1. Introduction:

Influenza A virus (IAV) is a seasonal epidemic virus which infects between 10-15% of the global population each year [1, 2]. Infection results in illness typically lasting between 5 and 15 days, characterised by respiratory distress, fever, headache and myalgia. In at-risk patients such as the elderly, infants and the immunocompromised, IAV can cause significant morbidity resulting hospitalisation and death [3]. IAV exists in several different subtypes that can be classified on the basis of the 18 variants of the hemagglutinin (HA) and 11 variants of the neuraminidase (NA) proteins expressed by the virus. Further minor variation in these proteins can be used to differentiate strains of IAV. In immune-competent individuals IAV infection stimulates an immune response that confers lasting protection that is strain specific. However, immunity to one strain rarely confers protection against other strains and there is even less cross-protection between sub-types. Thus, minor seasonal variations in IAV antigenic composition (antigenic drift) can result in individuals experiencing repeated bouts of influenza due to different strains, while changes in the prevalent seasonal IAV subtype (antigenic shift) poses an even greater immunological challenge. In addition to seasonal epidemics, novel strains of the virus arise periodically which can cause global influenza pandemics associated with increased mortality in all patient groups [4]. Current influenza vaccines are designed to protect against the predominant circulating strains of IAV in a given year, identified through epidemiological surveillance. The vaccine efficacy relies on accurate prediction of the circulating seasonal strains and these recommendations are reviewed and updated annually. However, there is often a mismatch between the predicted vaccine target strains and the prevalent strain that causes a seasonal epidemic resulting in low vaccine efficacy [5, 6]. Furthermore, current vaccines are unlikely to protect against newly emergent pandemic strains that may arise following recombination between strains such as avian and human IAV [7, 8]. Current IAV vaccines target immunodominant regions of the virus that

are subject to particularly high mutation rates and so protection is frequently short-lived since variant strains emerge which escape immune memory and recognition. To overcome this variation an alternative strategy is to target highly conserved regions of the virus, mutation of which may severely compromise virus viability. Despite the high variability of proteins such as hemagglutinin and neuraminidase, the major targets of existing vaccine strategies, previous work has shown that some protein domains within IAV are highly conserved between different subtypes, strains and clades [9-11]. These conserved domains represent potential targets for a universal IAV vaccine which could generate broad immunity against many strains of IAV and thus remove the need to use epidemiological surveillance to predict the likely prevalent strains. Moreover, such a vaccine may have the advantage of conferring longer lasting immunity, obviating the need for annual vaccinations, because a vaccine based on conserved antigens will not be rendered obsolete by mutations in the variable parts of IAV. The protein targets within IAV that show a good degree of conservation include nucleoprotein (NP), matrix protein 1 and 2, some epitopes of neuraminidase (NA), and the stalk domains of hemagglutinin (HA) [12-14]. Previous attempts to exploit these antigens in vaccines have had only moderate success because usually the conserved domains are poorly immunogenic in the context of trivalent or quadrivalent inactivated vaccines. This may be because they have not been presented in the correct conformation or because the immunological context of presentation was not sufficient to generate protective immunity [15-19]. We have attempted to harness the immunogenic properties of hepatitis B core protein VLP to enhance the immunogenicity of conserved IAV antigens that fail to elicit effective immune responses through natural infection or split virus vaccines. We have adapted hepatitis B tandem core [20] sub-units to generate a virus-like particle (VLP) vaccine incorporating 3 variants of the Matrix protein 2 ectodomain (M2e) and a conserved antigen from group 1 HA-stalk to create an IAV vaccine candidate.

Tandem core (TC) is composed of two hepatitis B core molecules linked by a poly-glycine-serine linker that confers stability and facilitates assembly of multiple TC subunits into a virus-like particle. Each TC has two independent major insertion regions (MIRs) each of which can accommodate exogenous antigens in the correct conformation for antigenic recognition, and within the immunogenic context of a VLP [21-23]. In our vaccine, the first major insertion region (MIR) has been engineered to incorporate a HA-stalk structural antigen from H1N1 corresponding to an alpha-helix within HA2 domain of hemagglutinin stalk, sometimes referred to as long alpha-helix (LAH) [24]. The sequence encoded in the MIR is based on an extended sequence from the A/Luxembourg/46/09 pandemic IAV strain. The second major insertion site in our VLP incorporates three variants of the influenza matrix protein 2 conserved ectodomain (M2e) as a triplet sequence insert based on the predominant variants found in H1N1, H5N1 and H11N9 subtypes. Thus the final vaccine candidate, named Tandiflu1, bears a total of 4 conserved IAV antigens contained within a single VLP (Fig.1). The conservation, cross-protection and functionality of these IAV antigens has been amply characterised [11, 15, 18, 25-28] but to our knowledge, their incorporation into a single recombinant protein vaccine has not been developed or tested previously. In this study we report the expression of Tandiflu1 VLP in *Escherichia coli*, followed by immunisation of mice to assess its immunogenicity and the evaluation of vaccine efficacy in a mouse model of live influenza virus infection.

