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Research paper

# Development and characterization of a free therapeutic ligand binding assay with assistance from kinetics modeling

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ABSTRACT

Bioanalytical data from early human studies conducted in normal volunteers are often used for building pharmacokinetic/pharmacodynamic models that can predict outcomes of future studies in diseased patients. Thus, it is important to develop and validate reliable and accurate bioanalytical assays that instill confidence that the intended therapeutic species (total or free) are being measured. Assays quantifying the free therapeutic species, the partially bound (for multivalent therapeutics) and unbound species, require much more characterization than assays that quantify the total therapeutic species. We have developed an immunoassay to measure free BMS-962476, an Adnectin protein therapeutic against soluble proprotein convertase subtilisin kexin (PCSK)-9, and performed an in-depth characterization of the accuracy of this assay with the assistance of modeling. The experimental data correlates with modeled data within 15% at all clinically relevant levels of PCSK9 in normal and diseased populations.



## 1. Introduction

Pharmacokinetic (PK) data are used to assess drug exposure and safety, characterize the PK/pharmacodynamic (PD) relationships, and aid in dose selection. Data from early clinical trials are used to model PK/PD effects for new dosing regimens and populations. Accurate assessments and successful modeling depend upon the quality and specificity of the bioanalytical assays being used to measure the therapeutic drug species in samples. Thus, a thorough understanding of what species are being detected in the assay is essential. For therapeutics against circulating targets (either soluble or shed from the cell surface), there may be multiple therapeutic species in the clinical sample that may include bound, partially bound, and unbound. It is widely accepted that free therapeutic species, both partially bound and unbound, are the pharmacologically active species, and that calculated PK parameters could differ depending on whether data from the total assay (which measures all species) or the free assay (that measures unbound and partially bound species) is used for the evaluation (Ahene, 2011; Fischer et al., 2012). However, the necessity to have free and total therapeutic levels for PK analysis and the challenges of having an accurate free therapeutic assay are topics of much discussion in the industry (Staack et al., 2012; Tang and Prueksaritanont, 2011; Kuang et al., 2010; Lee et al., 2011; Roskos et al., 2011). The necessity for free and total PK data will vary for each program. But for novel protein therapeutics, it might be very important to collect free and total drug data to ensure proper characterization of the PK/PD relationship and associated parameters in the non-clinical or first in human clinical trials.

There are many factors to be considered when developing an assay to detect only unbound or partially bound (free) therapeutic species. Some of those factors include: valency of the therapeutic (drug) and circulating target, endogenous concentration of circulating target, $K_D$ value, minimum required dilution (MRD), sample dilution into the quantifiable range of

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the assay, assay conditions (including temperature and incubation times), and available reagents (Staack et al., 2012; Kuang et al., 2010; Lee et al., 2011; Roskos et al., 2011; Ternant et al., 2006). Fig. 1 illustrates the different species that could be in circulation with mono- and divalent therapeutics as well as mono- and divalent targets. The MRD, additional sample dilutions required, and assay conditions will depend upon the reagents available, assay platform, and format. Free therapeutic assays are commonly developed using the target to capture or detect the partially bound or unbound species. However, lot-to-lot variability of the target could be an issue that might require more than just a few qualification experiments to bridge across lots. Alternatively, the therapeutic protein could be captured or detected using anti-drug and/or anti-idiotypic antibodies. The use of antibodies to capture or detect in a free therapeutic assay requires that one antibody bind to the same epitope on the therapeutic as the target. Once the appropriate reagents are selected and the assay is developed, confirming that the assay is indeed measuring free therapeutic offers additional challenges.

A fundamental, and challenging, issue in developing a free therapeutic assay is to determine whether there is detectable assay interference in the presence of the target. Further, it is also important to assess whether the level of interference is consistent with what is expected based on the binding kinetics. Computer modeling has proven to be useful to predict *in vivo* concentrations of free drug under various conditions (Fischer et al., 2012). We have developed an immunoassay to detect the free therapeutic species using two specific antibodies against the therapeutic and utilized computer models to confirm the accuracy of the assay in the presence of high concentrations of target. The therapeutic, BMS-962476, is a monovalent ~11 kDa Adnectin conjugated to polyethylene glycol (PEG) that binds to PCSK9 with a $K_D$ of 0.85 nM (measured by surface plasmon resonance at 25 °C pH 7.4) (Mitchell et al., 2014). An assay was developed using a blocking anti-idiotype antibody to capture free BMS-962476 and then a polyclonal anti-drug antibody specific for the protein scaffold was used to detect the captured drug. The performance of this assay was evaluated by using or preparing samples that had PCSK9 concentrations ranging from 0.5 nM to 50 nM and BMS-962476 concentrations ranging from 5 to 200 nM. Samples were tested in the assay and concentrations input into an appropriate model. The values were then compared to assess the accuracy of the assay. The characterization and desirable performance of the free therapeutic assay are described in the following sections.

## 2. Materials and methods

### 2.1. Free BMS-962476 assay procedure

A free BMS-962476 assay was developed using the meso scale discovery (MSD) platform. Streptavidin coated microtiter plates were blocked with 5% bovine serum albumin (BSA) in PBS with 0.05% Tween 20 (PBST) for 1 h at 4 °C. The plates were then coated for 1 h at room temperature with a biotinylated mouse anti-idiotype monoclonal antibody at 2 μg/ml in assay buffer (1% BSA in PBST). The capture antibody has an affinity of 37 nM for BMS-962476 and competes with PCSK9 for binding to BMS-962476. After washing the plate, the samples (which refer to standards, quality controls (QCs), individual, and/or interference samples) were diluted 10 fold (MRD) in assay buffer, added to the plate, and incubated for 1 h with shaking at room temperature. The plate was washed, then incubated with 1 μg/ml of a ruthenium labeled rabbit polyclonal antibody against the scaffold of BMS-962476 for 1 h at room temperature with shaking. After a final wash, a buffer containing tripropylamine was added to the wells and the plate was subsequently read on a MSD reader.

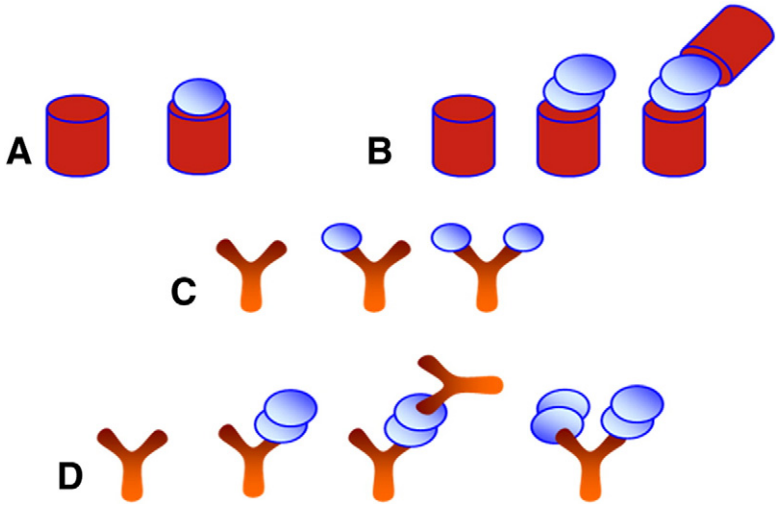


**Fig. 1.** Illustration of different unbound, partially bound, and totally bound therapeutic species that can exist in circulation with (A) a monovalent therapeutic to a monovalent target; (B) a monovalent therapeutic to a divalent target; (C) a divalent therapeutic to a monovalent target; and (D) a divalent therapeutic to a divalent target.