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Progress in the development of human parainfluenza virus vaccines

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Abstract

In children under 5 years of age, human parainfluenza viruses (HPIVs) as a group are the second most common etiology of acute respiratory illness leading to hospitalization, surpassed only by respiratory syncytial virus but ahead of influenza viruses. Using reverse genetics systems for HPIV serotypes 1, 2 and 3 (HPIV1, 2 and 3), several live-attenuated HPIVs have been generated and evaluated as intranasal vaccines in adults and in children. Two vaccines against HPIV3 were found to be well tolerated, infectious and immunogenic in Phase I trials in HPIV3-seronegative infants and children and should progress to proof-of-concept trials. Vaccines against HPIV1 and HPIV2 are less advanced and have just entered pediatric trials.

Keywords

acute respiratory illness; clinical trial; intranasal; live-attenuated; parainfluenza virus vaccine; pediatric; vaccine

Epidemiology & clinical disease

Acute lower respiratory illness (ALRI) is a major cause of morbidity and mortality. Globally, ALRI remains the most important cause of postneonatal mortality in children under 5 years of age, accounting for approximately 1.6 million deaths every year [1]. In addition to bacterial pathogens such as *Streptococcus pneumoniae* and *Haemophilus influenzae* type b, which were estimated to account for approximately half (36 and 16%, respectively) of the global pneumonia mortality in children under 5 years of age in 2000 [2,3], the major viral contributors to childhood ALRI are respiratory syncytial virus (RSV), human parainfluenza viruses (HPIVs), human metapneumovirus (HMPV) and influenza viruses [4-6]. Whereas licensed vaccines against invasive pneumococcal and *H. influenzae*

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Financial & competing interests disclosure

Alexander C Schmidt, Emmalene J Bartlett and Peter L Collins are inventors on PIV patents and patent applications. NIAID and MedImmune have a cooperative research and development agreement in place for the delvelopment of RSV, PIV and HMPV vaccines. NIAID receives royaltits (not related to P/V vaccints) from MedImmune. Alexander C Schmidt is an Associate Scientist at NIAID and the Project Officer responsible for translational and clinical PIV vaccine development. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter materials discussed in the manuscript apart from thou disclosed.

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type b disease are available and increasingly accessible, vaccines against RSV, the HPIVs and HMPV are still in development.

Globally, RSV is the most common cause of childhood ALRI [5] and the HPIVs as a group are the second most common etiology, responsible for more hospitalizations in children under the age of 5 years (1/1000 per year) than influenza [7-9]. A recent population-based burden of hospitalization study conducted by the New Vaccine Surveillance Network estimated that, in the USA, HPIVs accounted for approximately 7% of all hospitalizations for fever, acute respiratory illness (ARI) or both in children under 5 years of age [7]. This estimate translates into 23,000 HPIV-attributable hospitalizations per year in the USA, with HPIV3 responsible for half of that burden and HPIV1 responsible for the majority of the remainder [7].

Of the four HPIV serotypes, types 1, 2 and 3 (HPIV1, 2 and 3) are common causes of respiratory illness in infants and young children [6,10]. HPIV3, like RSV, frequently causes bronchiolitis and pneumonia in young infants. HPIV1 and HPIV2 are responsible for epidemics of croup, with HPIV1 being the most common etiologic agent of that disease [7,11]. Although HPIV1 and HPIV2 disease is most commonly seen in 1-6 year olds, hospitalization rates for all three HPIVs are highest in the first 6 months of life, with bronchiolitis, fever/possible sepsis, upper respiratory illness, pneumonia, croup and apnea as the most frequent discharge diagnoses. In infants and children 6 months of age and older, asthma and croup are the most common discharge diagnoses [7]. The use of corticosteroids and nebulized epinephrine to treat croup requiring urgent medical care has decreased croup-related hospitalization significantly and also explains a reported decrease in the contribution of HPIV1 to overall HPIV-attributable hospitalization [11-13].