2. Materials and methods:

2.1 VLP construct design

Tandem core (TC) is based on the *wild type* Hepatitis B core (HBc) protein (Fig.1A) that self-assembles into a virus-like particle (VLP). In one TC, two HBc monomers are molecularly

linked together to yield a more stable dimer that spontaneously assembles to form a VLP [20]. Each dimer bears two major insertion sites. TC VLP without any inserts (empty core) was used as a control not containing IAV antigens (Fig.1B). The Tandiflu1 VLP incorporates an antigen from the hemagglutinin stalk domain (HA-stalk) based on the pandemic H1N1 A/Luxembourg/43/2009 influenza virus sequence [29] inserted into the first major insertion region (MIR1). This intermediate construct was expressed and tested to determine the immunogenicity of HA-stalk as a single insert VLP; referred to in the text as HA-stalk VLP (Fig.1C). The second insertion region of Tandiflu1 contains a triplet antigen insert, corresponding to three different variants of a conserved region of the matrix-protein 2 ectodomain that are found in the majority of IAV strains. To determine the immunogenicity of this triplet insert as a single MIR insert VLP, the intermediate construct was generated containing the triple M2e antigen insert in MIR2 with the other MIR1 left empty, referred to in the text as 3M2e VLP (Fig.1D). The complete Tandiflu1 capsomere contains the HA-stalk antigen in MIR1 and the triplet M2e antigen in MIR2 (Fig.1E). Tandiflu1 assembles into a VLP comprised of 90-120 individual capsomeres. The full genomic sequences for Tandiflu1 inclusive of the IAV inserts has been previously published in patent application PCT/GB2015/053699 with publication number WO2016087863 A1 [30].

2.2 Cloning and Expression

The constructs described above (TC VLP, 3M2e VLP, HA-stalk VLP and Tandiflu1) were synthesised via GeneArt (Life Technologies) and included the restriction sites XbaI and XhoI at the 5' and 3' ends respectively. Using these restriction sites, the constructs were cloned into the pET28b(+) plasmid (Novagen #69865) and used to transform *E. coli* DH5 alpha cells (NEB #C2987H). Positive clones were identified via colony PCR and sequencing and used for the extraction of plasmids which were then used to transform *E.coli* BL21 cells (NEB #C2527I) for VLP expression. Bacterial expression was performed by culturing *E.coli* BL21

(DE3) clones in Luria-Bertani broth containing 50 μg/ml of kanamycin at 37°C at 250 rpm in an orbital shaking incubator up to an optical density (OD 600) of 0.6. Induction was triggered by adding 1 mM Isopropyl β-D-1-thiogalactopyranoside and incubating the culture for 17 Hrs. at 12°C in a shaking incubator set to 250 rpm.

2.3 Purification and Analysis of VLP

Briefly *E.coli* cells were harvested from liquid culture by centrifugation at 15,000 g for 30 min. in a bench-top centrifuge. The cell pellet was then re-suspended in lysis buffer (20 mM Tris, 5 mM 1,4-dithiothreitol, 2 mM 4-benzenesulfonyl fluoride hydrochloride, 125 U/g Benzonase, pH 8) at a density of 0.125 g wet weight/ml. Cells were then disrupted by micronisation (APV Gaulin Micron Lab40 homogeniser, 1200 bar, 3 passes), followed by detergent extraction (0.05% Tween 20, 1 Hr. on ice) and clarification by ultra-centrifugation (20,000 g, 30 min., 4°C). VLP were further solubilised by addition of an equal volume of 20 mM TRIS, 5 mM EDTA, 2 M urea buffer (pH 8), and concentrated 40-fold using tangential flow filtration with a 1 MDa nominal molecular weight limit, Pellicon-XL 50 cassette (Millipore #PXB050A50). The resulting concentrate was then applied to 2 subsequent gel-filtration chromatography columns; a Sepharose CL-4B column (GE #17015001) was run in a buffer consisting of 20 mM Tris pH 8.4, 5 mM EDTA, 1 M urea. The void volume containing VLPs and larger components was subjected to a second round of size exclusion chromatography using a Sephacryl S-1000 SF resin (GE #17047601) (in 20 mM Tris pH 8.4, 5 mM EDTA). The fractions containing VLP were collected and analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), Western Blot and electron microscopy to confirm the presence and identity of the VLPs. For SDS-PAGE, samples were heated to 95°C for 5 min. in Laemmli buffer and loaded on Mini Protean Any kD tgx gels

