

## Background

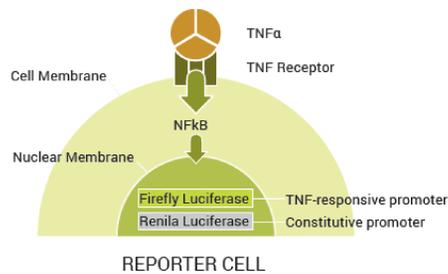
Use of TNF- $\alpha$  inhibitors has revolutionized the treatment of patients with several non-infectious inflammatory disorders, including Crohn's Disease and Ulcerative Colitis. Unfortunately, up to 50% of patients experience treatment failure. There is currently no established standard for handling patients with treatment failure to TNF- $\alpha$  inhibitors. One approach is to monitor drug levels and anti-drug-antibodies (ADA). Current methods for ADA detection are complicated by the fact that most TNF- $\alpha$  inhibitors are antibodies, and the complexity of measuring antibodies against antibodies in non-functional binding assays. More importantly, all current methods fail to differentiate binding from neutralizing ADA.

## Aim

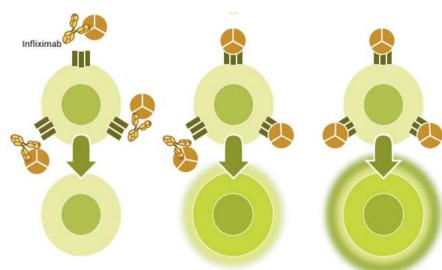
To validate a clinical test to measure infliximab's biological capacity to inhibit TNF- $\alpha$  and detect neutralizing antibodies to infliximab responsible for treatment failure.

## Methods

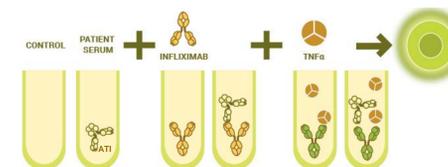
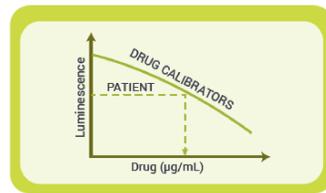
This cell function assay uses the principles of iLite™ method (Figure 1).



Reporter cells carry a TNF- $\alpha$  inducible NF $\kappa$ B regulated firefly luciferase reporter-gene construct. When TNF- $\alpha$  is added to the cells, the reporter gene turns on and generates firefly luciferase that is measured by a luminometer. Results of firefly luciferase expression are normalized relative to the expression of renilla luciferase gene, under the control of a constitutive promoter, also carried by the same reporter cell.



Serum of a patient taking infliximab is mixed with TNF- $\alpha$  and added to the cells. Infliximab blocks the activity of TNF- $\alpha$ . The amount of infliximab inversely correlates to the amount of luminescence. The amount of infliximab in serum can be calculated from the amount it inhibits TNF- $\alpha$  compared to calibrators of known infliximab concentrations.



Some patients develop antibodies to infliximab (ATI). In the presence of neutralizing ATI, the reporter gene is turned on despite the presence of exogenous infliximab in the assay. ATI titer is obtained by identifying the dilution point of patient's serum where blocking of infliximab activity is no longer observed.

## Results

•Both infliximab activity levels and titers of neutralizing antibodies to infliximab (ATI) could be quantified with a high degree of precision (intra and inter assay coefficients of variation of 20% or less), and without interference from the following cytokines: IFN- $\gamma$ , IL-10, IL-12, IL-13, IL-17, IL-1b, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-8, and sCD40L.

•Normalization of TNF- $\alpha$ -induced firefly luciferase activity relative to renilla luciferase expression allowed results to be independent of cell number or differences in cell viability, and allowed correction for serum matrix effects.

•Ninety-six sera from healthy individuals were used to determine clinical reference intervals. The following cutoff values were found: 0.65  $\mu$ g/mL (for infliximab activity), and 1:20 dilution (for neutralizing ATI).

•Trough serum levels of infliximab activity and titers of neutralizing ATI were measured in 268 serum samples of patients on infliximab therapy. Patient results can be divided in 3 groups (Figure 2).

