A CONSIDERATION OF H1N1/09pdm AND NEW VARIANT H3N2/13 AS AGENTS FOR HUMAN CHALLENGE TRIALS

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Seasonal epidemics of mild or attenuated influenza sweep the globe twice per year – typically from December to February and April to September in the in the Northern and Southern Hemispheres respectively – resulting in 111 million days of working days lost to illness p.a. in the USA alone. The regular symptoms of influenza: temperature, sore throat, headaches and aching limbs may be inconvenient and unpleasant but rarely prove fatal. However, every decade or so a more virulent strain may arise due to antigenic shift (associated with a reassortment of genes coding for the HA and NA surface proteins), leading to sustained escape from acquired immunity to influenza present in the general population and resulting in a more profound pathology with high case fatality rates. Such reassorted viruses may go on to cause global pandemics.

THE HISTORY OF H1N1 AND H3N2

Influenza appears to have arisen as a zoonosis; new phylogenetic evidence suggests influenza originated in bats and spread sequentially to horses, poultry and swine before entering humans about 6000 years ago. Seasonal epidemics have likely been around for as long as influenza has been transmissible between humans. Pandemic influenza has been documented from as early as 876 A.D. when an influenza-like illness followed Charlemagne's army across Europe with waves of disease continuing to occur, spreading in a Northerly direction from Italy, in a pattern seen to be repeated until 1760.

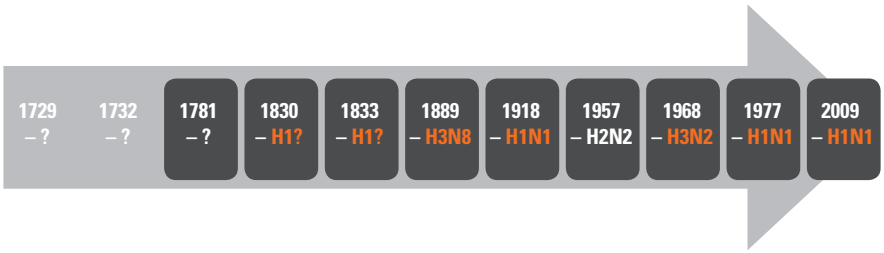
Latterly influenza settled into a pattern of avian and swine / human strain reassortment with three pandemics per century coincident to major antigenic changes. The outbreaks of 1580 and 1830, 1833 being notable for their severity and thus prominence in contemporary literature.

The most virulent influenza strain seen in recent history was that of 1918 when a new strain of H1N1 emerged and is estimated to have killed 50-100 million people worldwide during its two year dominance. It is the ability of the influenza virus to continually modify its immunogens by drift and shift that makes flu vaccine development so challenging and causes such concern to international healthcare organisations. Reassorted viruses tend to persist, replacing or displacing more attenuated strains and may become the prevalent serotype for centuries following a global pandemic.

There are three major serotypes or strains of Influenza: A, B, and C. Influenza A has the largest host range and has been responsible for all the major, documented flu pandemics since the first quarter of the 19th Century. Although influenza B is responsible for approximately one quarter of all influenza infections worldwide and is associated with a higher severity index in the tropics (positively correlated to <age), vaccine research has naturally focussed on influenza A due to its potential for pandemic spread accompanied by a dramatic increase in case fatality rates. The viral envelope of Influenza A contains two large glycoproteins: haemagglutinin (HA), which binds the virus to target cells, and neuraminidase (NA), which facilitates the release of progeny viruses from infected cells. Both HA and NA are strongly antigenic but also highly variable.

There are currently 18 HA and 11 NA serotypes known. Reassortment or drift (mutation) of HA and NA genes lead to new serotypes of influenza. H1, H2, H3, N1, and N2 have evolved sustained transmission into humans. However, only

FIGURE 1: THE 11 PANDEMICS SINCE 1700, LISTED BY THE YEAR THEY STARTED



two serotypes, H1N1 and H3N2 have responsible for the majority of pandemics during the 20th and 21st Centuries with pandemic strains originating towards the end of the 19th century.

