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Research paper

Inhibition of interleukin-5 induced false positive anti-drug antibody responses against mepolizumab through the use of a competitive blocking antibody

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ABSTRACT

Mepolizumab, a humanized IgG1 monoclonal antibody that blocks native homodimeric interleukin-5 (IL-5) from binding to the IL-5 receptor, has recently been approved for treatment of severe eosinophilic asthma. Our initial immunogenicity assay method for phase I and II studies utilized a bridging electrochemiluminescence format with biotin and ruthenium-labelled mepolizumab linked by anti-drug antibodies (ADA). We discovered that IL-5 significantly increased in dosed subjects from a phase II study and that the increased IL-5 was in the form of a drug-bound complex. We demonstrated that the elevated drug-bound IL-5 produced false-positive response in the in vitro ADA assay, in which drug-bound IL-5 dissociated and then bridged mepolizumab conjugates to yield positive signal. To eliminate the IL-5 interference, we compared two strategies: a solid-phase immunodepletion of IL-5 and an in-solution IL-5 immunocompetition. We identified the best competitive antibody for each purpose. We found both methods demonstrated similar effectiveness in reducing the false positive signal in IL-5 spiked samples; however, the in-solution immunocompetition for IL-5 had fewer false positives in study samples. Additionally, the in-solution immunocompetition method was experimentally simpler to execute. We modified the ADA assay by adding a pre-treatment step with a mepolizumab competitive anti- IL-5 antibody. Using this new method, we retested clinical samples from two phase II studies (MEA112997 and MEA114092). The confirmed ADA positive incidence was reduced from 29% and 61% to 1% and 8% with the modified in-solution immune inhibition method. Target interference is a fairly common problem facing immunogenicity testing, and target-induced false positive cannot be distinguished from true ADA response by the commonly used drug competitive confirmation assay. The approach and method used here for resolving target interference in ADA detection will be useful for differentiating between a true ADA response and target induced false positive as well as similar challenges in other programs.

1. Introduction

Mepolizumab is a humanized IgG1 monoclonal antibody that blocks human IL-5 from binding the IL-5 receptor and has been an effective treatment to reduce the risk of asthmatic exacerbations in patients with severe eosinophilic asthma (Bel et al., 2014; Ortega et al., 2014; Pavord et al., 2012; Pouliquen et al., 2015). However, the administration of a therapeutic protein (biologic) is often associated with unwanted immune responses. Potential clinical consequences of immunogenicity range from no or little effect, altered PK or efficacy, to severe adverse events (Koren et al., 2002; Schellekens and Casadevall, 2004; Wolbink et al., 2009; Casadevall et al., 2002; Li et al., 2001). The strategy for measuring ADA involves clinical sample analysis with a sensitive screening assay, followed by a confirmation assay. Those clinical samples confirmed as positive are further characterized with titration, isotyping, or neutralization analysis. Ligand binding immunoassay platforms, including enzyme-linked immunosorbent assay (ELISA) and electrochemiluminescent immunoassays (ECLIA) are often used as screening assays to detect antibodies directed against biotherapeutics. The bridging immunoassay with the electrochemiluminescent (ECL) detection is an efficient approach with superior sensitivity and good drug tolerance for the detection of anti-drug antibodies. However, the ECL bridging immunoassay is susceptible to interference from biological components that can inhibit or crosslink the labelled drug to generate either a false negative or false positive result, respectively. In the ADA screening assay, the most common interference that can lead to a false negative is the drug itself. On the other hand, the interference factors that lead to a false ADA positive result can be of many sources, including rheumatoid factor - human antibodies against the Fc region of human IgG (Tatarewicz et al., 2010; Araujo et al., 2011), multivalent target interference (Zhong et al., 2010; Schwickart et al., 2014), soluble target receptors (Dia et al., 2014), and membrane

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bound protein on cellular fragments (Chen et al., 2013). It should be kept in mind that aforementioned drug targets can also result in false-negative ADA detection, depending on the assay format as well as the concentration, structure, and chemical and biological properties of the targets. Therefore, it is important to evaluate and understand the impact of drug intended targets and other relevant biological components for each ADA assay.

IL-5 is produced by a number of cell types and regulates multiple major eosinophil functions, including cellular proliferation, maturation, and mobilization from the bone marrow into the peripheral circulation (Takatsu and Nakajima, 2008; Schrezenmeier et al., 1993; Mishra et al., 2002; Kuo et al., 2001). Overexpression of IL-5 has been associated with the increase of eosinophils in allergic diseases, including asthma (Hamid et al., 1991; Yamamoto et al., 2003). For example, normal subjects had less than 1 pg/mL IL-5, while asthma patients exhibiting acute asthmatic attacks had approximately 5 fold more (4.8 pg/mL) (Lee et al., 2001). During the early development of the ADA assay for mepolizumab, we tested for the interference of endogenous IL-5 in serum and observed that IL-5 levels in asthma subjects tested negative (i.e. they did not give a false positive result in the ADA assay). In the early phase I and II clinical studies with mepolizumab, the observed ADA positive rate was close to 50%. However; these elevated ADA positive incidences did not correlate with decreased PK/PD or increased adverse events. Since IL-5 levels were not evaluated in early mepolizumab clinical studies, the impact of mepolizumab on free and drug-bound IL-5 levels was unknown until the two phase II studies (MEA112997/NCT01000506, MEA114092/NCT01366521).