(BIO-RAD #4569035) together with the Precision Plus Protein molecular weight marker (BIO-RAD #161-0363), and run at 160 V for 40 mins. After protein transfer onto a 0.45 μm nitrocellulose membrane using standard methods, the presence of protein of interest was detected using anti-HBc antibody - clone 10E11 (Abcam #Ab8639)- in combination with goat anti-mouse IgG-Peroxidase (Sigma #A4416) and Super Signal chemiluminescent substrate (Fisher #10743105).

2.4 Immunisation and infection

Groups of at least 5 female BALB/c mice (Envigo, U.K.) 6-8 weeks old were immunised with 15 μg of purified VLP mixed with 20 μl of Imject Alum (Thermo Scientific #77161) and 20 μl of Sigma Adjuvant System (Sigma #S6322) made up to a volume of 100 μl with sterile saline solution. Primary immunisation was delivered intra-peritoneally (i.p.), secondary and tertiary immunisations were delivered sub-cutaneously (s.c.). Immunisations were performed 1 week apart. 3 weeks after the final immunisation mice were infected intra-nasally (i.n) with 5x MLD50 of influenza A virus (A/PR 8/34 H1N1). MLD50 was calculated using the Reed and Muench method [31] and translated as TCID50 by the Pearman & Kärber algorithm as described in Hierholzer & Killington Virology Methods Manual [32] where 1x MLD50= $10^{3.11}$ TCID50. Weight loss and clinical score was monitored daily after infection with a maximum weight loss limit of 20% in compliance with U.K. Home Office approved animal protocols under licence PPL 70/7376.

2.5 Antibody titres

Tail bleeds were performed to check seroconversion at weekly intervals following immunisation. Detection of anti-HA or anti-M2e antibodies was performed by Enzyme-linked immunosorbent assay (ELISA). Briefly, 96-well Nunc Maxisorp plates (Sigma

#M9410-1CS) were coated with 1μg/ml recombinant hemagglutinin (rHA Life Technologies)) from influenza virus (subtype as indicated in the text) in carbonate bi-carbonate buffer, or 6.25 μg/ml of the M2e consensus peptide sequence MSLLTEVETPIRNEWGCRCNGSSD-OH (Activotec) in 1 M NaCl buffer. Coated plates were washed 3x with PBS-Tween20 0.05% (PBST) and blocked with 10% skimmed milk solution (Sigma #1.15363.0500) for 1 Hr. at 37°C. Pooled serum from 5 mice per group was diluted in 2.5% milk was added to the test wells and plates were incubated at 37°C for 1 Hr. then washed 3x as before. Goat-anti-mouse IgG –peroxidase secondary antibody (Ab) was added at 1/2500 dilution (Sigma #12-349) and incubated at 37°C for 1 Hr. then washed 3x as before. 3,3′,5,5′-Tetramethylbenzidine substrate (Sigma# T0440) was added to each well for 20 min. and reaction was stopped with 1 M H_2SO_4. Anti-M2e monoclonal antibody 14C2 (Abcam #ab5416) was used a positive control for M2 peptide detection. Serum from mice inoculated with a sub-lethal titre of A/PR 8/34 H1N1 virus (PR8 immune +) was used as a positive control for rHA detection. Absorbance (Abs) at 450 nm with a 630 nm correction was read using the SpectraMAX 190 plate reader (Molecular Devices, USA). All wells were run in duplicate with at least 3 dilution repeats.

2.6 Cell binding assays

Madin Darby canine kidney (MDCK.2) cells (ATCC #CRL-2936) were cultured in Roswell Park Memorial Institute medium and infected at a multiplicity of infection of 2, with H1N1 (A/PR 8/34) for 2-3 days. Infected cells were then harvested and fixed, together with uninfected controls. Cells were then incubated with serum from Tandiflu1 immunised mice for 30 min. at room temperature. After 5x washes with PBST, a secondary goat-anti mouse IgG- FITC (Abcam #ab7064) conjugate was used to stain the cells for 30 min. at room temperature on an orbital shaker with gentle rotation. The ability of Tandiflu1 serum to bind

infected cells was then determined by Flow cytometry using the Epics XL-MCL (Beckman Coulter) flow cytometer by gating on cells which were positive for bound IgG on FL1 FITC (Fluorescein isothiocyanate) green fluorescence channel.