In the USA, HPIVs can be isolated throughout the year, but HPIV3 circulation tends to peak in the spring, HPIV2 in autumn and HPIV1 in the autumn of odd-numbered years [14]. Reinfections with the HPIVs are frequent, although usually associated with milder illness and restricted to the upper respiratory tract (URT) [15]. Indeed, most HPIV-associated clinical illness is mild, even in primary infection. Rhinitis, pharyngitis, coryza and fever are common, whereas otitis media, croup, bronchitis, bronchiolitis and pneumonia are only observed in a minority children. Therefore, most HPIV-associated illness is treated on an outpatient basis and is not diagnosed with regard to viral etiology, leading to an underestimation of the HPIV-attributable burden of disease.

In addition to infants and young children, immunocompromised patients and the elderly are also at increased risk for severe HPIV disease. However, our understanding of the burden of disease in the elderly is very limited since most epidemiologic studies in this population focus on RSV and influenza. In a prospective study of healthy elderly individuals and of adults with chronic heart or lung disease, RSV infection was responsible for 11% of hospitalizations for pneumonia, 11% for chronic obstructive pulmonary disease, 5% for congestive heart failure and 7% for asthma [16]. In patients hospitalized with acute cardiopulmonary conditions, mortality was similar in RSV and influenza-infected patients [16]. If one assumes that the HPIVs behave like RSV in rhe aforementioned population and that their relative contribution to the burden of disease is similar to that observed in children, then the impact of HPIVs may be significant. However, data to substantiate this assumption are not available. In the immuno-compromised, especially in hematopoietic cell transplant (HCT) and in lung transplant patients, HPIVs can cause severe morbidity and mortality [17]. HPIVs are known ro be responsible for ALRI outbreaks in HCT units and outpatient clinics, with high transmission rates and high mortality (up to 45%) [18-21]. In HCT patients, HPIVs are as common a cause of viral pneumonia as RSV and high viral loads are found in bronchoalveolar lavage fluid from these patients [22].

Is there a need for HPIV vaccines?

Studies conducted decades ago (reviewed elsewhere [6]), as well as recent epidemiologic studies [7], indicate that the HPIVs as a group cause at least as much ARI in infants and young children as influenza. Whereas universal influenza vaccination of children is recommended, no licensed vaccine against the HPIVs exists. Since cross-protection between HPIV serotypes is very short-lived or insignificant, a decision is needed as to which serotypes should be included in HPIV vaccines. As previously indicated, HPIV3 is responsible for more hospital admissions than HPIV1 and HPIV2 combined. Therefore, a HPIV3 vaccine for infants is desirable and would ideally be given to infants as young as 1 or 2 months of age since much of the severe illness occurs in the first several months of life. As both RSV and HPIV3 can infect very early in life and in the presence of maternally-derived serum antibody, a combined RSV/HPIV3 vaccine that can induce protective immunity in this young infant population is probably the most important goal in pediatric respiratory viral vaccine development. A recent study conducted in rural Kenya indicated that the prevention of RSV-associated severe pneumonia alone might reduce all-cause (i.e., including bacterial) pneumonia hospital admissions of children under 13 years of age by a third [23]. A HPIV1/HPIV2 vaccine could be given later than an RSY/HPIV3 vaccine because clinical disease from these serotypes is less common in the first 6 months of life. Since the relative contribution to the burden of disease in infants and young children is estimated at approximately 4:2:1 for HPIV3, HPIV1 and HPIV2, respectively [6,24], a HPIV2 vaccine is not an attractive target as a stand-alone vaccine. However, if a HPIV1/HPIV2 vaccine could be developed as a single vaccine or be combined with a boost against RSV, it might prove to be a worthy and medically meaningful target.

