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Research paper

Comparison of cell-based and non-cell-based assay platforms for the detection of clinically relevant anti-drug neutralizing antibodies for immunogenicity assessment of therapeutic proteins

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ABSTRACT

Anti-drug neutralizing antibodies (NAbs) formed due to unwanted immunogenicity of a therapeutic protein point towards a mature immune response. NAb detection is important in interpreting the therapeutic's efficacy and safety *in vivo*. *In vitro* cell-based NAb assays provide a physiological system for NAb detection, however are complex assays. Non-cell-based competitive ligand binding (CLB) approaches are also employed for NAb detection. Instead of cells, CLB assays use soluble receptor and conjugated reagents and are easier to perform, however have reduced physiological relevance. The aim of this study was to compare the performance of CLB assays to established cell-based assays to determine the former's ability to detect clinically relevant NAbs towards therapeutics that (i) acted as an agonist or (ii) acted as antagonists by binding to a target receptor.

We performed a head-to-head comparison of the performance of cell-based and CLB NAb assays for erythropoietin (EPO) and two anti-receptor monoclonal antibodies (AMG-X and AMG 317). Clinically relevant NAb-positive samples identified previously by a cell-based assay were assessed in the corresponding CLB format(s). A panel of 12 engineered fully human anti-EPO monoclonal antibodies (MAbs) was tested in both EPO NAb assay formats. Our results showed that the CLB format was (i) capable of detecting human anti-EPO MAbs of differing neutralizing capabilities and affinities and (ii) provided similar results as the cell-based assay for detecting NAbs in patient samples. The cell-based and CLB assays also behaved comparably in detecting NAbs in clinical samples for AMG-X. In the case of anti-AMG 317 NAbs, the CLB format failed to detect NAbs in more than 50% of the tested samples. We conclude that assay sensitivity, drug tolerance and the selected assay matrix played an important role in the inability of AMG 317 CLB assays to detect clinically relevant NAbs.

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1. Introduction

All therapeutic proteins are expected to induce a certain level of unwanted immune response in patients; therefore it is important to monitor their immunogenic potential during drug development. Immunogenicity monitoring provides important information that allows interpretation of pharmacokinetic, pharmcodynamic and safety studies (Radstake et al., 2009). A risk based approach has been proposed for immunogenicity testing (Koren et al., 2008).

Abbreviations: ADA, anti-drug antibody; MAb, monoclonal antibody; NAb, neutralizing antibody; CLB, competitive ligand binding; MRD, minimal required dilution; PC, positive control; PHS, pooled human serum; MAb, monoclonal antibody; IC_{50}, half of maximal inhibitory concentration; RT, room temperature; h, hour; min, minute; SPRIA, surface plasmon resonance immunoassay; ECL, electrochemiluminescence; rHu, recombinant human; Bio, biotinylated; EPO, epoietin alfa; NESP, darbepoietin alfa; FU, followup; IL-4, interleukin-4.

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The immune response generally consists of anti-drug antibodies (ADAs) that can bind to the therapeutic and depending upon the magnitude of the immune response could also neutralize its activity *in vivo*. ADAs with the latter property are classified as neutralizing antibodies (NAbs). A tiered approach has been adopted industry wide for antibody testing. A sample designated for ADA testing is first screened and confirmed for the presence of binding antibodies via an immunoassay. Samples that test positive for binding antibodies are expected to be assessed for any drug-specific neutralizing ability in a NAb assay. There are two major platforms for NAb assays: cell-based or non-cell-based (Civoli et al., 2012). EMA and FDA immunogenicity guidance documents indicate a preference for cell-based NAb assays due to their physiological relevance (European Medicines Agency, EMEA, 2007; US Food Drug Administration, FDA, 2014) especially for therapeutic proteins that resemble an endogenous protein (e.g. EPO) where cross-reactive NAbs could result in an autoimmune state, thereby impacting patient safety. A non-cell-based assay appears to be acceptable when a drug's mechanism of action is to solely neutralize a soluble target. Non-cell-based assays may also be employed (in consultation with regulatory agencies) when substantial efforts to develop a sensitive, specific, reliable cell-based assay fail due to unavailability of a cell line or due to assay related issues (e.g. serum toxicity, impaired assay precision, robustness, sensitivity, etc.) that render a cell-based NAb assay unreliable.