2.7 Passive serum transfer

100 μl of serum collected from mice immunised with Tandiflu1 (or serum collected from control mice immunised with empty core VLP containing no IAV insert) was injected i.p. into naïve BALB/c female mice 6-8 weeks old. On the same day, these mice were infected with 3 x MLD50 of influenza A virus (A/PR 8/34 H1N1) virus intra-nasally. Three days post-infection a second identical serum injection was given to both groups of mice. Weight loss and clinical scores were monitored daily after infection. Animals were euthanized if they exhibited extreme distress or weight loss exceeding 20% from baseline weight following approved Home Office U.K. animal protocols under licence PPL70/7376.

2.8 Infected cells in the lung

The degree of IAV infection was determined by measuring the relative proportion of lung cells expressing influenza nuclear protein (NP) in lung homogenate from infected and control mice. Briefly the lungs from infected mice (or naïve controls) were homogenized to single cell suspension by pressing them through a 100μm nylon mesh strainer and incubated with red blood cell lysis buffer (Roche #11814389001) for 5 minutes at 4°C. Cell viability was assessed using Live/Dead stain (Thermofisher L23102), with monoclonal mouse anti-influenza A virus nucleoprotein antibody conjugated to FITC (Abcam #ab20921) to determine infection. The percentage of live lung cells infected with IAV was determined by using flow cytometry (BC Epics XL-MCL flow cytometer) by gating on live cells expressing

nucleoprotein on the FL1 FITC channel. We determined the percentage of lung cells expressing influenza NP; and therefore infected with influenza A virus.

2.9 Statistical Analysis

Prism version 7.03 (Graphpad) was used to perform statistical analysis. For Kaplan-Meier survival plots the Gehan-Breslow Wilcoxon test was used. Welch's t-test was applied to compare averages of group body mass between infected and control groups using a 5% threshold for significance. The Mann-Whitney test was used for rank comparison of viral infectivity between 2 groups adopting a 5% threshold for significance.

3. Results:

3.1 Tandem core carrying multiple influenza conserved domains forms a virus-like particle (VLP)

The TC VLP containing a HA-stalk and three M2e inserts (Tandiflu1) was expressed in *E. coli* and purified using a combination of tangential flow filtration and gel filtration chromatography. Western blot analysis shows a band at 68.8 kDa corresponding to the full length Tandiflu1 recombinant protein (Appendix1. D). Size exclusion chromatography (SEC) was used to select larger protein complexes between 3 to 6 MDa found in the eluted fractions, and electron micrographs confirmed the presence of purified VLP conforming to a 30-40 nm diameter size (Appendix1 Fig.1). This purified material was used for all subsequent immunisation experiments in mice.

3.2 Immunisation with Tandiflu1 VLP generates antibodies to conserved influenza A antigens

In addition to generating the final Tandiflu1 construct with multiple inserts (Fig.1e) we first created two single insert VLP with IAV antigens inserted into only one of the two TC MIRs; HA-stalk VLP and 3M2 VLP (Fig.1c and 1d respectively). Mice vaccinated with the HA-stalk VLP generated polyclonal antibodies that bind recombinant HA (H1), and similarly, mice vaccinated with 3M2 VLP generated an anti-M2e specific IgG response (Fig.2A/B). Mice vaccinated with Tandiflu1 generated polyclonal antibodies against both HA-stalk and M2e simultaneously (Fig.2A/B). Seroconversion to both antigens (M2 and HA) in response to vaccination with Tandiflu1 was confirmed independently by the Luxembourg Institute of Health, using Tandiflu1 manufactured at iQur using a classical two-week interval immunisation schedule and MF59 adjuvant (Appendix 2).

We tested the ability of anti-Tandiflu1 antibodies to bind MDCK cells infected with influenza virus by flow cytometric analysis. We found that the sera from Tandiflu1 vaccinated mice can bind influenza antigens expressed by cells infected with H1N1 virus, and no binding was observed to uninfected cells (Fig.3A). Binding of HA or M2 expressed by MDCK confirms that the antibodies generated by vaccination can bind the antigenic targets in the context of influenza infected cells. This is important since many antibody mediated effector mechanisms rely on targeting infected cells instead of direct virus neutralisation, especially when the HA globular head domain is not a vaccine component.