Viruses

The HPIVs are enveloped, nonsegmented, negative-sense RNA viruses belonging to the *Paramyxoviridae* family in the order *Mononegavirales.* HPIV1 and HPIV3 are members of genus *Respirovirus*, while HPIV2 is a member of genus *Rubulavirus.* The genomes of all three HPIVs are approximately 15,500 nucleotides in length and encode six common proteins in the invariant order N-P-M-F-HN-L (Figure 1). Infection of a host cell by HPIV is initiated by binding of the hemagglutinin-neuraminidase (HN) glycoprotein on the virion envelope to sialic acid on cellular membrane proteins [25,26]. The fusion (F) protein then mediates the fusion between the viral envelope and the host-cell plasma membrane. This releases the viral nucleocapsid, which consists of the viral genome tightly bound by the nucleoprotein (N) and associated with the phosphoprotein (P) and the large RNA-dependent RNA polymerase L protein. The nucleocapsid-bound polymerase directs copying of the viral genes into separate mRNA transcripts and also directs replication of the RNA genome [27]. Progeny nucleocapsids assemble and are packaged into virions that bud from the plasma membrane. The matrix (M) protein coats the inner surface of the envelope and the spike-like glycopcoteins F and HN project from the outer surface of the envelope [6,28].

In addition to these six common proteins, each of the HPIVs encodes at least one additional nonessential protein from alternate open reading frames in the P gene. HPIV1 and HPIV3 encode short C proteins, while HPIV2 encodes a V protein that is distinguished by having a C-terminal domain that contains a conserved cysteine-rich motif. HPIV3 also encodes a D protein and may express small amounts of a V protein. The V and C proteins primarily act as inhibitors of the host innate immune response, whereas the function of D is unknown [29-32]. Although the C and V proteins are unrelated in either sequence or mechanism of action, they have a common function: to suppress the antiviral activity of type I interferons (IFNs) by blocking both their induction and their ability to signal amiviral responses by host cells.

Tropism

Replication of HPIV1, 2 and 3 occurs in the superficial epithelial cells lining the respiratory tract. Infection typically starts in the mucous membranes of the nose and throat but an infectious virus may also be transmitted directly to the lower respiratory tract (LRT). Within the LRT, the ciliated and alveolar cells but not the basal cells of the bronchial epithelium are infected [26]. Whether disease progresses from the URT to the LRT depends on several factors, including previous exposure to that serotype, virus titer in the URT and genetic susceptibility to severe disease [27,33,34). HPIV infection, unlike influenza, does not cause extensive cytopathic effect or tissue destruction in an *in vitro* model of the respiratory airway epithelium [26,32,35]. This suggests that the host immune response may contribute significantly to pathogenesis. HPIV infection is generally limited to the respiratory tract and does not spread systemically unless the infected individual is severely immunocompromised. Newly formed virions are primarily released from the apical surface of ciliated respiratory cells into the lumen of the respiratory tract [26,32,35]. This directional budding is thought to play a role in limiting HPIVs and RSV to the respiratory tract. However, RSV RNA can be detected in the blood of 10% of severely immunocompromised patients with RSV pneumonia, suggesting that, in addition to directional budding, some degree of host immunity might be needed to prevent systemic spread [22].

Immune response & correlates of protection

Human parainfluenza virus infections can induce potent humoral and cellular immune responses in the infected host. Innate immune responses, local and systemic IgG and IgA responses and CDS^+ and $CD4^+$ T-cell responses are known to be induced [36,37]. Although cellular responses are important in restricting virus replication and clearing primary HPIV infection, neutralizing antibodies targeting the HN and F glycoproteins of HPIV play a major role in conferring long-term protection from HPIV disease [6,38]. Both serum and mucosal neutralizing antibodies can provide lasting protection against disease. The presence of nasal antibody was found to be a good correlate of protection whereas serum antibody titers needed to be high in order to confer protection [43,44]. Thus, the induction of neutralizing antibodies is thought to be essential for a successful HPIV vaccine. As discussed earlier, virus-encoded IFN antagonists permit HPIVs to replicate efficiently *in vivo*; however, IFN production and activity are usually not completely blocked and probably contribute to host defense. Many patients with primary HPIV infections develop a detectable IFN response during the acute stage of illness [39]. The induction of T-cell responses also contributes to viral clearance [40,41]. Cellular immunity confers some resistance to reinfection but this effect wanes over a period of weeks to months [42].

