Novel drug and soluble target tolerant antidrug antibody assay for therapeutic antibodies bearing the P329G mutation

Aim: Bridging immunoassays for detection of antidrug antibodies (ADAs) are typically susceptible to high concentrations of residual drug. Sensitive drug-tolerant assays are, therefore, needed. **Materials & methods:** An immune complex assay to detect ADAs against therapeutic antibodies bearing Pro329Gly mutation was established. The assay uses antibodies specific for the Pro329Gly mutation for capture and human soluble Fc γ receptor for detection. **Results:** When compared with a bridging assay, the new assay showed similar precision, high sensitivity to IgG1 ADA and dramatically improved drug tolerance. However, it was not able to detect early (IgM-based) immune responses. **Conclusion:** Applied in combination with a bridging assay, the novel assay serves as orthogonal assay for immunogenicity assessment and allows further characterization of ADA responses.

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Keywords: antidrug antibody • drug tolerance • ELISA • Fc γ RI • immune complex assay • immunogenicity • safety assessment • suppressed Fc effector functions • target interference

Bridging immunoassay (Figure 1A) is currently the predominant assay format for immunogenicity assessment in preclinical and clinical studies [1–3]. The assay allows for high-throughput testing with high sensitivity and easy implementation [4]. However, the bridging assay can be interfered by the presence of oligomeric soluble target, which can serve as a ‘bridge’ thus reducing specificity by providing a false-positive result [5–8], or residual drug, which can form an immune complex with antidrug antibodies (ADAs), thereby reducing sensitivity by providing a false-negative result [6,9,10]. Thus, there is a need for alternative assay formats that can overcome these potential weaknesses of the bridging design [11].

We recently described a novel assay with an orthogonal detection method based on digoxigenin (Dig) labeled ectodomain (extracellular ligand-binding domain) of the human Fc γ receptor I (hsFc γ RI; see

Figure 1B) [12]. In this assay, the secondary signal detection provides evidence that the assay signal is caused by an IgG1 antibody – a clear advantage over the bridging assay, which would detect any entity that is able to bind two drug molecules, such as an oligomeric soluble target. The hsFc γ RI-based assay reliably detected human and Cynomolgus monkey IgG-ADAs against therapeutic monoclonal antibodies (mAbs) with suppressed Fc effector functions, such as those bearing Pro329Gly (PG) or Leu234Ala and Leu235Ala (LALA) substitutions in the Fc region [12–14]. The assay requires only one free ADA Fab fragment, as opposed to two in the bridging format, and hence it was less susceptible to the presence of residual drug. Yet, the interference by residual drug cannot be completely excluded by the use of a hsFc γ RI-based detection method because the capture method still requires a free drug-binding site on the ADA molecule.

Uwe Wessels^{*1}, Eginhard Schick², Mirko Ritter³, Frank Kowalewsky³, Julia Heinrich¹ & Kay Stubenrauch¹

¹Pharmaceutical Sciences, Pharma Research & Early Development, Roche Innovation Center Munich, Nonnenwald 2, 82377 Penzberg, Germany

²Pharmaceutical Sciences, Pharma Research & Early Development, Roche Innovation Center Basel, Grenzacherstrasse 124, 4070 Basel, Switzerland

³Roche Diagnostics GmbH, Roche Innovation Center Munich, Nonnenwald 2, 82377 Penzberg, Germany

*Author for correspondence:

Tel.: + 49 885 660 3814

Fax: + 49 885 660 3268

uwe.wessels@roche.com

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Therefore, we pursued the further development of the hsFcγRI-based assay, aiming at achieving even better tolerance toward high concentrations of residual drug. The improved assay format presented in this report can complement the bridging assay in immunogenicity assessment of therapeutic mAbs bearing the PG-substitution in the Fc region, which are used in several clinical development projects [12,14,15]. The assay principle, which, in similar design has been previously described for the preclinical assessment of drug–ADA complexes [11,16–18], involves the capture of a drug–ADA complex by an immobilized antibody that is specific for the PG-mutagenized Fc region. Bound immune complexes are detected using hsFcγRI (Figure 1C). Since the hsFcγRI does not bind to the PG-modified Fc region of the drug (Figure 1D; [12]) and the anti-PG capture antibody, in turn cannot bind to the wild-type IgG (wtIgG; Figure 1E), the detection is specific for the drug–ADA complex. We therefore expect that the assay is intrinsically insensitive to even high drug concentrations in the matrix. Moreover, the absence of residual drug may negatively affect the assay sensitivity, as it may lead to low levels of drug-bound ADA in the sample. The presented assay format therefore includes a sample pre-incubation step with an excess of free drug, which ensures the presence of drug–ADA complexes. Like the previously described assay, the new hsFcγRI-PG assay benefits from using hsFcγRI as detection reagent, ensuring that the serum factor that is responsible for a signal is indeed an antibody.

