# Evaluation of a fully human monoclonal antibody against multiple influenza A viral strains in mice and a pandemic H1N1 strain in nonhuman primates

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## ARTICLE INFO

## ABSTRACT

Influenza virus is a global health concern due to its unpredictable pandemic potential. Frequent mutations of surface molecules, hemagglutinin (HA) and neuraminidase (NA), contribute to low efficacy of the annual flu vaccine and therapeutic resistance to standard antiviral agents. The populations at high risk of influenza virus infection, such as the elderly and infants, generally mount low immune responses to vaccines, and develop severe disease after infection. Novel therapeutics with high effectiveness and mutation resistance are needed. Previously, we described the generation of a fully human influenza virus matrix protein 2 (M2) specific monoclonal antibody (mAb), Z3G1, which recognized the majority of M2 variants from natural viral isolates, including highly pathogenic avian strains. Passive immunotherapy with Z3G1 significantly protected mice from the infection when administered either prophylactically or 1–2 days post infection. In the present study, we showed that Z3G1 significantly protected mice from lethal infection when treatment was initiated 3 days post infection. In addition, therapeutic administration of Z3G1 reduced lung viral titers in mice infected with different viral strains, including amantadine and oseltamivir-resistant strains. Furthermore, prophylactic and therapeutic administration of Z3G1 sustained $O_2$ saturation and reduced lung pathology in monkeys infected with a pandemic H1N1 strain. Finally, de-fucosylated Z3G1 with an IgG1/IgG3 chimeric Fc region was generated (AccretaMab® Z3G1), and showed increased ADCC and CDC in vitro. Our data suggest that the anti-M2 mAb Z3G1 has great potential as a novel anti-flu therapeutic agent.

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## 1. Introduction

Influenza virus infections are a major public health concern and cause approximately 20,000–40,000 infection related deaths and more than 100,000 hospitalizations annually in the US alone. High-risk populations, such as infants, young children, the elderly, and immunosuppressed persons usually develop more severe disease after the infection (Simonsen et al., 2000; Thompson et al., 2003). The 2009 H1N1 pandemic and the human cases of avian H7N9 in China in 2013 clearly demonstrate the periodical emergence of new viruses that infect people with little or no immunity. In addition, previous outbreaks of avian influenza infections caused as severe disease and high mortality in healthy young adults as in people at risk (Prawira et al., 2012; Santillan Salas et al., 2013; Uyeki, 2008). Therefore, the pandemic threat poses serious concerns for public health considering the fact that there is no effective universal therapeutic for this virus.

Vaccines are an important strategy to counter the threat of influenza epidemics and pandemics. The annual influenza vaccine aims to stimulate the generation of anti-HA neutralizing antibodies, which confer protection from homologous strains (Jegerlehner et al., 2004; Palladino et al., 1995; Virelizier, 1975). However, the influenza strains for the vaccine must be selected based on prediction before the influenza season, and this has become challenging, in particular if a threatening pandemic strain emerges. Efforts to develop universal vaccines targeting conserved proteins of

influenza virus continue. Influenza virus M2 protein has been a focus of vaccine studies for more than a decade (De Filette et al., 2005; Denis et al., 2008; Fan et al., 2004; Fiers et al., 2004; Mozdzanowska et al., 2003; Neirynck et al., 1999). M2 ectodomain (M2e) seems to be a good candidate antigen for a vaccine because of its high degree of conservation (Fiers et al., 2004). Several approaches have been taken to formulate the M2 vaccine in order to stimulate strong antibody responses, but have met with limited success that required multiple injections of high dose immunogens with one or two adjuvants to achieve high levels of anti-M2e antibody titers. (Fan et al., 2004; Jegerlehner et al., 2004; Mozdzanowska et al., 2003; Neirynck et al., 1999). The challenge in this field remains to formulate a vaccine that is able to elicit robust antibody responses that protect against a wide spectrum of viral strains in humans, especially in young children, the elderly and immunocompromised populations, who in general benefit less from active immunization.

NA inhibitors, such as Tamiflu®, are the primary therapeutics, but they have limited efficacy, in particular when administered after 2 days of infection. In addition, the number of resistant viral strains increases along with the widespread use, especially in the treatment of H5N1 infections (Collins et al., 2008; de Jong et al., 2005).

We, and a few other groups, have taken a different approach of targeting M2, a highly conserved influenza virus transmembrane protein, for therapeutics (Beerli et al., 2009; Grandea et al., 2010; Wang et al., 2008). Previously, we reported that we generated a fully human anti-M2 mAb, Z3G1 (Wang et al., 2008), which recognized a broad spectrum of M2e variants, including those with distinctive mutations in the middle region of M2e (Liu et al., 2005; Zebedee and Lamb, 1988; Zharikova et al., 2005), and provided significant protection from influenza A infection in mice through the mechanisms of antibody-dependent cell-mediated cytotoxicity, (ADCC) and complement-dependent cytotoxicity (CDC). Here we report that Z3G1 significantly protected mice from a lethal infection when administered at 3 days post infection, and reduced lung viral loads from infections of multiple different viral strains, including amantadine and Tamiflu®resistant strains. In addition, Z3G1 prevented lung damage and sustained $O_2$ saturation in monkeys infected with a 2009 H1N1 pandemic strain when administered prophylactically and therapeutically. We generated defucosylated Z3G1 using POTELLIGENT® technology (Shinkawa et al., 2003) and genetically engineered the antibody to possess an IgG1/IgG3 chimeric Fc region using COMPLEGENT® technology (Natsume et al., 2008). The modified Z3G1, AccretaMab® Z3G1, showed dramatically enhanced ADCC and CDC activity *in vitro*. Our data imply that Z3G1 has a strong potential as an influenza therapeutic agent against a broad spectrum of strains, and its AccretaMab® version may lead to a higher efficacy at a lower dose.

