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Research paper

Identification and elimination of target-related matrix interference in a neutralizing anti-drug antibody assay ☆

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ABSTRACT

Biopharmaceuticals administered to the human body have the potential to trigger the production of anti-drug (also called anti-therapeutic) antibodies (ADA) that can neutralize the therapeutic activity. For antibody therapeutics, cell-based neutralizing ADA assays are frequently used to evaluate ADA in clinical studies. We developed a method to detect neutralizing antibodies against MEDI-575, a fully human IgG2κ antagonistic antibody against PDGFR-α. We evaluated three assay formats, two of which measured late responses, cell proliferation and apoptosis, whereas the third assay detected an early signaling event, phosphorylation of PDGFR-α. Measuring phosphorylation provided a superior assay window and therefore was developed as a neutralizing ADA (NAb) assay. Matrix interference, however, was significant, and could be identified to be caused by PDGF-AA and PDGF-AB, apparently the two most abundant ligands of PDGFR-α present in human serum samples. A simple pre-treatment step, addition of an inhibitory antibody to PDGF-A, a subunit present in PDGF-AA and PDGF-AB, was found to eliminate matrix interference, increasing assay reliability and sensitivity. We integrated the pre-treatment step into assay development and qualified a robust NAb assay.

1. Introduction

Biopharmaceuticals have the potential of inducing the generation of anti-drug antibodies (ADA) that could cause undesired effects ranging from loss of drug exposure to serious adverse events (Food and Drug Administration, 2013; Vugmeyster et al., 2012; Wadhwa et al., 2003). Testing for ADA is a regulatory requirement when conducting clinical studies and samples are commonly tested in a tiered approach to evaluate and confirm ADA. Only samples that are confirmed positive for ADA are subjected to testing by more laborious and time-consuming neutralizing ADA (NAb) assays.

NAb assays can be categorized by their format: ligand-binding assays that measure neutralization of binding and cell-based assays that measure the neutralization of a biological effect of the drug. Cell-based assays, in contrast to ligand-binding assays, are considered to provide a readout that is most representative of the biological effect elicited by neutralizing antibodies (Food and Drug Administration, 2009). However, there are technical challenges to cell-based NAb assays. The sensitivity is often inferior and results tend to be more variable. NAb assays are often influenced by sample components other than neutralizing ADA, and if not addressed during assay development, these components can result in false-positive or false-negative results during clinical sample testing. This so-called sample or matrix interference is caused by often unknown factors that either generate nonspecific signals or inhibit the specific signal. Matrix interference depends much on assay design. NAb assays can detect either early or late biological responses caused by the drug. Early responses are signaling pathway activation events that occur soon after

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drug binding. Late biological responses are located much more downstream, such as cell proliferation, cell death, cell differentiation and secretion of cellular products (Gupta et al., 2007). Late responses tend to be the consequence of complex cellular responses potentially influenced by pathways unrelated to the drug and therefore more amenable to matrix interference.

Matrix interference in cell-based NAb assays and also cell-free NAb assays can be eliminated by purification of total antibodies from the sample (McCutcheon et al., 2010; White et al., 2008). Such purification steps, however, have the potential to influence the concentration or activity of the purified antibodies, leading to an underestimation of NAb in clinical samples. Therefore, the matrix interference and strategies to eliminate such interferences are routinely evaluated during method development. Relevant assay development considerations are described in detail in the literature (Gupta et al., 2007).

This study describes the development of a NAb assay to detect neutralizing ADA directed against MEDI-575, a monoclonal fully human antibody to PDGFR-α.