In this article, we describe the developed hsFcγRI-PG assay format and compare it with a standard bridging assay, including assay qualification, assessment of drug tolerance and the applicability of the hsFcγRI-PG assay to immunogenicity testing in clinical samples.

Materials & methods

Chemicals, reagents & equipment

All antibodies were produced by Roche Diagnostics GmbH (Penzberg, Germany) and were stored in aliquots at -80°C until use [19]. Anti-X mAb is a recombinant human PG-modified IgG1 targeting protein X. The capture antibody against the PG modification was generated by immunization of mice with human monoclonal IgG1 bearing the PG and LALA substitutions within the Fc domain. Briefly, hybridomas were generated by fusion of splenocytes of immunized mice with P3 × 63-Ag8.653 myeloma cells. Hybridomas were screened for production of mAbs binding to human IgG containing the PGLALA mutation using ELISA. Cross-reactivity with human wild-type (WT) IgG was tested by ELISA and cross-reactive mAbs were excluded from further evaluation. hsFcγRI was

purchased from R&D Systems (MN, USA). Biotin (Bi)- and Digoxigenin (Dig)-labeling of antibodies and hsFcγRI were performed in-house.

Anti-Dig polyclonal sheep Ab-Fab-fragments coupled with horseradish peroxidase (anti-Dig-S-Fab-HRP), peroxidase substrate 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and phosphate-buffered saline were from Roche Diagnostics GmbH. Low Cross Buffer® was from Candor Bioscience GmbH (Wangen, Germany). Washing buffer contained 0.05% Tween-20 (Calbiochem, Darmstadt, Germany) and 0.0002% 5-bromo-5-nitro-1,3-dioxane (Bronidox; Sigma-Aldrich, Steinheim, Germany) in phosphate-buffered saline. Human pooled serum (HPS) and individual human serum samples collected in all cases from healthy individuals were obtained from Sera Laboratory International Ltd. (Haywards Heath, UK).

Streptavidin (SA) coated 96-well ELISA microtiter plates (SA-MTP) were provided by Roche Diagnostics GmbH. Biacore® T200 instrument, CM5 sensor chip and amine coupling kit were obtained from GE Healthcare Life Science (Uppsala, Sweden).

Specificity of anti-PG & hsFcγRI binding

The specificity of hsFcγRI binding was determined by surface plasmon resonance using a Biacore T200 instrument. The anti-PG antibody and hsFcγRI (diluted to 10 µg/ml in 10 mM acetate buffer, pH 4.5) were directly immobilized to a CM5 sensor chip using amine coupling kit (achieved immobilization levels were approximately 8000 relative units for the anti-PG antibody and 5000 relative units for the hsFcγRI). After immobilization, an antibody sample (10 µg/ml) was injected for 60 s at a flow rate of 10 µl/min and temperature of 25°C. Resonance signal was measured 80 s after the start of injection. Following the analysis, the chip was regenerated by injection of Tris-HCl buffer, pH 2.0 for 30 s.

Bridging ADA assay

ADAs against mAb anti-X were detected by a one-step ELISA (Figure 1A). Affinity-purified anti-idiotypic polyclonal rabbit antibody (Ab) directed against the complementarity determining region of mAb anti-X served as calibration standards and QC samples (prepared in HPS). Reagents and samples were incubated at room temperature with shaking at 450 rpm.