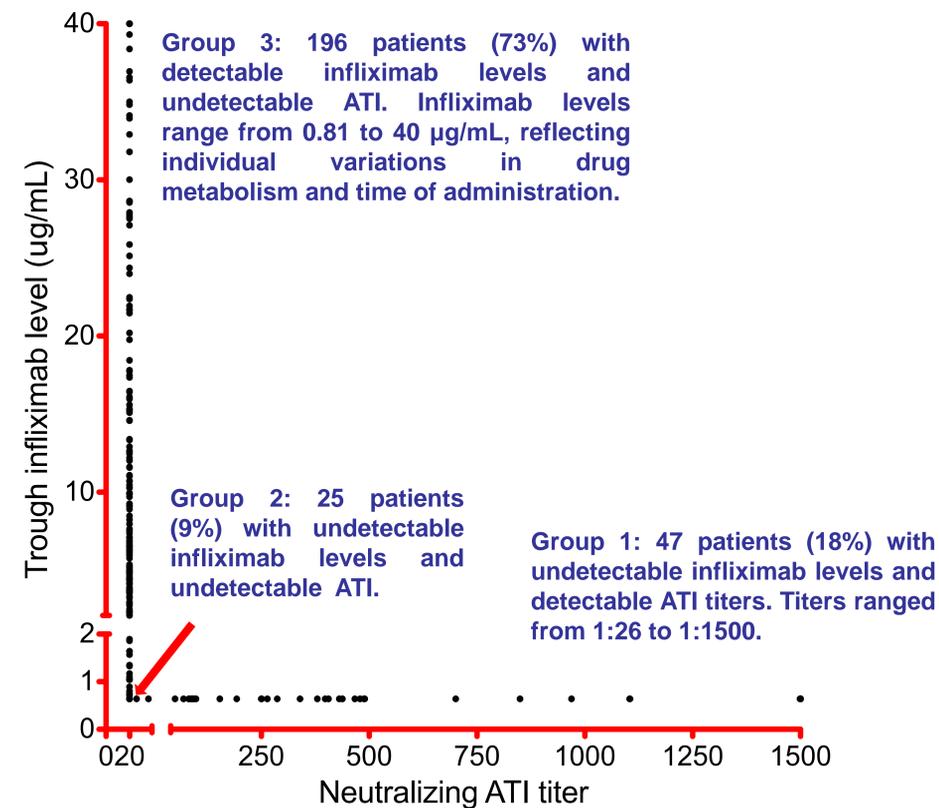


Figure 2. Trough serum levels of infliximab (y-axis), and titers of neutralizing ATI (x-axis) measured in 268 patients. Dotted lines represent low detection limit for infliximab level (0.65  $\mu$ g/mL) and ATI titer (1:20)

- Performance of the cell function assay was compared with Prometheus® Anser™ IFX (Figure 3).

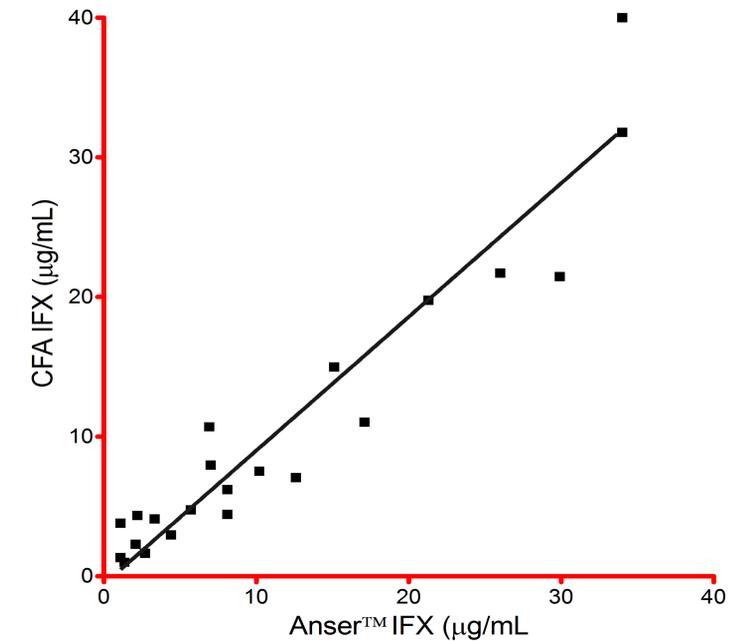


Figure 3. Correlation of serum levels of infliximab in 22 specimens using Anser™ IFX and cell function assay (CFA IFX). Very good correlation was observed between Anser™ IFX and CFA IFX (Pearson r value of 0.95 (95% CI: 0.86-0.97, p< 0.0001). One sample with undetectable infliximab levels and detectable ATI by both methods was not plotted.

## Conclusions

- A cell function assay was clinically validated for diagnosis and monitoring of infliximab treatment failure.
- At this time, this is the only clinical assay available for detection of biological TNF- $\alpha$  neutralization (infliximab activity) and ATI with drug-neutralizing function, as recommended by the FDA.
- The assay resembles the *in vivo* conditions in tissue and circulations under which TNF- $\alpha$  antagonists are believed to function, and can be easily adapted for all known anti-TNF- $\alpha$  drugs.

### Concluding remarks:

- Testing for immunogenicity to biologicals is one of the fastest growing areas in clinical laboratory testing.
- Multiple methods are available for measuring drug levels and ADA, few allowing important differentiation between neutralizing and non-neutralizing ADA.
- Future longitudinal prospective studies are necessary to establish testing intervals and clinical decision-making cut-offs. Collaborations in this field are being pursued and can be discussed with Julio Delgado (julio.delgado@aruplab.com).