Mucosal antibodies that neutralize virus infectivity appear to be the best correlates of protection against HPIV disease in adults [43] and may also correlate with protection in children, though measurement of mucosal immunity in children has been difficult. IgA has the advantage of being specifically transported through the epithelium to the lumenal surface and IgA is also able ro neutralize the virus within infecred epithelial cells. Although local IgA plays a key role in resistance against reinfection, this protective effect is also relatively short-lived and two or more infections might be needed in order for mucosal IgA to persist long term [43,44]. Long-term resistance in the URT is less complete than resistance in the LRT and most individuals experience multiple HPIV and RSV infections in the URT throughout life [45]. Serum neutralizing antibodies seem to provide long-term resistance to virus replication. By analogy with RSV, protection conferred by serum antibodies is likely to be more effective in the LRT than in the URT [46]. In addition, young infants possess maternally-derived serum IgG antibodies that are transferred across the placenta during the last trimester of pregnancy and provide some protection during the first months of life.

Immune responses in infants are reduced in magnitude, effectiveness and durability compared with older children due to immunologic immaturity and the immunosuppressive effects of maternal antibodies, as reponed below [47-49]. Immunity induced by primary infection with HPIV often does not prevent symptomatic reinfection for more than a few months, especially in infants. However, illness upon reinfection is generally milder than with primary infection, with restricted virus replication and infrequent progression co ALRI [15].

Approaches to vaccine development & obstacles along the way

The first attempts at developing a HPIV vaccine were undertaken in the early 1960s, not long after the discovery of these viruses. It was shown that protection against challenge with wild-type (WT) HPIV1 correlated with the presence of neutralizing nasal mucosal antibodies [50]. Serum antibodies also contributed to protection, but only when the serum antibody titer was high. A formalin-inactivated (FI) HPIV1 vaccine that induced neutralizing antibody titers in the serum, but not the nasal mucosa, did not confer protection [43,50]. FI vaccines against RSV and measles were also evaluated clinically in the 1960s and conferred only transiem (measles virus) or insignificant (RSV) protection; instead, enhanced and atypical disease was observed following natural infection of vaccinees with WT virus. Of the 23 FI-RSV vaccinees who were infected with WT RSV during an outbreak following vaccination, 18 needed to be hospitalized and two infants died of pneumonia (reviewed elsewhere [6]). As a result of the failure of these FI vaccines, new attempts at developing HPIV and RSV vaccines for use in children have focused, for many years, on live-attenuated approaches. This is because preclinical and clinical data demonstrate that disease enhancement is not associated with live vaccines [51]. Only very recently has the interest in developing protein-based nonlive vaccines re-emerged and several vaccine developers have invested in the development of nonlive RSV vaccines.

At first consideration, HPIVs appear to be an easy target for vaccine development because these viruses cause acute, self-limiting disease and do not establish persistent infection. However, vaccine development has proved to be far from trivial and several obstacles need to be overcome. First, immunity induced by a single infection with WT HPIV does not prevent symptomatic reinfection. Most children and adults experience multiple symptomatic infections. However, reinfections generally induce milder disease and significant LRI is infrequent. Second, severe HPIV3 disease often occurs in young infants under 6 months of age and a robust antibody response to the viral surface glycoproteins is induced less frequently in this age group than in older infants [52,53]. In addition, young infants have a less diverse B-cell repertoire and less efficient antibody affinity maturation [54-56]. Although they can mount a protective immune response, as indicated by restriction of a second dose of live vaccine, measurable correlates of protection are not well defined [47]. Third, maternal antibodies can suppress the immunogenicity of both parenterally-administered nonlive and mucosally-delivered live vaccines [49,57,58]. In summary, one would want an ideal HPIV vaccine to be immunogenic in young infants in the presence of maternal IgG, to protect against LRI during the first infection with WT virus and to be well-tolerated and safe. A live-attenuated intranasally-administered HPIV or RSV vaccine would probably need to be given in twO or three doses, perhaps at 2, 4 and 6 months of age to fit the pediatric vaccination schedule.