Samples were diluted tenfold in Low Cross Buffer® containing Bi- and Dig-labeled mAb anti-X (each at 1.0 µg/ml), incubated overnight and then transferred to a SA-MTP (2 × 100 µl of each sample in duplicate wells). After 1-h incubation, the plate was washed three-times followed by aspiration, 100 µl anti-Dig-

Figure 1. Principles of the bridging, human soluble Fc γ receptor I based and human soluble Fc γ receptor I-Pro329Gly assays. (A) In the bridging assay, the two antigen-binding sites of ADA couple immobilized drug (drug-B) with digoxigenylated drug (drug-Dig) used for detection. (B) In the hsFc γ RI-based assay, ADA is captured by immobilized drug and then detected using digoxigenylated hsFc γ RI (hsFc γ RI-Dig). (C) In the hsFc γ RI-PG assay, PG-modified drug-ADA complex (IC) is captured by immobilized anti-PG antibody and the bound ADA is detected using Fc γ RI-Dig. (D) Anti-PG antibody but not hsFc γ RI can bind to a PG-modified drug. (E) hsFc γ RI but not anti-PG antibody can bind to wild-type IgG (e.g., ADA). ADA: Antidrug antibody; B: Biotin; Dig: Digoxigenin; hsFc RI: Human soluble Fc receptor I; PG: Pro329Gly substitution; SA-MTP: Streptavidin-coated microtiterplate.

S-Fab-HRP conjugate (25 mU/ml) was added to each well and the plate was incubated for 1 h. Thereafter, the plate was washed three-times followed by aspiration, ABTS (100 μ l per well) was added and the color reaction was photometrically assessed at 405 nm (reference wavelength 490 nm) at a time when the highest standard reached an optical density (OD) of 1.8–2.2. Samples were measured in duplicates and mean absorbance values were calculated. Measurements were considered valid if the precision of the assay signal per duplicate was $\leq 20\%$ of the coefficient of variation (CV).

The screening and the confirmatory cut-point was determined nonparametrically according to Shankar *et al.* [20] by analyzing 100 individual blank serum samples (in the absence and the presence of spiked

mAb anti-X). To calculate plate-specific cut-points, the plate specific blank value was multiplied by the assay specific normalization factor. In this study, two plates were used to generate data. The corresponding cut-points were 0.061/0.062 OD. Each sample was analyzed three-times in duplicates. Clinical sample analysis followed the three-tiered approach described in the literature [20].

hsFc γ RI-PG assay

The assay was developed as a three-step solid phase ELISA (Figure 1C). A conjugate of human wtIgG and human PG-modified IgG was used as calibration standard and for preparation of QC samples. (prepared in HPS). Reagents and samples were incubated at room temperature with shaking at 450 rpm.

First, Bi-labeled anti-PG antibody (2 µg/ml, 100 µl per well) was bound to a SA-MTP. In the meantime, study and QC samples were pre-incubated under shaking (500 rpm) for 30 min in Low Cross Buffer® containing 1 µg/ml mAb anti-X. After threefold washing and aspiration of the microtiter plate, two-times 100 µl of these pre-incubated samples were added to adjacent wells. After 1-h incubation, the plate was washed threefold followed by aspiration and 100 µl of Dig-labeled hsFcγRI (0.5 µg/ml) were added to each well. After an 1-h incubation and threefold washing followed by aspiration, 100 µl anti-Dig-S-Fab-HRP conjugate (50 mU/ml) were added to each well and the plate was incubated again for 1 h. After threefold washing and aspiration, ABTS (100 µl per well) was added and the color reaction was followed in the microplate reader.

Readout of the microtiter plate was performed as described for the bridging assay.

The cut-point was evaluated by analyzing 25 individual blank serum samples of either sex (a total 50 samples) in duplicates. Calculation of the cut-point was performed using a non-parametric approach according to Shankar *et al.* [20]. To calculate plate-specific cut-points, the plate-specific blank value was multiplied by the assay-specific normalization factor. In this study, two plates were used to generate data. The corresponding cut-points were 0.188/0.187 OD.

Analysis of drug tolerance

Bridging and hsFcγRI-PG assays were performed in the presence of mAb anti-X using affinity-purified anti-idiotypic polyclonal rabbit Abs directed against

Figure 2. Specificity of anti-Pro329Gly monoclonal antibody and human soluble Fcγ receptor I binding as assessed by surface plasmon resonance. The binding specificity of anti-PG mAb and hsFcγRI was tested for purified human wild-type IgG1, wild-type IgG4 and IgG subtypes with suppressed Fc effector functions (mutations PG, LALA, and PGLALA).

