GYROS PROTEIN Technologies

Gyrolab Affinity Software enables routine affinity analysis in the development of immunoassays and therapeutic antibodies

Case Study

Assays that accurately and precisely measure therapeutic monoclonal antibodies (mAb) are key to successful preclinical and clinical pharmacokinetic (PK) characterization. Dosing studies aimed at delivering high target coverage require measurement of soluble target levels, but this can be challenging due to the complex dynamics of binding equilibriums between bivalent antibodies, corresponding target and antibody-target complexes. Having observed low target coverage of humanized mAbs targeting connective tissue growth factor (CTGF), a team at Boehringer Ingelheim Pharmaceuticals used Gyrolab® system combined with Gyrolab Affinity Software to characterize the binding properties of their investigational mAbs and associated reagents. They concluded that this combination workflow would be very valuable in routine assay development.

The promise of CTGF-targeting agents

CTGF, also known as CCN2, plays an important role in the control of many biological processes, such as cell proliferation, differentiation, adhesion and angiogenesis, as well as multiple pathologies, such as tumor development and tissue fibrosis (1). This has led to multiple preclinical and clinical studies on CTGF-targeting compounds, including mAbs, aimed at treating a number of pathologies. Two investigational humanized mAbs (mAb 1 and mAb 2) targeting soluble CTGF are being evaluated at Boehringer Ingelheim Pharmaceuticals.

CTGF-targeting mAbs deliver less than 100% target coverage in preclinical study

The team at Biotherapeutics Discovery, Boehringer Ingelheim Pharmaceuticals had already performed a preclinical study of mAb 1 and mAb2 on cynomolgus monkeys using Gyrolab assays to determine the PK properties and corresponding free and total target levels. Their approach was in line with the most recent recommendations for developing antibody and biomarker assays (2). The target coverage was calculated as:

$$\text{target coverage} = ((\text{total} - \text{free})/\text{total}) \times 100\%$$

The target coverage never reached the desired level of 100% but plateaued at 70 – 90%. Before they could use the data to determine the dose regime for adequate target coverage, the team needed to determine whether this was an inherent assay artifact, an effect related to the antibodies themselves, or the result of another phenomenon.

Gyrolab Affinity Software is a valuable tool in the investigation

Having already used Gyrolab system in the PK study, the team saw how Gyrolab Affinity Software would enable them to easily include steps into a workflow to screen, rank, and determine in-solution binding affinities of two interactants. This is an approach that would be very valuable in routine assay development. They therefore decided to evaluate Gyrolab Affinity Software by using it to investigate the performance of the two investigational antibodies targeting CTGF (3).

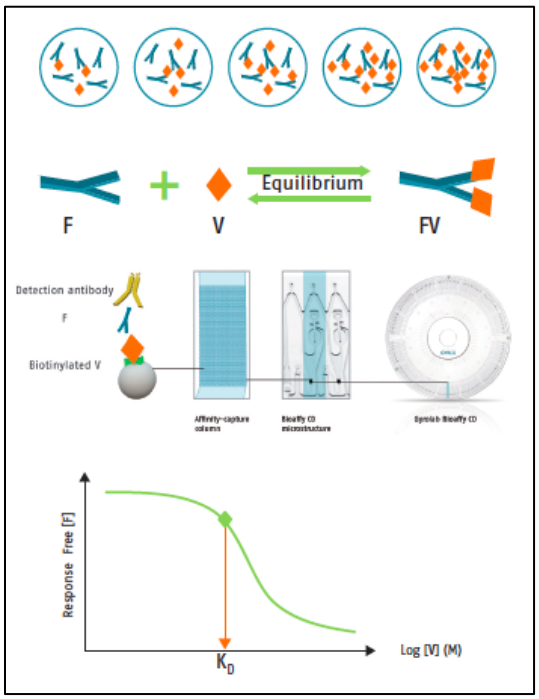


***Figure 1**. The principle of determining in-solution affinity using Gyrolab system in combination with Gyrolab Affinity Software. The software guides the experiment set-up that involves the affinity series, i.e. the number of interacting pairs and their variable concentrations, are defined as well as the method to run. The software generates a loading list to help setting up the affinity samples in the microplate.*

The affinity samples consist of one interactant that is diluted to form a concentration series [V] and the second interactant that is added at a fixed concentration [F] to each sample in the affinity series. The affinity samples are allowed to reach equilibrium on the bench.

The response from free Fixed interactant [F] is measured in Gyrolab Bioaffy 200 CD or Bioaffy 1000 CD run using an automated immunoassay procedure on Gyrolab systems. Gyrolab Affinity Software plots the response from free [F] interactant against the molar concentration of the variable [V] interactant and fits the affinity curve according to a selected interaction model to generate the K_D value. From Gyros Protein Technologies.