Individual vaccines in clinical development *HPIV3 vaccines*

HPIV3*cp*45 was derived from the JS strain of HPIV3 by 45 passages at low temperature (cold-passage [*cp*]) [59]. During this process, the virus acquired 20 nucleotide substitutions, 15 of which were considered significant because they occurred in or near RNA signals (five

mutations) or caused amino acid substitutions (ten mutations) (Figure 2) [60,61]. Six of the amino acid substitutions were found to contribute independently to the HPIV3*cp*45 attenuation phenotype. In Phase I trials, HPIV3*cp*45 was evaluated sequentially in adults, seropositive children, seronegative children and finally in infants at 1-2 months of age (Table 1). At a dose of 10^4 infectious units (expressed as plaque-forming units [PFU] or 50% tissue culture infectious doses [$TCID_{50}$]), HPIV3*cp*45 was well-tolerated and highly infectious (>90%) in 1–3 month-old seronegative infants. The vaccine virus was shed for 2–3 weeks. with a mean peak titer of $10^{3.3}$ PFU/ml of nasal wash fluid [47,62]. A second dose of vaccine was administered either 1 or 3 months after the first dose. Vaccinees who received dose two at 3 months after dose one were more likely to shed the vaccine virus and to shed higher titers of the vaccine virus than vaccinees who received dose two 1 month after dose one, indicating that protective immunity induced by the first dose had partially waned by 3 months. No reliable serological correlate of the observed protection could be identified in this youngest cohorr of vacinees. HPIV3-specific IgG responses were infrequent, potentially obscured owing to the presence of maternally-derived HPIV3-specific IgG. HPIV3 HN-specific serum IgA responses were detected in the majority of infants but did not correlate well with protection against replication of a second dose of vaccine [47]. In a separate trial involving 380 children aged 6–18 months that included 226 seronegative infants and children, a single dose of 10^5 PFU ofHPIV3*cp*45 was found to be well-tolerated, safe and immunogenic [63]. No significant difference in the frequency of adverse events (rhinitis, cough, fever or otitis media) was noted during the first 2 weeks after vaccination and 84% of seronegative vaccinees seroconverted to HPIV3, indicating that the vaccine was safe, appropriately attenuated and immunogenic in this age group [63]. Compatibility between live-attenuated RSV and HPIV3 components of an experimental bivalent vaccine was assessed by simultaneous intranasal vaccination with 10^5 PFU each of RSV cold-passaged and temperature sensitive [*cpts*]248/404 and HPIV3*cp*45 in 6–18 month-old seronegative children. In this trial, 92% of vaccinees were infected with HPIV3*cp*45 when the vaccine was given as a monovalem vaccine, whereas 76% were infected following co-administration with the RSV component, suggesting that the replication of *RSVcpts*248/404 might have interfered with that of HPIV3*cp*45. Nonetheless, antibody responses against HPIV3 were similar in subjects receiving monovalent versus bivalent vaccine [64]. The above mentioned trials were performed with biologically-derived virus. Subsequently, the vaccine virus was rederived from cDNA using a reverse genetics system and recombinant (r)HPIV3*cp*45 is the drug substance used in current clinical development efforts. Derivation of the vaccine virus from cDNA provides a preparation with a short, well-defined passage history that minimizes the risk of potential biological contamination of the vaccine seed virus. This technology also enables rederivation of the vaccine virus from cDNA at any time. The clinical development of rHPIV3*cp*45 is conducted in a cooperative research and development agreement between the National Institute of Allergy and Infectious Diseases (NIAID) and MedImmune, LLC. Currently, two Phase I trials are being sponsored by NIAID. The first protocol (ClinicalTrials.gov identifier: NCT00308412) [101] enrolled a total of 45 children 6–36 months of age into two cohorts, both randomized 2:1 to receive two doses of rHPIY3*cp*45 (10^5 $TCID_{50}$) at placebo 4–10 weeks apart. In the first cohort of 24 unscreened infants 6–12 month of age, frequent nasal washes were performed for quantitative virology. In this unscreened cohort, all ten seronegative vaccinees had the vaccine virus detected in nasal washes for approximately 2 weeks, with a mean peak titer of $10^{3.6}$ $TCID_{50}$/ml, whereas only two out of five seropositive vaccinees had the vaccine virus detected for a single day each, with a mean peak titer of $10^{0.9}$ $TCID_{50}$/ml. Only one of the 15 vaccinees shed the vaccine following dose two, again suggesting that a protective immune response restricted replication of the vaccine virus. An additional 21 seronegative children 6–36 months of age were enrolled into this study to expand the safety and immunogenicity data and the findings from the first cohort were confirmed with regard to safety, infectivity and immunogenicity. rHPIV3*cp*45 was found to

