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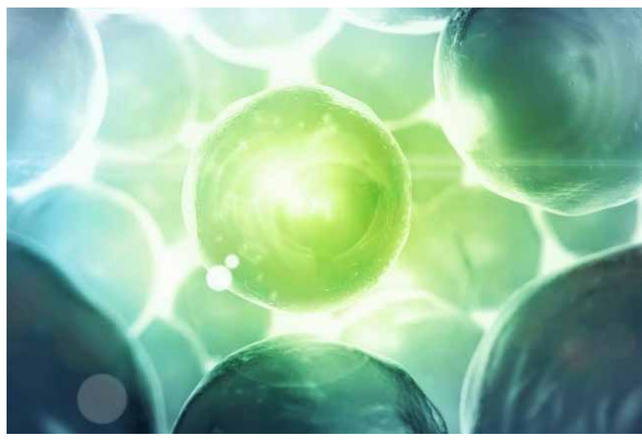
iLite™ Reporter Gene Assay for the Quantification of the Activity and Neutralizing Antibody Response to TNF-α Antagonists

Christophe Lallemand and Michael G. Tovey. Biomonitor SAS, Villejuif, France



Introduction

Antagonists of tumor necrosis factor alpha (TNF-α) are used widely for the treatment of a number of chronic inflammatory or autoimmune diseases such as rheumatoid arthritis (RA), psoriasis, and Crohn's Disease. Such antagonists include; infliximab (Remicade®), a chimeric monoclonal antibody against TNF-α, adalimumab (Humira®) and golimumab (Simponi®), fully human monoclonal anti-TNF-α antibodies, etanercept (Enbrel®), a fusion protein comprising the p75 chain of the TNF-α receptor and the Fc moiety of human IgG1, and certolizumab (Cimzia®), pegylated Fab' fragments of a humanized anti-TNF-α monoclonal antibody.

Method

A cell-based assay has been developed for the quantification of the activity of TNF-α antagonists that signal through the NFkB pathway (Figure 1) based on human erythro-leukemic K562 cells transfected with a 5 x tandem repeat of a non-canonical NFkB recognition sequence regulating expression of the Firefly luciferase reporter-gene (Figure 2).

The use of a TNF-α specific reporter gene construct (Figure 2) together with cells engineered not to respond to other factors that signal through the NFkB pathway renders the assay specific for TNF-α (Figure 3).
The assay is rapid and can be completed within 4 hours, is simple to perform (Figure 4), highly sensitive (Figure 5) and yields a dynamic range of some 90-fold (Figure 3).

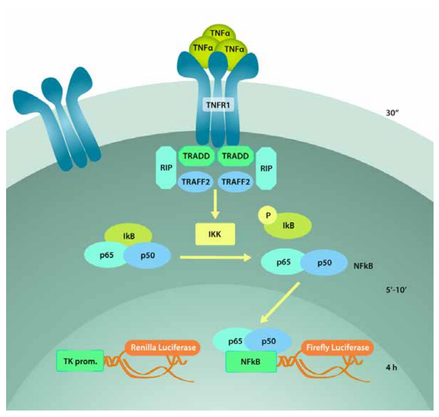


Figure 1. TNF-α signal transduction pathway.

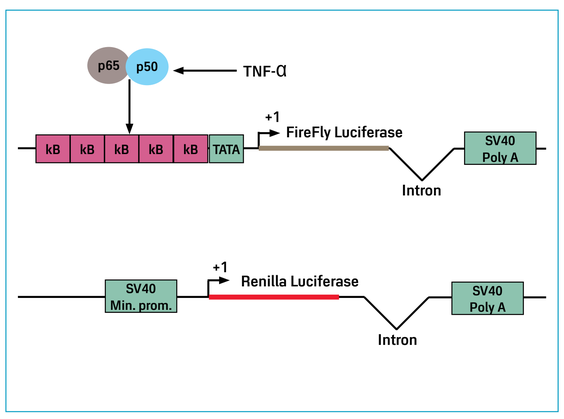


Figure 2. TNF-α normalized reporter-gene construct.

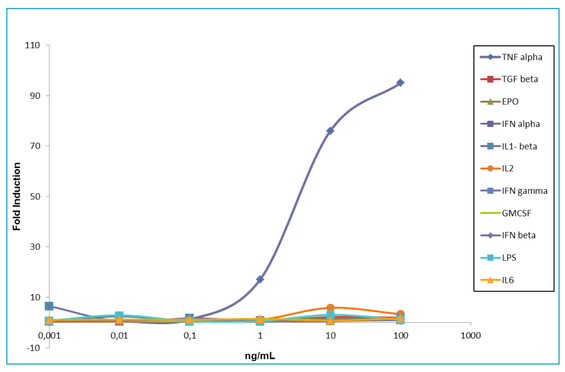


Figure 3. Specificity of the response of the iLite™ TNF-α responsive cells..

Characteristics	*iLite*™ TNF-α responsive cells
Time of incubation	4 hours
Normalization	Yes
EC50	2 ng/mL
LLQ	500 pg/mL

Figure 4. TNF-α Dose response curve - 4 hour assay, 25 000 cells/well.