In order to assess the cross-reactivity of the anti-HA antibodies induced by Tandiflu1, the serum from immunised mice was tested against a panel of recombinant HA from various subtypes by ELISA. Figure 3B shows that there is binding to all of group 1 hemagglutinins tested (H1, H5, H9, H11 and H16), but not group 2 hemagglutinins (H3, H7), demonstrating

broad cross-reactivity of Tandiflu1 antibodies to HA within Group 1 but not Group 2 IAV strains. Positive binding to various group 1 hemagglutinins confirms the conservation of the selected antigen vaccine target across multiple subtypes.

3.3 Vaccination with Tandiflu1 VLP confers 100% survival in challenge with H1N1 IAV

Mice were immunised with either Tandiflu1 or the single insert HA-stalk VLP, 3M2 VLP or with the negative control empty TC VLP containing no influenza inserts (Fig.1). Mice immunised three times with Tandiflu1 showed minimal signs of disease as measured by weight loss after viral challenge with H1N1 IAV, whereas the mice in the negative control group reached the lethal severity limit (Fig.4A). By day 7 post-infection survival in the control groups was 0%, compared to 100% in the Tandiflu1 immunised group (Fig.4B). In addition, mice vaccinated with the simpler VLPs containing only HA-stalk or M2 inserts also showed increased group survival (Fig.4B). The HA-stalk VLP vaccinated group provides further evidence of a cross-protective immune response since the VLP antigen was derived from the H1N1 2009 pandemic strain HA-stalk sequence template and the live challenge administered was a heterologous A/PR8/1934 H1N1 virus. Further confirmation of efficacy was obtained when Tandiflu1 immunised mice were also 100% protected from a challenge with H1N1 A/Luxembourg/43/2009 pandemic strain, in a protection experiment carried out independently at the Luxembourg Institute of Health (Appendix 2).

3.4 Tandiflu1 immunised mice have protective antibodies and reduced viral titres

Mice immunised with Tandiflu1 showed reduced signs of infection and increased survival, but vaccination did not induce sterilising immunity. This is consistent with previously published data which show HA-stalk and M2e immunity permits infection but protects

against severe manifestations of influenza [18, 25, 28, 33]. In order to investigate the mechanism by which Tandiflu1 confers protection against IAV we harvested the lungs of immunised mice 3 days after viral challenge with PR/8 H1N1 virus. The vaccinated group presented with a decreased percentage of IAV infected lung cells equivalent to a 6-fold decrease in viral infection compared to sham vaccine controls (Fig.5).

In order to determine whether passive serum transfer from Tandiflu1 immunised mice was sufficient to confer protection we injected 100µl of Tandiflu1 immune serum into naïve mice at day 0 and 3 post-infection. All the mice that received the anti-Tandiflu1 serum survived challenge with a lethal dose of H1N1 compared to 40% of control animals. Immunized mice exhibited less weight loss during H1N1 viral challenge, whereas recipients of control serum had diminished survival and increased weight loss (Fig.6).

4. Discussion:

In the face of the deficiencies of current seasonal influenza vaccines that confer strain-specific immunity a universal influenza vaccine is highly desirable and, as a result, has been the focus of significant research effort. Over the past two decades research groups have endeavoured to identify the conserved and functional protein targets within influenza virus that could make this a reality. There has been previous moderate success using purified single or conjugated antigens, but as data accumulate it is becoming clear that a broader multi-target approach may be more effective than a vaccine directed at a single target. Here we present an IAV vaccine candidate (Tandiflu1) that combines four conserved antigen sequences from two different influenza proteins on a single VLP display platform, representing the first successful insertion of different antigens into dual MIR sites for a HBc based VLP. The tandem core VLP platform was chosen because hepatitis B virus core is a well-studied

protein that has been shown to be a safe and well-tolerated vaccine subunit in human trials (NCT00819013). Additionally, the well-documented propensity of hepatitis B virus core to confer immunogenicity to associated antigens is required when targeting epitopes with low natural immunogenicity [22, 23].