Measurement of total and free CTGF for target and titration assays

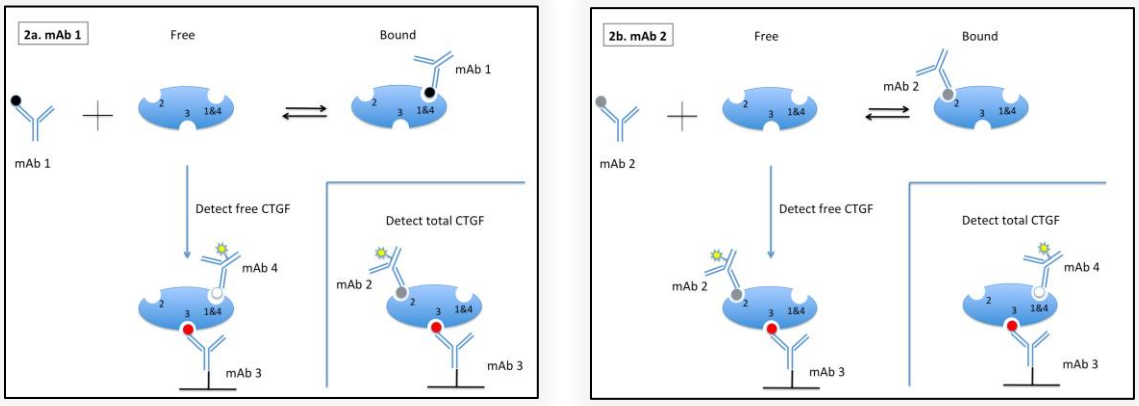
The properties of the two investigational humanized mAbs monoclonal antibody (mAb) and the other mAbs used as reagents in the study are summarized in Table 1. All four antibodies cross-reacted with human and cyno CTGF. The team also prepared recombinant forms of human and cyno CTGF in-house.

Table 1. *Monoclonal antibody reagents used in the study.*

Monoclonal antibody	Source and type	Epitope*
mAb 1	Human IgG1	Same as mAb 4
mAb 2	Human IgG1	Specific
mAb 3	Rat IgG2b	Specific
mAb 4	Mouse IgG1	Same as mAb 1

*As determined by Surface Plasmon Resonance (SPR) spectroscopy

The antibody reagents were used to detect free or total recombinant CTGF in samples, and also to measure endogenous levels in baseline control and diseased samples (Figures 2a and 2b). The reagents were chosen such that free CTGF could be measured using a capture antibody that does not compete with the binding domain of the dosed anti-CTGF antibody (mAb 1 or mAb 2) whereas the detection antibody competes. Total CTGF was measured using a detection antibody that did not bind to the epitope of the dosed anti-CTGF antibody. Commercially available anti-species antibodies were used to detect free antibody for the affinity series. The binding affinities of the CTGF reagents are shown in Table 2.



Figures 2a *(mAb 1)* ***and 2b*** *(mAb 2). Schematic representations showing how free, bound and total levels of CTGF (blue oval) were measured with mAb reagents 1–4 targeting epitopes 1&4, 2, and 3. The detection antibodies are shown with a '*-' label. mAb 3 was used as the capture antibody in all assays.*

Table 2. *Binding affinities (K_D) of CTGF antibodies. From Table 2, Myzithras et al, 2017.*

Anti-CTGF antibody	Cynomolgus CTGF K_D (nM)	Human CTGF K_D (nM)
mAb 1	2.6	0.6
mAb 2	20.3	6.0
mAb 3	0.3	2.5
mAb 4	0.8	0.2

The key findings were:

- mAb 1 and mAb 2 have a four-fold higher affinity to human CTGF than cyno CTGF
- mAb 2 has the weakest affinity to cyno CTGF
- mAb 4, which was used as detection reagent for free target levels for mAb 1 and total target levels for mAb 2 has a higher affinity to both cyno CTGF and human CTGF compared to dosed mAbs 1 and 2

These differences suggested that mAb-target complexes from dosed animals could be dissociating in cyno plasma matrix during assay preparation and run time, and preferentially re-binding the excess reagent due to higher affinity. This would then lead to the low target coverage observed in the earlier study.

Differences between cyno- and human CTGF detection indicate lack of coverage is not an assay artifact

The team tested this hypothesis by running a titration experiment using increasing concentrations of mAbs 1 and 2 with a fixed concentration of hCTGF or cCTGF in corresponding species plasma. The results are summarized in Figure 3.