THE CHALLENGES OF DEVELOPING & TESTING INFLUENZA VACCINES

Whilst the current, circulating H1N1 and H3N2 strains are ancestors of major epidemic and pandemic serotypes, influenza viruses mutate so readily that multiple strains continually develop within each serotype. Mutations within the HA, NA and non-structural (NS) genes may serve to make the virus more or less virulent (The H1N1 responsible for the 1918 flu pandemic had a case fatality rate (CFR) of 2%; when a reassorted strain re-emerged during the 2009 pandemic (H1N1/09pdm), it had attenuated and the CFR was only 0.03%) and to effect surface epitope changes making seasonal vaccines less effective.

Just as healthcare organisations and vaccine manufacturers must attempt to predict which strains will predominate in the forthcoming flu season, sero-surveillance programs are also required to ensure that their assays are capable of identifying new, emergent serotypes of influenza. With the loss or diminution of haemagglutination by the HA antigen in many circulating viruses since 2010, a new generation of assays has been necessitated e.g. neutralisation testing (MNT), to identify the seroprevalence of mutated influenza strains within populations. Together with novel markers for severity of disease e.g. PB2 E627K, NS-1 gene mutations, such assays may allow for a better prognosis of pandemic potential.

Within the field of influenza research, there is becoming an increasingly large place for Human or Viral Challenge Trials (HCT). Challenge trials allow for the direct measurement of vaccine or drug performance against a manufactured virus inoculated into healthy volunteers. Such studies can circumvent the need for large scale field trials in early phase, providing high quality efficacy and safety data.

TABLE 1: VIRAL DYNAMICS

In a meta-analysis of human clinical trials, viral shedding was noted in 93.1% of H1N1/09pdm subjects and 92.5% of H3N2
Peak viral loads differ little between H1 and H3 studies
vAUC is observed to be greater in H3N2 challenge studies
No significant correlation has been observed between pandemic (H1N1) 2009 or seasonal influenza viral loads and clinical severity of illness
Viral loads in pandemic H1N1 viruses are characterised by lower copy numbers than seasonal H3N2 viruses (~1 $\log^{10}$)

TABLE 2: IMMUNOLOGY

H1N1	H3N2
Promotes a strong protective antibody response to surface hemagglutinin and neuraminidase antigens (?H7N9)	Since 2010, all circulating influenza strains are showing a decreased ability to agglutinate
Full-length H1 HA antigens induce a profound HI and NAb response	Both full-length and secreted, transmembrane-truncated H3 HA antigens induce high-level HI and NAb responses
Severity of disease correlates to prior exposure	Severity of disease correlates to prior exposure
Immunity does not correlate to pre-seasonal HI titres	Immunity correlates to pre-seasonal HI titres
H1N1/09pdm immunity increases with age	H3 HA genes mutate more rapidly than H1 – H3 has a greater propensity to vaccine failure or escape

TABLE 3: SEVERITY AND SEQUELAE

Influenza A H3N2 infection is more severe than influenza A H1N1 in terms of fever, leucopoenia, and CRP.
Mean ages for attack are 33 +/- 8.4 (H1N1) and 41 +/- 15.2 years (H3N2).
A greater number of hospitalizations occur during years that influenza A H3N2 is predominant.
Pneumonia is positively associated with vaccination.
Seasons with predominant circulation of influenza A H3N2 have 2.7 times more deaths than years with influenza A H1N1.

Owing to the heterotypic nature of influenza it is necessary that pharma companies or clinical research organisations work to develop a broad menu of strains as challenge agents. The use of cGMP manufactured H1 or H3 viruses in such trials can emulate high incidence disease, negating the limitations of prevalence and seasonality of disease in the wild.