Tandiflu1 is the first vaccine application of the tandem core VLP, and immunisation with Tandiflu1 leads to simultaneous generation of antibodies to the M2 ectodomain as well as cross-reactive antibodies to group 1 hemagglutinin stalk. Matrix protein 2 ectodomain has been extensively characterised as a potential immunogen in universal influenza vaccines. De Filette et. al and others have previously shown that complete survival can be achieved with M2e vaccination administered in various forms [27, 28, 34]. Yet, mice effectively protected from lethal infection still experienced significant weight loss and exhibited signs of influenza. This indicates that immune protection targeting the M2e alone is not sufficient to prevent symptomatic influenza. Similarly, the LAH domain within the HA-stalk has long been known to be a conserved domain of IAV [35]. Wang and colleagues characterised this epitope and found that antibodies to this stalk domain are protective in the face of lethal IAV challenge [36]. Since then, many groups have tried to target this region with mixed success. While some groups demonstrated broad cross-protection others found only modest protection levels to the homologous viral strain [24, 37-40]. However, to our knowledge, a single vaccine platform which simultaneously targets both HA-stalk and M2 protein antigens has not previously been produced or tested. We showed that a VLP containing the M2e triplet antigen was sufficient for 100% group survival but targeting two conserved antigens simultaneously increases the probability of broad IAV coverage and decreases the likelihood of escape variants arising from a single gene mutation.

Our data suggest that immunisation with Tandiflu1 combining three M2e variants and HA-stalk antigen results in significant protection against influenza mortality. We have

demonstrated that the tandem core platform allows the integration of several functional vaccine antigens into a single VLP, with implications for the generation of a multi-valent immune response, as well as an impact on lowering cost of production by producing multiple subunit antigens by a single process.

The precise immune mechanisms for M2 and LAH vaccines remain a source of discussion. Several groups have shown that administration of anti-M2e antibodies before or during infection can confer protection against IAV associated illness [41, 42]. Equally, others have shown that anti-LAH antibody injections are sufficient to protect mice from IAV infection [36]. Antibodies which bind the HA stalk have been shown to mediate protection by Fc receptor mediated neutrophil degranulation and phagocytosis, and NK-cell antibody-dependent cellular cytotoxicity (ADCC) [43, 44]. The protection conferred by Tandiflu1 vaccination is also mediated by antibody dependent mechanisms since protection was observed during passive serum transfer experiments, although the full role of cellular immunity requires further investigation. For antibodies which target M2 ectodomain, Saelens and colleagues showed that immune complement and ADCC play a small role, but most of the protective activity derives from antibody dependent cell-mediated phagocytosis (ADCP) [45]. This is mediated by alveolar macrophages and is transduced through their Fc receptors. The effector mechanism is dependent on anti-M2e antibodies binding influenza infected cells that are subsequently destroyed by phagocytic cells. We showed that serum from Tandiflu1 immunised mice can bind IAV infected MDCK cells, thus antibody mediated effector functions targeting infected cells may explain the reduction in influenza infected lung cells at day 3 we observed. Future work will attempt to address the specific mechanisms of protection.

The anti-HA antibodies failed to recognise HA from group 2 viruses, this is not unexpected since the template sequence for the Tandiflu1 HA insert is from a group 1 H1N1 subtype. In

the future, we plan to design a second VLP that incorporates HA stalk inserts from group 2 influenza A subtypes and possibly influenza B, towards the development of a multi-valent universal influenza vaccine candidate. Tandiflu1 has been generated at laboratory scale and shown to be bioactive *in vivo*, however, as Tandiflu1 particles tended to aggregate and exhibited limited stability some optimisation will be required before this vaccine can be manufactured and formulated as a viable vaccine for human or animal use.

5. Conclusion:

The prospect of a universal influenza vaccine seems to be within reach and in recent years many potential candidates have been proposed. Guided by the recent success of commercial and experimental VLP vaccines which have been shown to be safe and effective, we have combined four well documented influenza universal antigen targets within a single VLP vaccine. The development of tandem core allowing larger inserts to be carried at multiple sites in chimeric virus-like particles [20] has enabled us to harness the immunogenic properties of hepatitis B virus core as a vaccine platform[22, 23]. We present an IAV vaccine candidate that generates cross-reactive and protective antibody responses to multiple conserved influenza A virus antigens simultaneously. Vaccination with this VLP (Tandiflu1) results in 100% protection from two H1N1 strains in a mouse model of influenza infection.

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Declaration of Interest

The vaccine candidate described in this paper is covered by patent application # PCT/GB2015/053699 filed by iQur Ltd. Authors Ramirez, Crescente and Whelan are named inventors in this application. Authors Ramirez, Crescente and Rosenberg are current employees of iQur Ltd.

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