Hu: Human; hsFc RI: Human soluble Fc receptor I; LALA: Leu234Ala and Leu235Ala; mAb: Monoclonal antibody; PG: Pro329Gly substitution; RU: Relative units; WT: Wild-type.

Table 1. Qualification data for the human soluble Fc γ receptor I-Pro329Gly and bridging assays.

QC (assay concentration)	hsFc γ RI-PG assay		Bridging assay	
	75 ng/ml (HQC)	5.73 ng/ml (CPQC)	154 ng/ml (HQC)	1.89 ng/ml (CPQC)
Intra-assay precision (% CV)	2	2	2.9	1.7
Interassay precision (% CV)	12	6	7.4	2.5
Drug tolerance factor	833		6	
Dilution factor	50 (2% serum)		10 (10% serum)	
Normalization factor	0.118 OD (additive)		0.054 OD (additive)	
Assay signal at screening cut point (two plates)	0.188/0.187 OD		0.061/0.062 OD	
Estimated cut point sensitivity in serum	5.73 ng/ml		1.89 ng/ml	

CPQC: Cut point quality control; CV: Coefficient of variation; HQC: High concentration quality control; hsFc γ RI: Human soluble Fc γ receptor I; n: Number of dataset/replicate; OD: Optical density; PG: Pro329Gly; QC: Quality control.

the complementarity determining region of mAb anti-X as a control ADA for the bridging assay and a conjugate of human wtIgG and human PG-modified IgG for the hsFc γ RI-PG assay. The drug tolerance factor was calculated as the maximal ratio between the concentrations of the control ADA and mAb anti-X, at which a positive screening signal was still observed.

Quantification of mAb anti-X

Drug levels were measured with a solid-phase ELISA with a quantification range of 1.5–60 ng/ml. Briefly, a biotinylated anti-idiotypic antibody was immobilized on a streptavidin-coated MTP to capture the anti-X antibody. Bound drug was detected by a dioxxygenylated anti-X detection reagent and anti-Digoxigenin antibody peroxidase conjugate. ABTS was used as a substrate to generate a color reaction, which was photometrically assessed at 405 nm (reference wavelength 490 nm).

Human study samples

Samples were obtained from eight patients who had received weekly or biweekly administrations of mAb anti-X (10 mg each dose). Blood samples were collected before and once daily after each dose. Blood samples were clotted for about 30 min at room temperature and were centrifuged within 1 h after blood collection at 1500 $\times$ g. Serum fractions were collected and stored at -70°C until analysis.

Results & discussion

Specificity of anti-PG mAb & hsFc γ RI

We tested the binding specificity of the anti-PG mAb raised against PGLALA IgG1 to purified human wt IgG1, wtIgG4 and human IgG1 bearing PG, LALA or PGLALA mutations (Figure 2, upper panel). The anti-PG mAb demonstrated a strong binding to PGLALA IgG1 and, to IgG1 carrying PG mutation only, with

the signal for PG IgG1 being only slightly lower than that for PGLALA IgG1. The binding to IgG1 with PG mutations in the Fc region was specific, as no signal was detected for wtIgG1, wtIgG4 or LALA IgG1. Thus, although the anti-PG mAb was raised against PGLALA IgG1, it can be used for capturing both PG and PGLALA IgG1.

As expected [12], hsFc γ RI binding to IgG1 bearing PG, LALA or PGLALA mutations was almost completely eliminated, whereas a strong signal was observed for human wtIgG1 (Figure 2, lower panel). Binding to wtIgG4 was weak in agreement to already published data [21,22].

Overall, these results demonstrate appropriate specificity of the used capture and detection reagents and that we can largely exclude interference due to unspecific capture of WT antibodies by the anti-PG mAb or unspecific hsFc γ RI binding to the captured drug.

Assay qualification parameters for bridging & hsFc γ RI-PG assays

The bridging and hsFc γ RI-PG assays demonstrated comparable intra and interassay precision in the assay qualification for the use in human serum samples (Table 1). The hsFc γ RI-PG had a slightly reduced interassay precision at high QC, yet all parameters were within the accepted deviation range ($\leq 20\%$). Both assays showed high and very similar intra-assay precision ($CV \leq 3\%$).

The dilution factor and the normalization factor for cut point (CP) were higher in the hsFc γ RI-PG assay than in the bridging assay, indicating a slightly lower sensitivity.

