR activation, it is frequently used in clinical trials to assess TLR agonist potency in vitro (tissue culture media) or in vivo (serum or plasma). In studies of novel TLR-based antivirals that employ IFN–α proteins as a component of their mechanism of action, increased IFN–α expression may be a key pharmacodynamic biomarker in treated individuals.

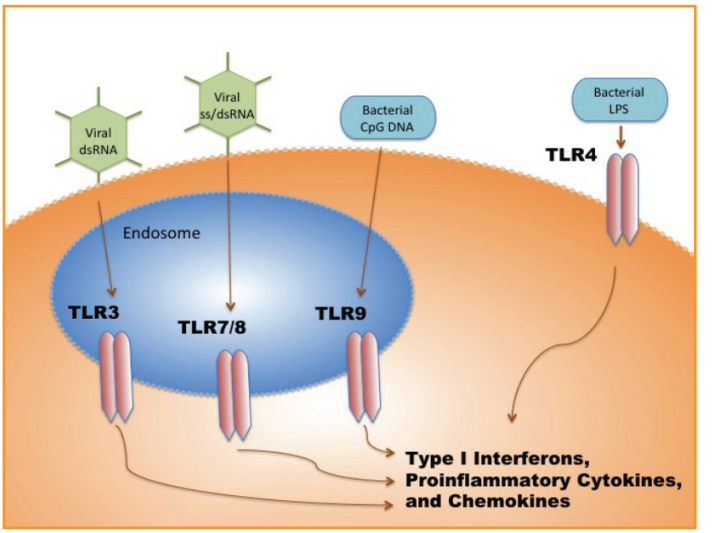

Figure 1. TLR signal transduction. TLR signaling in response to pathogens results in activation of the innate immune system.

IFN-α as a marker of toxicology

During pharmaceutical development, drug candidates with unintended off-target effects must be rapidly identified and discarded. Certainly, any drug inducing copious amounts of IFN–α could be detrimental, as a) many of the pathological consequences of autoimmune diseases such as SLE appear to be due to unchecked IFN–α production, and b) high doses of IFN–α therapeutics induce a hallmark set of side effects. Therefore, the ability to identify early in the development process those molecules that may spuriously result in induction of IFN–α is crucial to expediting delivery of the right lead molecule to the clinic. If identified late in the development process, such off-target effects of a drug may cause dose-limiting side effects in patients, resulting in decreased clinical applicability and a loss of financial investment.

VeriKine™ Human IFN-α Multi-subtype Serum ELISA Kit

The *VeriKine™* IFN–α Serum ELISA kit is a valuable tool for researchers and clinicians alike, as it can facilitate both pre-clinical drug development studies and patient evaluations in the clinic. The ELISA assay is a powerful technique that combines the specificity of antibodies with the sensitivity of a simple enzyme assay for the rapid detection and quantification of biological substances. The *VeriKine™* IFN–α Serum ELISA kit uses the 'sandwich' strategy in which antigen is captured by a plate-bound antibody and detected by second antibody, each recognizing particular epitopes on the IFN–α protein molecules. Because both antibodies are specific for the target protein, this method can increase the assay specificity two to five times over other direct or indirect detection methods, thereby eliminating the need for sample purification prior to analysis. Thus, the *VeriKine™* IFN–α Serum ELISA kit is suitable for analyzing samples with complex matrixes such as tissue culture medium, serum, plasma, cerebrospinal fluid, and urine.

The utility of the *VeriKine™* IFN–α Serum ELISA kit for analysis of serum and plasma samples was demonstrated during a spike recovery assay. Known concentrations of purified IFN (spikes) were added to IFN-free plasma, and the differences between the known IFN spike values and those detected by the ELISA (recoveries) were calculated. As seen in Table 1, the ELISA is able to detect IFN–α with a high degree of accuracy at these concentrations. It should be noted that for SLE patients treated with therapeutic IFN–α neutralizing antibodies under current clinical trial protocols, it is important to consider possible interference of these neutralizing antibodies when using any ELISA.

Spike Recovery *Matrix*	*Low (30 pg/ml)*	*Medium (300 pg/ml)*	*High (800 pg/ml)*
Serum	94%	100%	96%
Plasma (Na Citrate)	70%	112%	110%
Plasma (Na-Heparin)	95%	106%	101%
Plasma (Na-EDTA)	60%	86%	84%

Table 1. Percent recoveries of exogenously added IFN–α2a in normal human serum and normal human plasma with different anticoagulants; data are from experiments performed using serum and plasma from multiple donors, and standard curves run in comparable matrices.

Many currently available IFN–α ELISAs and multiplex formats are capable of detecting predominantly the two IFN–α2 protein subtypes, making these kits a poor choice for many of the applications discussed here, as these approaches can fail to detect even substantial amounts of other IFN–α proteins induced by a drug, virus, or other stimuli. In contrast, the *VeriKine™* Human IFN–α Serum ELISA Kit detects multiple IFN–α subtypes, thereby providing a better measure of global IFN–α production during sample analysis. Additionally, the *VeriKine™* IFN–α Serum ELISA Kit can be used to sensitively analyze large numbers of patient samples from clinical trials. Newer approaches for determining cytokine expression levels, including those that utilize bead-based technology and flow cytometry of individual cells, may be appropriate for identification of cell subsets responsible for IFN–α expression but can be cost-prohibitive when conducted on a large scale. In addition, the IFN–α subtype specificity of the antibodies utilized in bead-based assays may be unsuitable to the research goal. Thus, this colorimetric IFN–α ELISA remains among the simplest, most reproducible of assays by which to monitor expression of IFN–α protein subtypes.