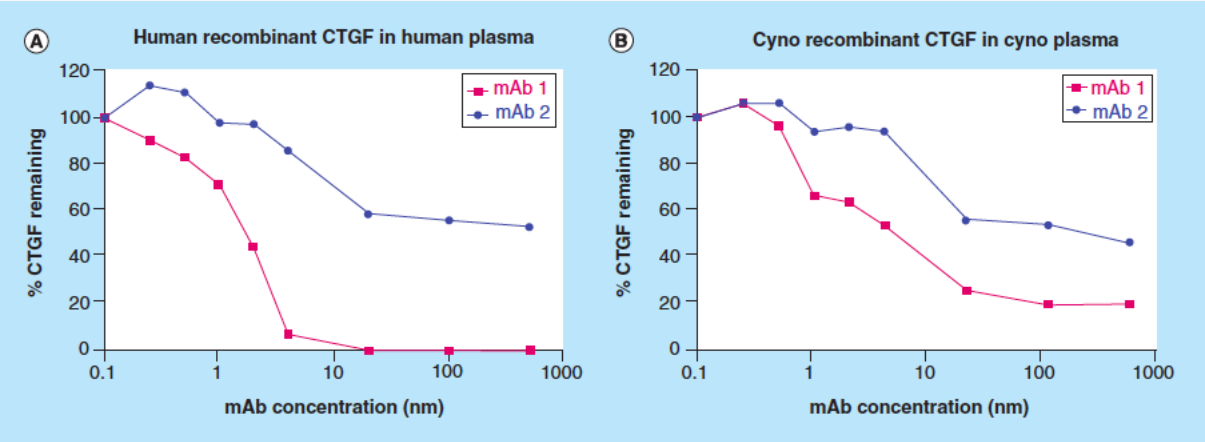


Figure 3*. Results of titrating mAb 1 or mAb 2 with CTGF. From Figure 3 in Myzithras et al, 2017.*

As expected from the PK/PD study, the levels of cyno recombinant CTGF did not go below 20% (mAb 1) or 50% (mAb 2) in the presence of excess antibody. But the situation was different in the case of human recombinant CTGF, where excess mAb 1 reduced the level of free CTGF to 0%. This titration experiment indicated that the low target coverage in the preclinical study using cynomolgus monkeys was *not* an assay artifact, despite the large differences in affinity between reagents. As the authors point out, this result indicates that care should be taken when developing assays for cynomolgus monkeys to determine target coverage and using the data to guide estimates for human dosage. The differences in affinity between human and cyno CTGF meant that the humanized anti-CTGF was not optimal for a cyno PK/PD study in helping guide dose predictions based on target coverage.

Gyrolab affinity determinations are comparable with SPR data

It always instills confidence when a new methodology gives results corresponding to those obtained with traditional technologies. The team was therefore encouraged by the high correlation between results obtained using Gyrolab system and SPR spectroscopy for the four mAbs used in this study (Figure 4).

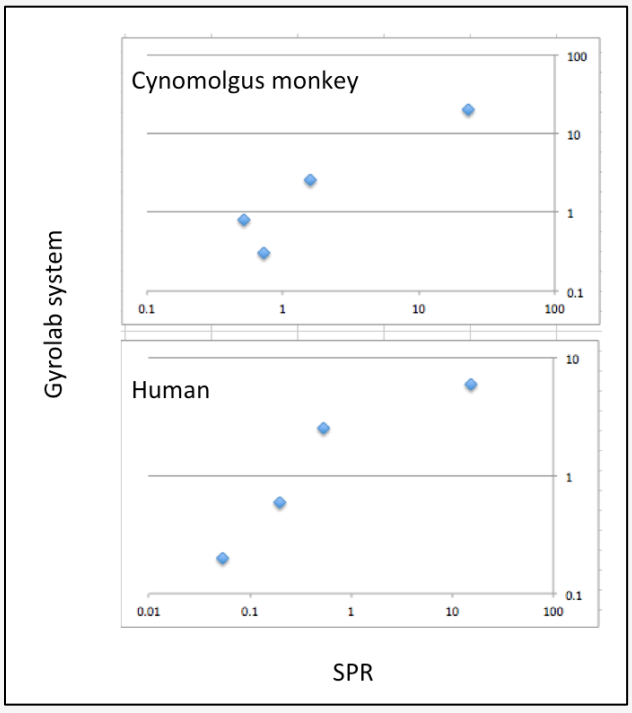


Figure 4. *SPR spectroscopy and Gyrolab system give similar K_D values (nM) for the four monoclonal antibodies used in the study vs cyno or human CTGF. Data taken from Table 3, Myzithras et al, 2017.*

A valuable tool for characterizing immunoassay reagents

The authors view Gyrolab system combined with Gyrolab Affinity Software as a powerful tool to improve workflows and screening compared with traditional SPR-based methods, and delivers comparable binding affinities using minimal reagent volume and with rapid assay development. They also point out that this combination provides a user-friendly experimental setup and data analysis with automated curve fitting and calculations. In addition, the broad dynamic range enables the characterization of extremely high affinity binders with K_D's in the sub-picomolar range, which can be challenging using SPR-based technology due to the slow off-rates.

In the study presented here, Gyrolab Affinity Software helped the team at Boehringer Ingelheim Pharmaceuticals to characterize the CTGF reagents to help explain the low target coverage in a PK/PD study using cynomolgus monkeys. They also noted that in another study, Gyrolab system enabled over 600 solution-based binding affinity determinations to be performed in two weeks (4). The authors conclude that, "the affinity software module on the Gyrolab xP workstation is useful in reagent characterization for developing and optimizing bioanalytical PK/PD assays."

References

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