Improved drug tolerance of the hsFc γ RI-PG assay

The drug tolerance factor for the hsFc γ RI-PG assay was 833 (30 ng/ml positive control was detected as positive in the presence of up to 25 μ g/ml mAb anti-X) as compared

with approximately 6 for the bridging assay (500 ng/ml positive control was detected as positive in the presence of up to 3 µg/ml mAb anti-X). Although these experimental data were generated with two different positive controls, which only mimic real samples, the dramati-

cally improved drug tolerance of the hsFcγRI-PG assay confirms our assumption that the assay setup is inherently resistant to interference by residual drug. Indeed, while the hsFcγRI-PG assay relies on the formation of a drug–ADA complex with the residual drug acting as a

Figure 3. Time course of antidrug antibody detection and drug concentration in three patients (cont. from facing page). Patients received biweekly (A & C) or weekly administration (B) of the therapeutic antibody mAb anti-X (10 mg each dose). Samples were tested for antidrug antibodies by both the bridging and the hsFcγRI-PG assays. -: ADA negative; +: ADA positive; ADA: Anti-drug antibody; C2: Second treatment cycle; C3: Third treatment cycle; mAb: Monoclonal antibody; OD: Optical density; pre: Predose.

signal enhancer, the bridging assay is negatively affected by residual drug, which can even lead to false-negative results at high drug concentrations (Figure 1).

Comparison of bridging & hsFcγRI-PG assays in human study samples

We assessed the performance of the assays in serum samples obtained from eight patients who had received weekly or biweekly administrations of 10 mg mAb anti-X (Table 2). In the bridging assay, seven patients were tested ADA-positive as compared with eight patients in the hsFcγRI-PG assay. Both assays demonstrated similar qualitative results for about 71% of the investigated samples. For 29% of the samples, different results were obtained with both assays (Figure 3). Below, we discuss possible interpretations and the impact on the applicability of the assays.

The onset of the immune response was detected earlier by the bridging assay (Table 2 & Figure 3) than by the hsFcγRI-PG assay in five of eight patients, with the especially striking difference being observed in Patient C (Figure 3C). This finding is in line with the previous observations [12] and most likely reflects early IgM-driven response [23], which cannot be detected by

the hsFcγRI-based assays [24]. Of note, the hsFcγRI-PG assay produced negative results for the late samples of Patient D (Table 2), suggesting a mixed IgM-IgG response with a predominant proportion of IgM in this patient without undergoing the usually observed class switch during immune response maturation [23].

At later time points, the hsFcγRI-PG assay showed a higher and more reliable detection rate than the bridging assay (Table 2). The reliability of the hsFcγRI-PG assay was most prominent in first post-dose samples after the second and third dosing, which were tested ADA-negative by the bridging assay (see also Figure 3). The failure of the bridging assay to detect ADA correlated with detectable drug concentration in these samples, thus again demonstrating high drug tolerance of the hsFcγRI-PG assay. It is worth noting that the presence of residual drug did not affect the qualitative results of the bridging assay in patients with high ADA concentrations (Table 2, patients C, G and H), because the ADA signal, although clearly decreased, remained above the cut point (Figure 3C & Supplementary Material).

The hsFcγRI-PG assay also showed a higher detection rate and higher signal in the late postdose