## 2. Materials and methods

### 2.1. Viruses

A/HK/1/68 (H3N2) virus, a prototype clinical isolate, was kindly provided by Dr. Jacqueline M. Katz at Center of Disease Control and Prevention (CDC) and Dr. Suzanne Epstein at FDA, who originally obtained the strain from Dr. Earl Brown at University of Ottawa (Brown et al., 2001). A/WSN/33 (H1N1) was kindly provided by Dr. Chris Benedict at La Jolla Institute for Allergy and Immunology, who originally obtained the strain from Dr. Adolfo Garcia-Sastre at Mount Sinai School of Medicine. A/Fort Monmouth/1/1947 (H1N1) and A/Puerto Rico/8/34 (H1N1) were purchased from ATCC (A/Fort Monmouth/1/1947: Cat # VR-97, Lot # 5040962; A/Puerto Rico/8/34: Cat # VR-95). The A/Hawaii/21/2007 (H1N1) was procured from CDC. All viruses were adapted in mouse lungs. The M2e

sequence in A/HK/1/68 (H3N2) and A/WSN/33 (H1N1) is the consensus sequence MSLLTEVETPIRNEWGCRCNDSSD, and in A/Fort Monmouth/1/1947 and A/Puerto Rico/8/34 is MSLLTEVETP̲T̲K̲NEW E̲CRCNDSSD (M2TKE), and MSLLTEVETPIRNEWGCRCN̲G̲SSD (M2G), respectively. A/Puerto Rico/8/34 (H1N1) and A/WSN/33 (H1N1) are amantadine-resistant strains containing the amino acid mutation (S31N) in the transmembrane domain of M2 protein, which has been reported to be a genetic marker of amantadine resistance (Abed et al., 2005; Bright et al., 2006). The A/Hawaii/21/2007 (H1N1) contains the consensus M2e sequence (MSLLTEVETPIRNE WGCRCNDSSD, Genbank Accession # ACA33507), and the H274Y mutation in NA sequence (Genbank Accession # ACA33553) (Hurt et al., 2009). The pandemic strain, A/Netherlands/602/2009 (H1N1), was from the stock at ViroClinics Biosciences. The M2e sequence in A/Netherlands/602/2009 (H1N1) is MSLLTEVETP̲T̲R S̲E̲WE̲CRCS̲DSSD (M2TSES).

### 2.2. Animals

C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were housed in the animal facility at the La Jolla Institute for Allergy and Immunology (LJI) under specific pathogen-free conditions. All animal studies were conducted according to the guidelines and approval of the LJI Institutional Animal Care and Use Committee (IACUC) and conformed to the principles outlined by the Animal Welfare Act.

Male cynomolgus macaques ($n = 20$) were obtained from Noveprim in Spain, and tested for the presence of serum antibodies against the 2010–2011 vaccination influenza strains A/H1N1 (A/ California/007/2009), A/H3N2 (A/Victoria/210/2009) and B (B/Brisbane/60/2008) using hemagglutination inhibition assay at maximal 4 weeks before they were transferred to the study site. Only animals that were negative for influenza were selected for the study. The animals were about 2 years old and had a body weight range of 2.15–2.81 kg at day 0. Animals were housed in the central animal facilities of the Erasmus MC in Rotterdam, the Netherlands. Studies were performed by ViroClinics according to a licensed study protocol approved by the animal ethics committee. Animals were acclimated for 9 days and 28 days before the start of prophylactic and therapeutic experiment, respectively.

### 2.3. Generation of conventional and AccretaMab® anti-M2 monoclonal antibodies

The conventional anti-M2 monoclonal antibody was generated as described previously (Wang et al., 2008). The AccretaMab® anti-M2 antibody was generated with the combined POTELLIGENT® and COMPLEGENT® technology (Natsume et al., 2008). Fully human isotype control anti-HSA (human serum albumin) and anti-DNP (dinitrophenol) monoclonal antibodies (IgG1, κ) were generated as described (Motoki et al., 2005). All recombinant anti-M2 antibodies were produced from stable Chinese hamster ovary cell lines.

### 2.4. Tamiflu®

Tamiflu® (Oseltamivir Phosphate, Roche; http://www.tamiflu.com) is an FDA approved antiviral drug for the treatment of uncomplicated influenza. Tamiflu® used for this study was taken out from the capsule, and ground using a mortar and pestle. The powder was weighed and dissolved in 0.2% Methyl Cellulose 4000 cP (Sigma M-0512) at 450 μg/mL.

### 2.5. Cell lines and human PBMC

Madin–Darby canine kidney (MDCK) cells were purchased from ATCC (Cat # CCL-34), and cultured with complete Minimum Essential Medium (MEM) supplemented with 10% fetal bovine