be safe, well-tolerated, immunogenic and bioequivalent to the biologically-derived vaccine virus, but an interval of 1–2 months was determined to be insufficient to allow for reinfection and boosting of the immune response. In order to test whether a longer interval between doses would increase the infectivity of a second dose of vaccine, a second NIAID-sponsored study (ClinicalTrials. gov identifiers: NCT01021397 and NCT01254175) [101] is currently enrolling seronegative children 6–36 months of age to evaluate the safety and immunogenicty of two doses of vaccine given 6 months apart. In addition to the above NIAID-sponsored studies. MedImmune has initiated a Phase I study evaluating the safety and immunogenicity of three doses of rHPIV3*cp*45 given 2 months apart (ClinicalTrials.gov identifier: NCT01150799) [101,65].

Bovine PIV3 & bovine/human HPIV3

Bovine PIV3 (BPIV3) and HPIV3 are closely related viruses that have evolved separately in their respective hosts. The two viruses are approximately 25% related antigenically, as determined by reciprocal cross-neutralization [66]. Compared with HPIV3, BPIV3 replication is restricted 100- to 1000-fold in the respiratory tract in rhesus monkeys [66]. BPIV3 was evaluated as a live-attenuated vaccine against HPIV3, a strategy similar to the use of cowpox as a vaccine against smallpox. In Phase I trials in seronegative infants and children, BPIV3 was found to be highly infectious, safe and immunogenic [67]. However, owing to antigenic differences between the BPIV3 and HPIV3 glycoproteins, the geometric mean hemagglutination-inhibiting (HAI) antibody titer in BPIV3 vaccinees was lower against HPIV3 than against BPIV3 and the seroconversion fate against HPIV3 was only 62% [67]. A Phase II trial of BPIV3 was conducted in 1922-month-old infants with four doses of 10^5 $TCID_{50}$ or 10^6 $TCID_{50}$ administered at 2, 4, 6 and 12–15 months of age [68]. With the exception of fever following dose two of the vaccine, the frequency of adverse events was equivalent in vaccinees and in placebo recipients. As in the Phase I study, seroconversion rates were satisfactory against BPIV3 but only modest against HPIV3 [68]. To improve immunogenicity against HPIV3, two independent but similar versions of a cDNA-derived chimeric bovine/human PIV3 virus (rB/HPIV3) were constructed by reverse genetics, one by NIAID and one by MedImmune. These chimeras were engineered to contain the WTHPIV3 HN and F genes in place of the respective BPIV3 genes [69,70]. In rhesus monkeys, rB/HPIV3 was as attenuated as BPIV3 but was more immunogenic against HPIV3 [69]. As expected for a live-attenuated vaccine, rB/HPIV3 was poorly infectious and highly restricted in replication in adults and in seropositive children. Single-dose studies in 6–36 month-old seronegative children are currently ongoing (ClinicalTrials.gov identifier: NCT00366782) [101] and preliminary data suggest that rB/HPIV3 is as attenuated and as infectious as rHPIV3*cp*45. Analysis of safety and immunogenicity data is pending.

