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A novel peptide-based pan-influenza A vaccine: A double blind, randomised clinical trial of immunogenicity and safety☆

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ABSTRACT

Background: FP-01.1 is a novel synthetic influenza A vaccine consisting of six fluorocarbon-modified 35-mer peptides that encapsulate multiple CD4+ and CD8+ T-cell epitopes and is designed to induce an immune response across a broad population.
Methods: FP-01.1 was evaluated for safety and immunogenicity in a randomised, double-blind, placebo-controlled, dose-escalation, phase I clinical study in healthy adult volunteers ($n = 49$). IFNγ ELISpot assays and multicolour flow cytometry were used to characterise the immune response.
Results: FP-01.1 was safe and well tolerated at all doses tested with a similar adverse event profile in actively vaccinated subjects compared with controls. Maximum immunogenicity was in the150 μg/peptide dose group where a robust response (243 spots/million PBMC above baseline) was demonstrated in 75% subjects compared with 0% in placebo controls. All six peptides were immunogenic. FP-01.1 induced dual CD4+ and CD8+ T cell responses and was shown to cross-recognise divergent influenza strains.
Conclusions: This first-in-human study showed that FP-01.1 has an acceptable safety and tolerability profile and generated robust anti-viral T cell responses in a high proportion of subjects tested. The results support the further clinical testing of FP-01.1 prior to clinical, proof-of-concept, live viral challenge studies.

1. Introduction

Influenza epidemics are associated with significant morbidity and mortality, particularly in at-risk groups such as the elderly [1]. Influenza A can be subjected to major antigenic shift in one or both surface antigens presenting a major risk of worldwide pandemic with a significant impact on human health and the economy [2,3].

Current inactivated trivalent influenza vaccine (TIV) strategies rely on the induction of an antibody-mediated immune response specific for the antigenically varying hemagglutinin (HA) surface antigen. TIV have only moderate efficacy or effectiveness [4–5] and is particularly impeded when the circulating strain has drifted significantly from the vaccine strain. A recent meta-analysis of TIV studies found that anti-HA antibodies are only partial correlates of protection in the general population and poor correlates of protection in the elderly [4].

There is a growing body of evidence supporting the protective contribution of cell-mediated immune (CMI) responses to influenza, in the absence of a specific antibody response, which are poorly induced by TIVs [6–8]. Cytotoxic T lymphocyte activity was associated with reduced virus shedding in a cohort of experimentally infected volunteers with no detectable antibody responses against the experimental strain [9]. Clinical studies demonstrated that T cell responses, as measured by the antiviral mediator granzyme B, were directly correlated with protection against influenza in the elderly [10,11]. Two recent studies have established a direct correlation between pre-existing T cell responses and reduced severity of influenza disease [12,13].

A major advantage of cell-mediated over humoral immunity is that T cells can recognise epitopes thoughout the virus proteome, including from internal antigens which are conserved across a range of influenza subtypes, and thus provide broad cross-protective immunity to a both seasonal strains and pandemics [14,15].

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We have developed a novel influenza vaccine strategy based on linking a fluorocarbon moiety to 35-mer peptides specific for conserved, internal influenza antigens. This vaccine, called FP-01.1 (Flunisyn™), is a chemically synthesized, freeze-dried preparation of six different synthetic peptides conjugated to an inert fluorocarbon chain. Linking a fluorocarbon chain to peptides promotes their half-life in vivo, thereby putatively allowing more prolonged exposure of the peptides to the immune system and enhancing immunogenicity.

We report the results of a first-in-human phase I clinical study in healthy adult volunteers assessing the safety, tolerability and immunogenicity of a novel, pan-influenza A fluorocarbon-modified peptide vaccine designed to induce oramplify pre-existing and cross-protective T cell responses.

2. Materials and methods

2.1. Vaccine design

Vaccine FP-01.1 comprises six different synthetic 35-mer peptides, each conjugated to the fluorocarbon moiety $C_8F_{17}(CH_2)_2$-COOH and are derived from influenza A internal proteins; nucleoprotein, matrix protein and polymerase basic 1 and polymerase basic 2 proteins:

P44, HMAIIKKYTSGRQEKNPSLRMKWMMAMKYPITADK;
P220, VAYMLERELVRKTRFLPVAGGTSSVYIEVLHLTQG;
P1100a, YITRNQPEWFRNVLSIAPIMFSNKMARLGKGYMFE;
P1116b, APIMFSNKMARLGKGYMFESKRMKLRTQIPAEMLA;
P3071, DQVRESRNPGNAEIEDLIFLARSALILRGSVAHKS;
P3845, DLEALMEWLKTRPILSPLTKGILGFVFTLTVPSER.