Clearly, IFN–α plays a pivotal role not only in the protective responses to many infections and diseases, but also in the generation of side effects and pathological sequelae when produced unchecked. The ability to precisely and comprehensively quantify the production of this family of cytokines is required not only in the clinical setting, but also for drug discovery and basic research. Effective quantification and titration of IFN–α is crucial to maximizing the therapeutic window where clinical benefits are achievable with minimal side effects. The *VeriKine™* Human IFN–α Serum ELISA kit is one of the most efficient methods for detecting IFN–α proteins available today.

References

1. Wussow, P.V., Jakschies, D., Freund, M., Hehlmann, R., Brockhaus, F., Hochkeppel, H., Horisberger, M. & Deicher, H. Treatment of anti-recombinant interferon-alpha 2 antibody positive CML patients with natural interferon-alpha. *Br J Haematol* 78, 210-216 (1991) PMCID# 2064959.
2. Ronnblom, L., Alm, G.V. & Eloranta, M.L. Type I interferon and lupus. *Curr Opin Rheumatol* 21, 471-477 (2009) PMCID# 19525849.
3. Niewold, T.B., Clark, D.N., Salloum, R. & Poole, B.D. Interferon alpha in systemic lupus erythematosus. *J Biomed Biotechnol* 2010, 948364 (2010) PMCID# 20652065.
4. Yao, Y., Richman, L., Higgs, B.W., Morehouse, C.A., de los Reyes, M., Brohawn, P., Zhang, J., White, B., Coyle, A.J., Kiener, P.A. & Jallal, B. Neutralization of interferon-alpha/beta-inducible genes and downstream effect in a phase I trial of an anti-interferon-alpha monoclonal antibody in systemic lupus erythematosus. *Arthritis Rheum* 60, 1785-1796 (2009) PMCID# 19479852.
5. Ronnblom, L. & Alm, G.V. Systemic lupus erythematosus and the type I interferon system. *Arthritis Res Ther* 5, 68-75 (2003) PMCID# 12718746.
6. Katze, M.G., He, Y. & Gale, M., Jr. Viruses and interferon: a fight for supremacy. *Nat Rev Immunol* 2, 675-687 (2002) PMCID# 12209136.
7. Brok, J., Gluud, L.L. & Gluud, C. Ribavirin plus interferon versus interferon for chronic hepatitis C. *Cochrane Database Syst Rev,* CD005445 (2010) PMCID# 20091577.
8. Li, L., Han, D.K. & Lu, J. Interferon-alpha induced severe thrombocytopenia: a case report and review of the literature. *World J Gastroenterol* 16, 1414-1417 (2010) PMCID# 20238410.
9. Dhillon, S., Kaker, A., Dosanjh, A., Japra, D. & Vanthiel, D.H. Irreversible pulmonary hypertension associated with the use of interferon alpha for chronic hepatitis C. *Dig Dis Sci* 55, 1785-1790 (2010) PMCID# 20411421.
10. Glue, P., Fang, J.W., Rouzier-Panis, R., Raffanel, C., Sabo, R., Gupta, S.K., Salfi, M. & Jacobs, S. Pegylated interferon-alpha2b: pharmacokinetics, pharmacodynamics, safety, and preliminary efficacy data. Hepatitis C Intervention Therapy Group. *Clin Pharmacol Ther* 68, 556-567 (2000) PMCID# 11103758.
11. Taliani, G., Gemignani, G., Ferrari, C., Aceti, A., Bartolozzi, D., Blanc, P.L., Capanni, M., Esperti, F., Forte, P., Guadagnino, V., Mari, T., Marino, N., Milani, S., Pasquazzi, C., Rosina, F., Tacconi, D., Toti, M., Zignego, A.L., Messerini, L. & Stroffolini, T. Pegylated interferon alfa-2b plus ribavirin in the retreatment of interferon-ribavirin nonresponder patients. *Gastroenterology* 130, 1098-1106 (2006) PMCID# 16618404.
12. Kiladjian, J.J., Cassinat, B., Chevret, S., Turlure, P., Cambier, N., Roussel, M., Bellucci, S., Grandchamp, B., Chomienne, C. & Fenaux, P. Pegylated interferon-alfa-2a induces complete hematologic and molecular responses with low toxicity in polycythemia vera. *Blood* 112, 3065-3072 (2008) PMCID# 18650451.
13. Kawai, T. & Akira, S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol* 11, 373-384 (2010) PMCID# 20404851.
14. Noppert, S.J., Fitzgerald, K.A. & Hertzog, P.J. The role of type I interferons in TLR responses. *Immunol Cell Biol* 85, 446-457 (2007) PMCID# 17667935.