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Journal of Immunological Methods 335 (2008) 8–20

JIM
Journal of Immunological Methods

www.elsevier.com/locate/jim

Research paper

Quantitative determination of humanized monoclonal antibody rhuMAb2H7 in cynomolgus monkey serum using a Generic Immunoglobulin Pharmacokinetic (GRIP) assay

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Received 14 September 2007; received in revised form 18 January 2008; accepted 23 January 2008
Available online 14 February 2008

Abstract

Preclinical pharmacokinetic (PK) assays are important to help evaluate the safety and efficacy of a potential biotherapeutic before clinical studies. The assay typically requires a biotherapeutic-specific reagent to minimize matrix effects especially when the host species are non-human primates such as cynomolgus monkeys and the biotherapeutic is a humanized monoclonal antibody (MAb). Recombinant humanized mAb 2H7 (rhuMAb2H7) binds to the extracellular domain of CD20 that is expressed on B cells and results in B cell depletion. It is currently being evaluated for its therapeutic potential in rheumatoid arthritis (RA) in clinical studies. During the early development of rhuMAb2H7, a cynomolgus monkey PK assay was needed to help assess the pharmacokinetic parameters of rhuMAb2H7 in a pilot cynomolgus monkey study. However, development of a cynomolgus monkey PK assay was challenging due to lack of rhuMAb2H7-specific reagents. Here we describe an alternative method for detection of rhuMAb2H7 in cynomolgus monkey serum using polyclonal antibodies against human IgGs. This assay quantifies rhuMAb2H7 in 10% cynomolgus monkey serum with high sensitivity, accuracy, and precision. This assay successfully supported the rhuMAb2H7 development,

Abbreviations: ADCC, antibody dependent cell-mediated cytotoxicity; BGG, bovine γ-immunoglobulin; CHAPS, 3-[(3-cholamidopropyl) dimethylammonio]-1-propane-sulfonate; CDC, complement dependent cytotoxicity; CPG, controlled pore glass; ELISA, enzyme-linked immunosorbent assay; ECD, extracellular domain; GRIP assay, Generic Immunoglobulin Pharmacokinetic assay; HRP, horseradish peroxidase; LLOQ, lower limit of quantification; MAb, monoclonal antibody; PK, pharmacokinetic; PD, pharmacodynamic; PBS, phosphate-buffered saline; rhuMAb2H7, recombinant humanized mAb 2H7; RA, rheumatoid arthritis; TMB, 3,3′,5,5′-tetramethylbenzidine; ULOQ, upper limit of quantification; CV, coefficient of variation; POC, proof of concept.

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doi:10.1016/j.jim.2008.01.016

and has the potential to be used to quantify other humanized MAb biotherapeutics in serum from a variety of non-human species.

Keywords: Pharmacokinetic assay; Cynomolgus monkey serum; Humanized IgG; ELISA; Anti-CD20; rhuMAb2H7

1. Introduction

rhuMAb2H7 is a humanized IgG1 kappa monoclonal antibody derived from the murine precursor 2H7 (Clark et al., 1985). The antibody is directed against the extracellular domain of the CD20 antigen, which is expressed on both normal and malignant B cells (Stashenko et al., 1980; Tedder and Schlossman, 1988; Clark and Ledbetter, 1989; Tedder and Engel, 1994; Riley and Sliwkowski, 2000). B cell depleting agents have been used successfully in treating B cell-mediated malignances such as non-Hodgkin's lymphoma (McLaughlin et al., 1998) and chronic lymphocytic leukemia (Jensen et al., 1998; Gopal and Press, 1999; von Schilling, 2003). In addition, B cells are also implicated in the pathophysiology of autoimmune diseases such as rheumatoid arthritis (Looney, 2002; Dorner and Burmester, 2003; Oligino and Dalrymple, 2003; Shaw et al., 2003), systemic lupus erythematosus (Anolik et al., 2003), and multiple sclerosis (Corcione et al., 2005). Treatment of non-human primates in vivo with rhuMAb2H7 results in binding of rhuMAb2H7 to the CD20 antigen on circulating B cells, a process that subsequently causes B cell depletion (Vugmeyster et al., 2005). Although the precise mechanism of B cell depletion by rhuMAb2H7 is not completely understood, it may include several different pathways such as antibody dependent cell-mediated cytotoxicity (ADCC), complement dependent cytotoxicity (CDC), phagocytosis and apoptosis. Both in vitro and in vivo studies suggested that rhuMAb2H7, an anti-CD20 therapeutic, has a therapeutic potential for B cell-mediated autoimmune disorders and hematologic indications (Gopal and Press, 1999; Kosmas et al., 2002; Moore et al., 2004; Keystone, 2005; Panayi, 2005; Tuscano et al., 2005; Vugmeyster et al., 2005; Isenberg, 2006).

Cynomolgus monkeys have been frequently used in preclinical studies due to their physiological and genetic similarities to humans (Da Silva et al., 2001; Hart et al., 2001; Kelley et al., 2001; Boon et al., 2002; Dodds et al., 2005; Ponce et al., 2005). During the early development of rhuMAb2H7 a proof of concept (POC) study was conducted in this species to assess the safety, pharmacokinetics (PK) and pharmacodynamics (PD) of the molecule. Therefore a sensitive bioanalytical method that could accurately quantify rhuMAb2H7 concentrations in cynomolgus monkey serum was needed.

In general, a PK assay that quantifies the concentration of a biotherapeutic in serum requires one or more biotherapeutic-specific reagents. Biological matrices tend to give high background due to non-specific interactions between matrix components and assay reagents (Selby, 1999). Such specific reagents are highly desired for rhuMab2H7, a humanized IgG1 that shares a high sequence identity with cynomolgus monkey IgGs (Lewis et al., 1993; D'Ovidio et al., 1994; Pace et al., 1996). Alignment of the heavy chain sequences of both rhuMAb2H7 and the cynomolgus monkey IgG reveals an overall of 80% of amino acid identity and 85% of sequence similarity. In addition, the circulating levels of cynomolgus IgGs are usually in the range of 10–16 mg/ml, which is much higher than the concentration of the therapeutic to be quantified (Biagini et al., 1988; Tryphonas et al., 1991). In the rhuMAb2H7 cynomolgus POC study, a sensitivity of 20 ng/ml in serum was required.

Quantitative enzyme linked immunosorbent assay (ELISAs) have been widely used to measure circulating levels of biotherapeutics in a biological matrix. These assays are typically based on analyte-specific reagents such as monoclonal and polyclonal antibodies directed against the therapeutics, target proteins or receptors (DeSilva et al., 2003). During the early development of rhuMAb2H7, the only available method that potentially could measure rhuMAb2H7 concentration was based on a WIL2-S cell line from American Type Culture Collection (Rockville, MD) that overexpresses CD20 on its surface (Hong et al., 2004). However, this assay was not suitable for developing a cynomolgus monkey PK assay with a high sensitivity and throughput. Specific polyclonal and monoclonal antibodies against rhuMAb2H7 were not available at the time of the POC pilot cynomolgus monkey study. CD20, the target of rhuMAb2H7, was only available in the form of peptides that resemble the C-terminal extracellular domain (ECD) of the molecule. The low binding affinities of these