Each peptide sequence was selected on the basis of a high level of conservation across H1–H9 influenza A strains isolated from humans, birds and pigs. For example, there is 99.0% and 97.1% identity between selected peptides and corresponding sequences from avian H5N1 and swine H1N1 (2009) respectively. The bioinformatics process also selected sequences that contain predicted HLA class II binding motifs across the most globally prevalent HLA class II molecules ($n = 13$) and HLA class I molecules ($n = 35$) in order to achieve a high population coverage. In addition, peptide selection avoid edanticipated large scale manufacturing constraints.

Each peptide was manufactured under current Good Manufacturing Practice (cGMP) conditions using solid phase Fmoc chemistry. FP-01.1 (considered as the Investigational Manufactured Product, IMP) was produced in accordance with cGMP by CarbogenAmcis, France and manufactured as a freeze-dried product containing 350 μg of each peptide and 31.5 mg mannitol. IMP was reconstituted in cGMP 28 mM L-histidine buffer, to create a homogeneous solution at a concentration of 500 μg/mL per peptide with close to neutral pH and an osmolality of 300 mOsm/L.

2.2. Subjects and study design

A randomised, double-blind, placebo-controlled, ascending dose study to assess the safety, tolerability and immunogenicity of repeated intramuscular administration of FP-01.1 in healthy volunteers was performed at Hammersmith Medicines Research Ltd., London (Fig. 1). The study was conducted in accordance with EU Directive 2001/20/ECand ICH GCP. The protocol was approved by the Medicines and Healthcare Products Regulatory Agency in the UK and the National Research Ethics Service Committee London (Brent). Written informed consent was obtained from 49 healthy male and female subjects aged between 18 and 55 years that were enrolled (Table 1). Detailed inclusion/exclusion criteria are described in clinicaltrials.gov under identifier NCT01265914. The primary safety endpoints were tolerability, incidence of treatment emergent adverse events (TEAEs), and clinically significant changes in laboratory safety tests, 12-lead electrocardiograms, vital signs and physical examination findings. Subjects were provided with diary cards to report AEs when not at the clinical site. Randomisation codes were computer-generated by an unblinded statistician and subjects were sequential assigned an unused randomisation number. With the exception of the pharmacist and statistician, all staff, subjects and employees of ITS remained blinded until the formal unblinding of the study. Three ascending dose cohorts (50, 150 and 500 μg/peptide) of 16 subjects (12 active: four placebo) administered test vaccine on Day 1, 29 (main study phase) and 99 (follow up phase). Each cohort started with a sentinel group of four subjects and safety data was reviewed before progression to the remaining subjects. Placebo subjects received 45 mg/mL mannitol in L-histidine buffer. Adverse events were recorded and presented from day 7 to day 113. No formal sample size calculations were performed. The study was conducted between August 2010 and March 2011 (up to day 113).

2.3. Peptides for in vitro testing

35-mer peptides, at ≥95% purity, corresponding to the antigens in FP-01.1 (but without the fluorocarbon chain) were used as *in vitro* recall antigens in ELISpot assays. A mixture of the six peptides (LPMIX6) and a mixture of 90 putative CTL (cytotoxic T lymphocyte) target peptides (SPMIX; 9-mer peptides predicted using SYFPEITHI (www.syfpeithi.de)) derived from the 35-mer peptide sequences were used as antigens for flow cytometric analysis and for multiplex cytokine assays.

2.4. PBMC isolation and cryopreservation

Within 8 h of collection, PBMC were separated from heparinised blood by density gradient centrifugation on Histopaque-1077 (Sigma). PBMC were cryopreserved at 1×10^7 cells/mL in 10% dimethyl sulfoxide/foetal calf serum and stored in liquid nitrogen. Samples were transferred between sites using a liquid nitrogen dry shipper. Only cell samples with >80% viability (as assessed by propidium iodide staining and flow cytometry) were analysed. Median cell viability and recovery were 96% and 79% respectively.

2.5. ELISpot assay

An IFNγ enzyme-linked immunosorbent spot (ELISpot) assay was utilised to measure the primary immunogenicity endpoint from samples collected up to day 43 and was conducted by Immune Health (Belgium) following Good Clinical Laboratory Practice guidelines. To minimise assay variability samples from each individual subjects were tested in parallel on the same day. PVDF plates (Millipore) were coated overnight at 4 °C with anti-human IFNγ antibody (R&D systems) and blocked for >1 h (1% BSA (PAA), 5% sucrose (Fisher), Dublecco's-PBS (Invitrogen)). Plates were washed with complete media (5% human serum (Sigma), RPMI Glutamax (Invitrogen) and gentamicin (Invitrogen)) prior to use. 2×10^5 PBMC were stimulated with individual 35-mer peptides (10 μg/peptide/mL). Negative (complete medium) and positive (phytohaemagglutinin and CEF (peptides from cytomegalovirus, EBV, and influenza)) controls were included. After 18 h of culture, plates were washed and incubated with a biotinylated secondary anti-human IFNγ (R&D systems) followed by streptavidin-HRP. Production of IFNγ was detected using the ELISpot blue colour module (R&D Systems) as per manufacturer's instructions. Plates

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