In a cell-based assay, NAbs are detected by their ability to interrupt the signaling events induced or impacted by the drug (Gupta et al., 2007). A variety of endpoints are available for cell-based NAb assays and their selection relies upon the signaling mechanism utilized or disrupted by the drug product. In contrast, non-cell-based NAb assays are based on the ability of NAbs to interrupt the binding between the drug and its target either of which may be immobilized on a surface. In the 3 examples shown in this paper, the indirect CLB assay uses immobilized receptor and the drug-induced interruption of the binding of ligand to the receptor forms the basis of NAb detection. The direct CLB format uses immobilized target (also the receptor for the selected products) and the inhibition of the ability of the drug to bind to the coated target allows the determination of NAbs.

In contrast to direct or indirect CLB assays, cell-based assays tend to be more complex and require a longer time for assay development, optimization and validation (Gupta et al., 2007, 2011). The ease of performing CLB assays is appealing; however the question still remains for these assays whether they provide a scientifically relevant system for detecting clinically relevant NAbs as compared to a cell-based assay. The value of using non-cell-based instead of cell-based NAb assays during routine immunogenicity testing is a frequently debated topic in industry forums especially if the drug is a monoclonal antibody therapeutic and the risk of NAbs is restricted to loss of efficacy. Finco et al. (2011) showed improved assay sensitivity for non-cell-based NAb assays, however no clinical data was provided. A recent report has shown a good correlation in NAb titers derived from cell-based and CLB assays (Cludts et al., 2013).

We present here the first report of a comparative assessment of cell-based assays and CLBs for three different drug products that had varied mechanisms of action (agonist, antagonist, etc). The drug products included EPO and two anti-receptor MAbs (AMG-X and AMG 317). Both EPO (Watowich et al., 1994) and AMG-X utilized or impacted signaling via receptors that underwent homodimerization. In contrast, AMG 317 impacted signaling of IL-4 that utilized a heterodimeric receptor for

Table 1
Cell-based assay methodologies.

Drug name	Cell line	Assay principle	Assay matrix (PHS)	Critical reagents (final in well)	Assay steps
EPO	32D cells (IL-3-dependent)	32D cells respond to EPO with proliferation that was blocked by anti-EPO NAbs.	5%	rHuEPO: 1 ng/mL 2×10^4 cells (Wei et al., 2004)	• Cells were deprived of IL-3 for 24 h. • Samples were incubated with EPO at RT for 45 min followed by addition of the remaining critical reagents. • Plates were incubated for 48 h at 37 °C. • 2 uCi/well of ^{3}H thymidine was added to each assay well and plates were incubated for 4 h. • Incorporated radioactivity was measured on TopCount.
AMG-X	Mo7e cells (GMCSF dependent)	Mo7e cells respond to Ligand A with tyrosine phosphorylation of Receptor-X. AMG-X blocked Ligand A's activity. Anti-AMG-X NAbs reverse the inhibition produced by AMG-X.	5%	AMG-X: 25 ng/mL Ligand A: 10 ng/mL 4×10^4 cells	• Samples were incubated with AMG-X for 1 h at RT, followed by incubation with Ligand A for 1 h. • The mixture was added to the cells and incubated at 37 °C for 10 min. • AMG-X receptor was immunoprecipitated with biotinylated anti-receptor-X Ab. • Phosphorylated AMG-X receptor was detected by 4G10 and anti-mouse IgG-sulfo-Tag on a MSD reader.
AMG 317	TF-1 cells (IL-4 dependent)	TF-1 cells respond to IL-4 with proliferation that was inhibited by AMG 317. Anti-AMG 317 NAbs reversed the inhibition produced by AMG 317.	5%	AMG 317: 20 ng/mL IL-4: 0.5 ng/mL 3×10^4 cells	• Cell were deprived of IL-4 for 24 h. • Samples were incubated with AMG 317 for 1 h at RT, followed by incubation with IL-4 for 1 h. • The mixture was added to the cells and incubated for 72 h at 37 °C. • The cells were lysed with Titer-Glo and the luminescence was measured on an EnVision reader.

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