Combined HPIV3 & RSV vaccines

The aforementioned chimeric rB/HPIV3 viruses were modified to express either the RSV F protein alone [71,72] or both the G and F proteins [73,74] from additional genes inserted into the B/HPIV3 genome. Each of these constructs is a bivalent vaccine virus expressing the major protective antigens of both RSV and HPIV3. These constructs were developed by MedImmune and NIAID, respectively, The MedImmune construct expressing the RSV F protein (Table 2), referred to as MedImmune, LLC (MEDI)-534, was well-tolerated in 1–9 year old seropositive children [75]. In 6–23 month-old children seronegative for both RSV and HPIV3, 67 and 100% of subjects who received 10^6 $TCID_{50}$ ofMEDI-534 seroconvetted to RSV and HPIV3, respectively [76]. The NIAID construct expressing both RSV F and G has not yet entered clinical trials.

HPIV1 vaccines

The NIAID has explored two approaches to developing a live-attenuated HPIV1 vaccine. Initially, rHPIV3*cp*45 was used as a vector for the HPIV1 F and HN antigens [77–79]. Replacement of the F and HN glycoproteins of rHPIV3*cp*45 with those of HPIV1 yielded a virus that was attenuated in hamsters and offered protection against challenge with WT HPIV1 at 1 month post-vaccination [78,79]. However, the chimeric virus was less immunogenic in HPIV3-immune animals. probably owing to restriction of replication mediated by cellular immunity against the internal HPIV3 proteins. Since infants would probably receive an HPIV3 vaccine prior to HPIV1 vaccination, an HPIV3-based HPIV1 vaccine was deemed suboptimal and this approach was abandoned. The current approach relies on a reverse genetics system for HPIV1 [80] that was used to import attenuating mutations from related viruses (HPIV3, BPIV3 and RSV) into homologous sites of the HPIV1 genome [81–84]. Mutations introduced into the P/C and L genes in several combinations yielded attenuated HPIV1 mutants. Single amino acid substitutions in the C proteins, such as the phenylalanine-to-serine substitution at amino acid residue 170 (F170S), were found to restrict HPIV1 replication of the respiratory tract of hamsters and of African green monkeys (AGMs) [82,84,81]. The F170S mutation in the C protein was subsequently stabilized by deletion of codons 169 and 170 (rHPIV1-$C^{\Delta 170}$) and this mutant was found to be as attenuated as rHPIV1-C^{F170S} in AGMs [84,85]. Another rHPIVl mutant with one substitution each in C and HN, rHPIVI-$C^{R84G}HN^{T553A}$, was also attenuated in AGMs. In addition, an attenuating substitution in the L protein, Y942A, was developed using a codon sequence chosen for stability against reversion. The live-attenuated HPIV1 vaccine that is currently being developed in clinical trials contains the three sets of attenuating elements previously mentioned and is called rHPIV1-$C^{R84G/\Delta 170}HN^{T553A}L^{Y942A}$ [83,86]. This vaccine candidate conferred protection against HPIV1 challenge in AGMs and is highly attenuated in adults and seropositive children [86]. A study in seronegative children is in progress (ClinicalTrials.gov identifier: NCT00641017) [101].

Similar to the use of BPIV3 as a vaccine against HPIV3, murine PIV1 or Sendai virus (SeV), is being evaluated as a live-attenuated xenotropic vaccine against HPIV1. Intranasal administration of SeV was well-toletated in a Phase I study in healthy adults [87] and an open-label Phase I dose-escalation study in children 1 to <6 years of age is currently ongoing (ClinicalTrials.gov identifier: NCT00186927) [101].