Biweekly dosed patients											
Patient A			Patient C			Patient E			Patient G		
Time point	hsFc γ RI-PG	Bridging Drug (ng/ml)	hsFc γ RI-PG	Bridging Drug (ng/ml)	Drug (ng/ml)	hsFc γ RI-PG	Bridging Drug (ng/ml)	Drug (ng/ml)	hsFc γ RI-PG	Bridging Drug (ng/ml)	Drug (ng/ml)
24 h	-	-	6250	-	-	1620	-	-	212	-	2350
72 h	n.d.	n.d.	-	-	43	n.d.	n.d.	n.d.	-	-	97
96 h	-	-	218	-	6	-	-	4	-	+	11
120 h	-	+	27	-	3	-	-	3	-	+	BLQ
168 h	+	+	2	-	2	-	-	2	n.d.	n.d.	n.d.
C2 Pre	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	BLQ	+	+	BLQ
C2 24 h	+	-	2430	-	1310	-	-	545	+	+	1010
C2 168 h	+	+	BLQ	+	BLQ	+	-	BLQ	+	+	BLQ
C3 pre	+	+	BLQ	+	BLQ	+	-	BLQ	+	+	BLQ
Weekly dosed patients											
Patient B			Patient D			Patient F			Patient H		
Time point	hsFc γ RI-PG	Bridging Drug (ng/ml)	hsFc γ RI-PG	Bridging Drug (ng/ml)	Drug (ng/ml)	hsFc γ RI-PG	Bridging Drug (ng/ml)	Drug (ng/ml)	hsFc γ RI-PG	Bridging Drug (ng/ml)	Drug (ng/ml)
24 h	-	-	885	-	-	722	-	-	2820	-	1800
72 h	-	-	3	-	-	62	-	-	262	-	70
96 h	-	+	4	-	-	7	-	+	3	-	6
C2 Pre	+	+	1	+	BLQ	+	+	BLQ	+	+	BLQ
C2 24 h	+	-	144	n.d.	n.d.	+	-	263	+	+	547
C2 96 h	+	-	BLQ	+	10	+	+	BLQ	+	+	BLQ
C3 pre	+	+	BLQ	-	1	+	+	BLQ	+	+	BLQ
C3 24 h	+	-	176	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	14
C3 96 h	+	-	1	-	8	n.d.	n.d.	n.d.	+	+	BLQ

Dark horizontal lines represent dosing.
 -: ADA negative; +: ADA positive; ADA: Anti-drug antibody; BLQ: Below lower limit of quantification; C2: Second treatment cycle; C3: Third treatment cycle; hsFc γ RI: Human soluble Fc γ receptor I; n.d.: No data; PG: Pro329Gly; pre: Predose.

samples with drug concentrations below quantification level (Figure 3), which was evident in patient B (Table 2 & Figure 3B) and especially in patient E (Table 2). Together with the higher dilution factor for the hsFcγRI-PG assay (Table 1), these results may indicate a better sensitivity of the hsFcγRI-PG assay despite the higher normalization factor. However, no definitive conclusion can be drawn on the basis of these data due to the variety of factors that can influence the sensitivity of the assays, such as drug concentration or patient's specific isotype composition of ADA.

Overall assay comparison

The standard bridging and hsFcγRI-PG assays differ in both the capturing and detection principle. In the bridging assay, drug-free ADAs are captured and detected by two differently labeled drug molecules, whereas the hsFcγRI-PG assay uses an antibody against a modified Fc region of the drug to capture a drug–ADA complex, which is then detected by the hsFcγRI specific for wtIgG1. The drug-dependent detection and capture makes the bridging assay susceptible to the presence of oligomeric soluble target, which can mimic ADA-bridge and thereby lead to false-positive results, and to the presence of residual drug, which can prevent detection by occupying binding sites of ADA [5–8]. In contrast, the hsFcγRI-based detection makes the hsFcγRI-PG assay tolerant to the presence of oligomeric soluble target [12], while the overall assay design that aims at the detection of drug–ADA complexes precludes the interference by residual drug. In fact, the assay format includes sample pre-incubation with free drug, which enables the formation of drug–ADA complexes and thus ensures the measurement of total ADA, especially in samples with low levels of *in vivo*-formed drug–ADA complexes. The assay may also be beneficial for detection of low affinity ADAs, because it does not require stable binding of two drug molecules.

However, the bridging assay is able to detect ADA of various Ig-subtypes, including IgM, and is applicable to all kinds of therapeutic antibodies [6,9], while the hsFcγRI-PG assay does not recognize Ig-subtypes other than IgG1 and therefore cannot detect less mature, potentially transient, immune responses of the IgM-type. The hsFcγRI-PG assay is also limited to therapeutic antibodies bearing the PG modification within the Fc domain.