H3N2 VS H1N1

H1 and H3 strains remain the influenza strains of choice for challenge trials as they have been dominant for more than 100 years; H1N1/09pdm being the agent responsible for the most recent human pandemic. The current, seasonal H1N1/09pdm is a naturally attenuated descendent of the original pandemic H1N1 and is associated with the most severe and well characterised symptomology of the all the recently circulating H1N1 serotypes; retaining elements of infectivity and pathogenicity but not the cytokine events and high CFR that characterised its emergence in 2009. H1N1/09pdm has seen a recent resurgence, becoming the predominant influenza A strain in North America and much of Europe for this past flu season (2015-2016).

Despite the prevalence of H1N1, H3N2 remains an important influenza strain due to some inherent properties of the virus e.g. a more exaggerated symptomology than the seasonal H1N1/09pdm. A new H3N2 variant was the predominant virus for 2014-2015 in the Northern hemisphere and drifted variants of the Switzerland/2013 strain were a leading cause of hospitalisation during that flu season.

Comparatively, H3N2 and H1N1 both have merit as challenge viruses. However, notable differences in viral dynamics and the immunology and pathology of these strains have been documented over the course of a large number of natural infections and also in over 1000 subjects enrolled in challenge trials (Tables 1, 2, 3).

A wider comparative review of H3 vs H1 as challenge agents would suggest that H3N2 remains the stronger candidate for challenge trials owing to a number of intrinsic factors including, but not limited to: a greater viral area under curve (vAUC) (increased titre and duration of shedding), increased symptomology (headache, rhinitis, sore throat and pyrexia), superior correlation to pathological and immunological markers (CRP, leucopoenia, serosusceptibility and antigenic responses) and a raised propensity for vaccine escape (higher mutation and age-related vaccine-failure). However, the role of H1N1 and influenza B should not be underestimated in the evaluation of novel agents for the prevention and treatment of disease.

THEN, NOW AND THE FUTURE

Influenza can be seen to have been the cause of epidemic and pandemic disease stretching back in documented history to at least the 9th century. H1N1 and H3N2 have been the dominant influenza strains for over 100 years and continue to cause seasonal, global outbreaks with 50,000 - 200,000 deaths per annum. As may be seen from comparative analyses, a seasonal H3N2 would appear to remain the challenge agent of choice given the advantageous features of enhanced symptomology and a greater vAUC – both essential outcome measures in any prophylactic or therapeutic trial. However, both H1N1 and H3N2 may both offer a cost-effective route to high quality PoC and safety data when compared to traditional field studies. Access to human challenge agents remains limited due the high cost of manufacture and the rigours of cGMP. Also seed stock may attenuate from wild-type during the course of manufacture due to passage or growth medium related issues reducing virulence and thus attack rates. Recent advances in technology have led to the production of a recombinant H1N1/09pdm available for use as a challenge agent, with the potential for other challenge agents to follow. Recombinant agents can be manufactured to cGMP without many of the contamination and allergenic issues associated with traditional manufacturing methods and in the future it may allow for genetic modifications to enhance or reduce pathogenicity and the tailoring of expressed surface epitopes to emulate domains seen in highly virulent or pandemic species without the downstream effects on cytokine production or increases in morbidity.

ABOUT SGS AND ITS HUMAN CHALLENGE PROGRAM

For several years SGS has been developing a dedicated viral challenge program which includes:

- A fully accredited Clinical Pharmacology Unit; an 8 single bed isolation rooms and a 12-bed ward style isolation unit facility. It is a dedicated Human Challenge Unit (Class II, negative pressure unit) with strict infection control including HEPA filtered air systems. There are typically two different testing modalities: subjects are infected with virus and given a test drug two days later, or they are vaccinated one month before challenge with a characterised cGMP virus.

- A challenge virus. SGS has recently isolated a drifted strain of the H3N2 A/Switzerland/2013 circulating locally in Belgium and this new strain is currently under cGMP manufacture for release in late 2016 as a novel challenge agent. As more strains are developed for use in the human challenge field it is hoped that the value of the model is enhanced.

REFERENCES

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