2. Material and methods

2.1. Reagents

Fully human monoclonal antibody IgG2κ (MEDI-575) was produced and purified by MedImmune. Rabbit antisera against MEDI-575 were produced at Eurogentec SA (Liege, Belgium). A polyclonal antibody directed against MEDI-575 (positive control pAb) was purified form terminal bleed antisera by precipitation with ammonium sulfate, followed by purification on MEDI-575 affinity columns, and depletion on isotype control affinity columns. Normal human serum and cancer serum samples were purchased from Bioreclamation (Westbury, NY) and informed consent was obtained from all donors. Human PDGF-AA affinity purified polyclonal antibody (Goat IgG), recombinant human PDGF-AB, PDGF-BB, PDGF-CC, PDGF-DD, and PDGFR-α were purchased from R&D Systems (Minneapolis, MN). Recombinant human PDGF-AA was purchased from Austral Biologicals (San Ramon, CA). 4G10® Platinum (anti-phosphotyrosine) was purchased from Millipore (Temecula, CA). Anti-PDGFR-α (D1E1E) XP™ rabbit mAb was purchased from Cell Signaling Technology (Danvers, MA). Sulfo-Tag labeled anti-rabbit antibody, Tris lysis buffer and 4× Read Buffer T were purchased from Meso Scale Discovery (Gaithersburg, MD). Phosphatase inhibitor cocktail 2 and phosphatase inhibitor cocktail 3 were purchased from Sigma-Aldrich (Saint Louis, MO). Complete protease inhibitor cocktail tablet was purchased from Roche Diagnostics (Indianapolis, IN). Caspase-Glo® 3/7 assay and CellTiter-Glo luminescent cell viability assay were purchased from Promega (Madison, WI). I-Block was purchased from Applied Biosystems (Carlsbad, CA), RPMI-1640 (+25 mM HEPES + L-glutamine), and characterized fetal bovine serum (FBS), trypsin 0.25% (1×) solution and penicillin–streptomycin solution (10,000 IU/mL penicillin, 10,000 μg/mL streptomycin) were purchased from Hyclone Laboratories, Inc. (Logan, UT). 1× phosphate buffered saline (PBS without Ca^{++}/Mg^{++} or phenol red) and water for cell culture applications were purchased from Lonza Group, Ltd. (Allendale, NJ).

2.2. Growth and maintenance of cells

The human squamous adenocarcinoma cell line, NCI-H1703, was obtained from the American Type Culture Collection (ATCC, Manassas, VA) and maintained in cell growth media (RPMI-1640 + 25 mM HEPES + L-glutamine medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin solution). Cells were cultured at 37 °C in a humidified 5% CO_2 incubator in a 75 cm^2 or 150 cm^2 cell culture flask (BD Biosciences, San Jose, CA). For routine cell culture, cells were plated at 3×10^6 cells in a T75 cell culture flask and passaged every 2–3 days.

2.3. Control samples and sample preparation for PDGFR-α phosphorylation assay

If not otherwise specified, all controls and samples were diluted to 50% with assay diluent (RPMI-1640 + 25 mM HEPES + L-glutamine medium supplemented with 0.4% FBS and 1% penicillin–streptomycin solution). Assay performance was monitored by two positive controls, a negative control, and an assay control. 2500 ng/mL (high positive control) and 1500 ng/mL (low positive control) of neutralizing pAb were prepared in assay matrix (50% normal human serum, 300 ng/mL MEDI-575 and 5 μg/mL anti human PDGF-AA). Negative control consisted of assay matrix. The assay control contained assay matrix without MEDI-575. To determine the LLOD, neutralizing pAb was titrated to a final concentration of 16,000, 8000, 4000, 2000, 1000, 500, 250, and 125 ng/mL in assay matrix. Controls and test samples were incubated at room temperature for 80 min with gentle agitation and then incubated on ice for approximately 30 min before addition to NCI-H1703 cells.

2.4. General procedure PDGFR-α phosphorylation assay

A cell suspension of 150,000 cells/mL in growth media was plated in each well of a clear 96-well flat bottom tissue culture plate (Corning, NY) at 100 μL/well. After approximately 24 h, cells were serum-starved by replacing the cell growth media with 100 μL/well of assay diluent. After approximately 12–20 h of incubation, the plate containing cells was incubated with samples and controls. Fifty microliters of the test samples and controls were transferred to the cells, and incubated at 2–8 °C for approximately 2 h. The supernatant was discarded and phosphorylation of PDGFR-α was stimulated with 150 ng/mL recombinant human PDGF-AA for 20 min at 37 °C in a humidified 5% CO_2 incubator. The cells were lysed with lysis buffer cocktail (Tris lysis buffer + complete protease inhibitor cocktail tablet + phosphatase inhibitor cocktail 2 and 3).

Phosphorylation of PDGFR-α in the cell lysate was measured with a plate-based immunoassay. Standard 96-well MSD plates were coated overnight at 2–8 °C with 35 μL of 4 μg/mL 4G10® Platinum (anti-phosphotyrosine) in 1× PBS. Coated plates were washed and blocked with 150 μL I-Block Buffer (0.2% I-Block, 0.5% Tween 20 in 1× PBS) at room temperature for 1 h. The plate was washed and cell lysate was added and incubated for 1 h at room temperature. After washing the plate with 1× wash buffer (PBS, 0.0% Tween 20), 35 μL of 0.5 μg/mL monoclonal anti PDGFR-α rabbit antibody prepared in I-Block