Overall, the hsFcγRI-PG assay represents a useful generic approach of immunogenicity testing for PG-modified antibodies. The assay can easily be adapted to different projects because it does not require labeled therapeutic mAb, which can especially be

advantageous for complex antibodies (e.g., multivalent antibodies or antibody–drug conjugates). The hsFcγRI-PG assay is extremely tolerant to the presence of any serum factor that is able to bind two drug molecules, such as an oligomeric soluble target, providing direct evidence that the observed signal is indeed an antibody. It is also insensitive to the presence of residual drug and may have increased sensitivity for the late immune response. Its inability to detect IgM response can be beneficial for immunogenicity assessment in special populations, such as in patients with high levels of rheumatoid factor – a composition of IgM antibodies that can be tested false positive in the bridging assays [25]. Although its specificity to IgG1 is a significant limitation, it is worth noting that a typical immune response always switches to IgG1. Therefore, we assume that there will be detectable ADA for this assay in most practical situations [23,26]. Finally, applied in a combination with the bridging assay, the hsFcγRI-PG assay can allow a more detailed characterization of the immune response.

Conclusion

The novel hsFcγRI-based assay for IgG1 ADA detection can be considered a platform assay applicable for all therapeutic antibodies bearing the PG mutation in the Fc domain. The assay does not require labeled drug, detecting drug–ADA complexes using two assay-specific reagents: a Bi-labeled antibody directed against PG modified therapeutic mAbs and Dig-labeled hsFcγRI, which specifically detects human wtIgG1. When compared with the bridging assay in qualification exercises and clinical studies, the hsFcγRI-PG assay showed similar precision and markedly improved drug tolerance. The assay can complement the bridging assay in immunogenicity assessment, increasing the overall reliability of the ADA detection and providing additional information about the nature of immune response.

Future perspective

The hsFcγRI-PG assay can complement the bridging assay as orthogonal assay, increasing the overall reliability of the immunogenicity assessment of therapeutic mAb with suppressed Fc effector function. The combination of both analytical methods, as suggested, is especially beneficial for the analysis of samples containing high concentration of residual drug or oligomeric soluble targets, which cannot be reliably tested by the bridging assay alone. Moreover, the IgG1-specificity of the hsFcγRI-PG assay can help to further characterize the immunogenicity profile of therapeutic mAbs, for example, by differentiating

between early IgM and late IgG responses. Performed without sample pre-incubation with free drug, the assay can detect pre-existing drug–ADA complexes (as opposed to total ADA detection described in this article) and thus contribute to a better understanding of adverse effects that result from the formation of immune complexes [27].

The assay can easily be adapted to any PG-modified therapeutic mAb, as it does not require the generation of drug-dependent capture and detection reagents. The high-sequence homology of the Fc region between human and cynomolgus monkey IgG [28] allows the usage of the same assay for the analysis of preclinical and clinical samples [12]. In addition to the PG, several other Fc modifications affect the affinity of mAbs to both the Fc receptors and complement [16,29–31]. We believe that the assay principle can be transferred to other Fc mutations that allow the generation of specific Abs to be used similarly to the anti-PG mAb.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.future-science.com/doi/full/10.4155/bio-2017-0048

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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Executive summary

- A novel assay to detect drug-antidrug antibody (ADA) complexes using anti-PG antibody for capture and human soluble Fc γ receptor for detection was established. The human soluble Fc γ receptor I-Pro329Gly (hsFc γ RI-PG) assay represents a platform solution for all therapeutic antibodies bearing PG modification, which does not require specifically labeled therapeutics.
- A bridging assay will detect anything that binds two molecules of drug, in contrast, the hsFc γ RI-PG assay confirms that the serum factor binding to drug is indeed an antibody.
- The hsFc γ RI-PG assay was compared with a standard bridging assay. Both assays showed adequate results during assay qualification. The hsFc γ RI-PG assay, however, demonstrated much higher drug tolerance than the bridging assay.
- The hsFc γ RI-PG assay had high sensitivity to IgG1 ADAs, but was not able to detect early (IgM-based) immune responses in the analysis of human samples from a clinical study. In contrast, the assay was superior to the bridging assay in detecting ADAs in the presence of residual drug.
- The hsFc γ RI-PG assay can be used as an orthogonal method complementary to the standard bridging assay, but not as a solitary assay.
- A combination of the standard bridging assay with the hsFc γ RI-PG assay can improve the reliability of immunogenicity assessment, especially in the presence of residual drug or oligomeric-soluble targets. The hsFc γ RI-PG assay provides evidence that the detected signal is caused by an IgG1 antibody. It can also allow a more detailed characterization of the immunogenicity response to therapeutic monoclonal antibodies containing PG mutation in the Fc domain with respect to the immunoglobulin subclass.

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