Results – Sensitivity

The cells also contain a Renilla luciferase reporter gene controlled by a constitutive promoter (Figure 2). This allows TNF-α induced Firefly luciferase activity to be normalized relative to Renilla luciferase expression thus rendering results independent of cell number (Figure 5) and providing an efficient means for correcting for serum matrix effects (data not shown).

Results - Quantification of Potency & NAbs with the same Assay

The *iLite*™ TNF-α responsive reporter gene cell line provides a means of directly quantifying both the potency (Figure 6) and neutralizing antibody response to TNF-α antagonists of diverse structure in the same assay. The *iLite*™ TNF-α responsive reporter gene cell line also provides a means of directly comparing both the potency and neutralizing antibody response of a biosimilar and innovator product in the same assay (data not shown).

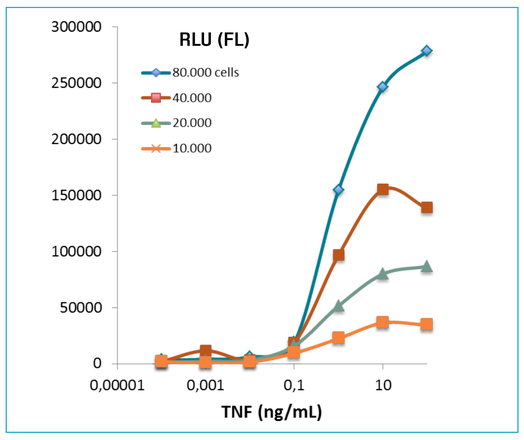


Figure 5a. Relationship between TNF-α induced FL expression and cell number without normalization.

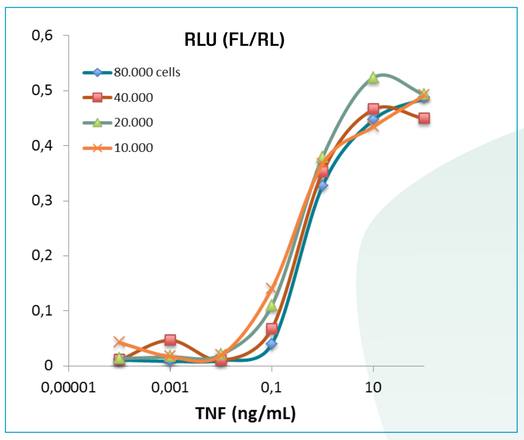


Figure 5b. Relationship between TNF-α induced FL expression and cell number using normalization with RL expression.

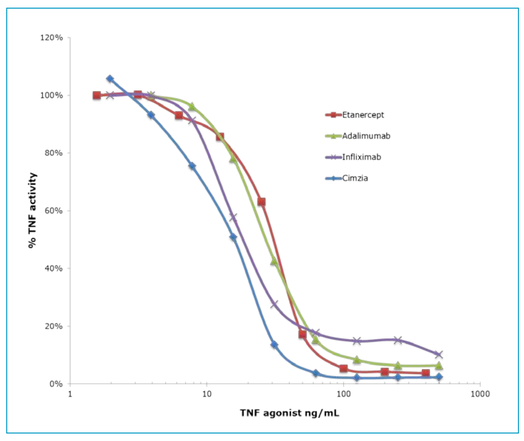


Figure 6. Quantification of the activity of TNF-α antagonists using *iLite*™ TNF-α responsive reporter gene cell cells.

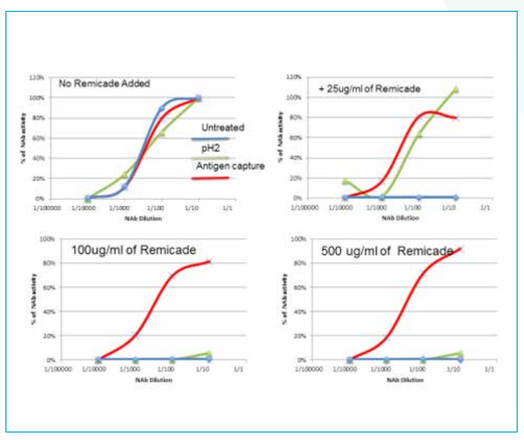


Figure 7. Quantification of the activity of anti-Remicade NAbs in presence of various concentrations of drug before and after antigen capture.

Results - Robustness

The use of TNF-α covalently bound to a solid support provides an efficient means of removing residual drug from patient sera and rendering the assay drug tolerant. Results presented here illustrate the robustness of the assay for the routine quantification of both drug activity and anti-drug neutralizing antibodies in serum samples from patients with inflammatory disease treated with TNF-α antagonists even in the presence of high concentrations of drug (Figure 7).

Conclusion

- The *iLite*™ TNF-α responsive reporter gene assay provides a precise, sensitive and rapid means of quantifying both drug activity and neutralizing anti-drug antibodies present in serum samples without serum matrix effects.
- Thus, the reporter gene assay described herein is ideally suited for high throughput quantification of residual drug activity and anti-drug NAb levels in samples of serum from patients with inflammatory disease treated with TNF-α antagonists.
- This single assay is applicable to the quantification of the activity or NAb response to a variety of different TNF-α antagonists including innovator products and biosimilars.
- Furthermore, the simple antigen capture procedure described herein allows neutralizing anti-drug antibodies to be detected even in sera containing high concentrations of drug and provides a means of detecting the onset of a NAb response prior to the clinical symptoms of drug resistance.