PF-03475952: a Potent and Neutralizing Fully Human Anti-CD44 Antibody for Therapeutic Applications in Inflammatory Diseases

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ABSTRACT

Introduction: CD44 is a cell adhesion molecule believed to play a critical role in T cell and monocyte infiltration in the inflammatory process. The reduction of CD44 expression or its ability to properly interact with its key ligand, hyaluronic acid (HA), inhibits migration and subsequent activation of cells within sites of inflammation. CD44-deficient mice exhibit decreased disease in a mouse arthritis model. Methods: Accordingly, we developed PF-03475952, a fully human IgG2 anti-CD44 monoclonal antibody (mAb). Results: Binding of PF-03475952 to CD44 inhibits binding of HA and induces loss of CD44 from the cell surface. PF-03475952 also passed a series of safety pharmacology assays designed to assess the risk of the mAb to bind Fc gamma receptors, stimulate cytokine release from human whole blood, and stimulate cytokine release from peripheral blood mononuclear cells (PBMC) using plate-bound antibodies. The latter assay was designed specifically to evaluate the risk of cytokine storm that had been observed with TGN1412 (immunostimulatory CD28 superagonist mAb). PF-03475952 exhibits high-affinity binding to both human and cynomolgus monkey CD44, but does not cross-react with rodent CD44. Thus, a rat anti-mouse CD44 mAb was used to demonstrate a dose-dependent decrease of disease in mouse collagen-induced arthritis. Importantly, efficacy was correlated with >50% loss of cell surface CD44 on circulating cells. Loss of CD44 expression on CD3+ lymphocytes was monitored following a single dose of PF-03475952 in cynomolgus monkeys as a pharmacodynamic marker. The recovery of CD44 expression was found to be dose-dependent. PF-03475952 doses of 1, 10, and 100 mg/kg reduced CD44 expression below 50% for 218, 373, and >504 hours,
respectively. **Conclusion:** Targeting of CD44 is a unique mechanism of action in the treatment of inflammatory diseases and is expected to reduce joint damage induced by inflammatory mediators, resulting in disease modification in inflammatory diseases such as rheumatoid arthritis.

**Keywords:** CD44; cytokine storm; inflammatory disease; PF-03475952; rheumatoid arthritis; TGN1412

**INTRODUCTION**

CD44 is a cell adhesion molecule that plays a critical role in T cell and monocyte infiltration in the inflammatory process.\(^1\,\,^2\) CD44 and its key ligand, hyaluronic acid (HA), also provide costimulatory signals for the activation of lymphocytes and monocytes, further supporting a role for CD44 in the inflammation process.\(^3\) The reduction of CD44 expression inhibits migration and subsequent activation of cells within sites of inflammation, such as synovial tissue, and is expected to decrease production of proinflammatory cytokines such as tumor necrosis factor (TNF)-\(\alpha\), interleukin-6 (IL-6), and IL-1\(\beta\) in the synovium. The genetic knock-out of CD44 expression in mice results in a decreased incidence and severity of disease in the collagen-induced arthritis (CIA) model.\(^4\) IM7 is a rat anti-mouse CD44 monoclonal antibody (mAb) that blocks CD44-HA interactions and induces the proteolytic cleavage of CD44 from the surface of various proinflammatory cells. Administration of IM7 to mice inhibits the formation of inflammatory arthritis in the collagen-induced arthritis model by blocking infiltration and activation of the inflammatory cells in synovial tissue.\(^1\,\,^2\)

PF-03475952 is a fully human IgG2 antibody that binds to the CD44 protein and functions as an antagonist. Alternative splicing of the CD44 gene gives rise to multiple CD44 isoforms (CD44v) with splice variants sharing a common HA binding domain but having additional sequences in the extracellular membrane-proximal region.\(^5\) The most prevalent form of CD44 is referred to as CD44H or CD44s, which represents the predominant form on immune cells. As PF-03475952 binds an epitope within the constant exons of CD44, PF-03475952 is expected to bind all CD44 isoforms. Targeting of CD44 with PF-03475952 is a novel approach to the treatment of inflammatory diseases and is expected to reduce the inflammation and joint damage in rheumatoid arthritis (RA),\(^6\) as well as having potential therapeutic benefits in other autoimmune diseases such as multiple sclerosis\(^7\) and asthma.\(^8\)

**MATERIALS AND METHODS**

**Generation of Human and Cynomolgus Monkey CD44-Ig Fusion Proteins**

The extracellular domain of human CD44 and cynomolgus monkey CD44 were expressed as human IgG1-fusion proteins. The complementary deoxyribonucleic acid (cDNA) encoding the mature extracellular domain of human and cynomolgus monkey CD44 were polymerase chain reaction (PCR) amplified from human spleen cDNA or cynomolgus monkey peripheral blood mononuclear cells (PBMC) cDNA, respectively, and cloned into mammalian expression vectors. These vector systems contained a CD5 leader sequence and a human IgG1 tag. The primer sequences are as follows: human AGTGAGACTAGTCAGATCGATT TGAATATAACCTGCGCCGTTTG and ATCACTGAGATCTTCTGGAATTTGGGTCT CCTTATAG, monkey ATCGGCGATCCAGATCGATT TGAATATAACC and CTGTGC-