Utilizing two biomarker assays, a total IL-5 biomarker assay which measured both unbound IL-5 (free) and drug-bound IL-5 and a free IL-5 biomarker assay which measured only the free IL-5, we found that IL-5 was significantly increased in dosed subjects compared to predose samples and the elevated IL-5 was in the form of drug-bound complex. With this new information, we suspected that the elevated total IL-5 levels in treated subjects contributed to the increased ADA positive incidence observed in early studies by forming bridges with conjugated drug in the assay. In this study, we tested our hypothesis of IL-5 induced false positive ADA responses with both spiked serum and clinical samples. We evaluated two distinct strategies to overcome the IL-5 assay interference: a solid-phase immunodepletion of IL-5 and an in-solution IL-5 immunocompetition. We resolved IL-5 interference in the ADA assay by adding a competitive therapeutic target antibody to the assay solution. With the improved ADA assay, we re-analysed last two phase II study samples. The ADA positive incidences were single digit percentages compared to values close to 50% observed with original ADA assay. This study highlights the importance of a parallel biomarker readout and a careful examination of drug target interference in both assay development phase and clinical sample testing phase.

2. Materials and methods

2.1. Reagents

Mepolizumab and recombinant human IL-5 were provided by GlaxoSmithKline (GSK) BioPharm CMC. The rhIL-5 was biotinylated at a biotin molar challenge ratio of 20:1 with EZ-Link™ Sulfo-NHS-LC-Biotin (Pierce, Waltham, MA) following the manufacturer's instruction; rhIL-5 conjugated beads for flow assay were generated using BD™ CBA functional beads and conjugation buffer set (BD Bioscience, San Jose, CA) following the manufacturer's procedures. Biotin (Pierce, Waltham, MA) and ruthenium (MSD, Bethesda, MD) labelled mepolizumab conjugates were produced according to the manufacturer's instructions (8:1 for biotinylation and 15:1 for ruthenium labelling, respectively). Read Buffer T (R92TC-1), ruthenium labelled streptavidin, streptavidin coated plates were purchased from MSD (Bethesda MD). Nunc 96-well plates, streptavidin agarose were purchased from Thermo Fisher Scientific (Waltham, MA). The positive control (PC) was affinity purified hyper-immunized rabbit sera at Covance (Chantilly, VA). The negative control (NC) was generated by pooling 65 normal human serum samples that had below cut point response in the initial ADA assay. Other buffer reagents including PBS, PBST (1× PBS with 0.1% Tween 20) was prepared by GSK Media Prep; BSA and azide were purchased from Sigma Aldrich (St. Louis, MO).

2.2. Human serum

A normal human serum panel was purchased from BioReclaimation. Study serum samples used for assay development purposes were from clinical study NCT01000506/MEA112997, and used within the ICF specifications. Additional studies include NCT01366521/MEA114092, NCT01691508/MEA115575, NCT01691521/MEA115588 and NCT01842607/MEA115661.

2.3. Anti IL-5 antibodies

A total of 16 different IL-5 antibodies were purchased from various vendors: LS-C50743 from LifeSpan BioSciences (Seattle, WA); CS-7887, SC-8433, SC-34812, and SC-34814 from Santa Cruz Biotechnology (Dallas, TX); MM550C and MM550CB from Thermo Fisher Scientific (Waltham, MA); MAB205, MAB605, and AB-205-NA from R&D Systems (Minneapolis, MN); 554491 from BD Biosciences (San Jose, CA); CSI10100 from Cell Sciences (Newburyport, MA); 10118-08, 10118-01,10119-01 and 10206-01 from Southern Biotech (Birmingham, AL).

2.4. IL5 biomarker assay

Total IL-5 was measured on the Meso Scale Discovery platform, and used commercial sources for both the capture (10118-01) and detection (clone TRFK5, cat#MM550CB) antibodies. Recombinant IL-5 was used as the calibrator. The assay was designed to detect both free IL-5 and drug-bound IL-5. Briefly, assay plates were coated overnight with capture antibody at 5 μg/mL with 1× PBS. Plates were then blocked at room temperature (RT) for 1 h with 3%BSA/PBS. Calibrators, controls, and samples were 1:1 diluted in buffer (6.4 mM EDTA, 5.1 mM EGTA, 50 mM HEPES, 149.2 mM NaCl, 1% Triton X-100, 1% BSA, pH 7.4), and then 50 μL of the diluted samples were added to the assay plate and incubated at RT for 2 h. After washing, 50 μL of detection solution containing 2 μg/mL of detection antibody (biotin labelled TRFK5) and 0.5 μg/mL ruthenium labelled streptavidin were added to each well and incubated for 1.5 h. Plates were washed and 2× Read buffer was added before reading on the MSD SECTOR Imager 6000. The measurement of IL-5 was tolerant to 100 μg/mL mepolizumab and therefore the assay was deemed fit-for-purpose for total IL-5 detection. The validated quantifiable range was 7.8–500 pg/mL. All samples and calibrators were run in duplicate, and a four parameter logistic curve was used to convert the MSD counts into concentration units (pg/mL).

2.5. Initial ADA screening assay

For the initial ADA screening method, serum samples were diluted 1:10 in assay diluent (0.5% BSA in PBS with 0.05% Tween 20 and 0.05% Azide) and then 1:1 with biotin and sulfo-tag drug conjugates (final concentrations for each conjugate were 0.25 μg/mL) for an overnight incubation at RT under gentle agitation (300 rpm). The sample/conjugate solutions (50 μL/well) were then added to a blocked (3% BSA in PBST) streptavidin-coated Meso Scale plate and allowed to incubate for an additional hour at RT with gentle agitation. Wells were washed with PBST (3 × 300 μL), and then received 1× Read Buffer T. Plates were read on a Meso Scale Sector Imager 6000. The mean signal from duplicate wells were normalized to the signal from the negative control on the plate, and referred to as relative ECL (RECL). This method was validated for clinical sample testing at a CRO. Samples above the cut point (RECL = 1.58) were further confirmed by free drug competition assay. Confirmation cut point (= 30.3%) was established to allow for 1% false positive