Human parainfluenza virus serotype 1 has also been evaluated as a vector for RSV and HMPV glycoproteins [88]. Interestingly, immunization of hamsters with an attenuated RSV vaccine virus, followed by a boost with HPIV1 expressing the RSV F protein, was substantially more immunogenic than two doses of the attenuated RSV strain [PETER COLLINS; UNPUBLISHED DATA]. This is probably because replication of the HPIV1 vector – and the immunogenicity of its expressed RSV F insert – would nor be restricted by prior immunization against RSV. Thus, a live-attenuated HPIV1 or HPIV2 vaccine in which the components are engineered to express the RSV F and G proteins might provide a more potent boost against RSV than a second dose of a live-attenuated RSV vaccine.

HPIV2 vaccines

The development of a live-attenuated HPIV2 vaccine is based on a cDNA-derived full-length HPIV2 [89,90]. In preclinical studies, the HPIV2 L protein was identified as a major target for mutagenesis to develop attenuated mutant HPIVs [82,91,92]. Several HPIV2 mutants were created by importing known arrenuating mutations from other paramyxoviruses into homologous sites in the L protein of HPIV2 [61,93–99]. This strategy effectively identified mutations at three sites in L – at amino acid positions 460, 948 and

1724 – that generated rHPIV2 mutants that were temperature sensitive *in vitro* and attenuated *in vivo* [99,100]. At these three amino acid positions, alternative codons that would require two or three nucleotide changes to revert to the WT amino acid assignment, as well as codon deletions, were employed to increase the genetic stability of these mutations [99]. In addition to the mutations in the L protein, a spontaneous T to C nucleotide substitution within the 3' extragenic leader region of the genome was found to attenuate HPIV2 [100]. An HPIV2 vaccine candidate called rHPIV2–15C/948L/Δ1724 was developed using this 15C leader mutation combined with an amino acid substitution (948L) and deletion (Δ1724) in the L protein. This vitus is highly attenuated in AGMs and provides significant protection against WT HPIV2 challenge [100]. In addition, the vaccine virus was found to be as attenuated in an *in vitro* model of primary human airway epithelium as in AGMs, suggesting that the *in vitro* model is useful for the identification of appropriately attenuated vaccines [32]. The vaccine was also highly attenuated in unscreened adult volunteers and is currently being evaluated in seropositive children (ClinicalTrials.gov identifier: NCT01139437) [100]. Additional HPIV2 mutants have been tested preclinically and are available for clinical development should the current investigational vaccine be unsatisfactorily attenuated. Specifically, viruses with mutations in the V protein that abrogate its inhibition of the type I IFN response and therefore induce a strong antiviral IFN response, were highly attenuated in AGMs [COLLINS P; UNIPUBLISHED DATA]. Some of these mucants might deserve evaluation in clinical trials.

Expert commentary & five-year view

The need for pediatric vaccines that protect against the HPIVs and RSV has long been recognized but progress toward such vaccines has been slow. This was partly owing to the negative consequences of early RSV and HPIV vaccine trials using FI vaccines, resulting in a lack of enthusiasm to test new vaccines, in academia, government and in industry. NIAID and partners in industry have pursued the development of live-attenuated vaccines but struggled to identify vaccine viruses that were immunogenic yet sufficiently attenuated for seronegative infants. Both reactogenicity and immunogenicity correlate with the magnitude of vaccine virus replication in the respiratory tract and the ‘therapeutic window’ for a suitably attenuated yet immunogenic vaccine virus seems quite narrow. However, the availability of reverse genetics systems has allowed a deliberate design of vaccine viruses from cDNA (instead of random mucation during *in vitro* passage) and yielded several promising live-attenuated vaccines that are now in Phase I/II clinical development. Of the three HPIV serotypes targeted. HPIV3 vaccine development is furthesr along and proof-of-concept trials are planned for two of the investigational vaccines, rHPIV3*cp*45 (or MEDI-560) and B/HPIV3–RSV–F (or MEDI-534). The target group for HPIV3 and RSV vaccines is ideally 1–2-month-old infants whereas HPIV1 and HPIV2 vaccines could be given at 6 momhs of age or even later. All of the live-attenuated HPIV (and RSV) vaccines will probably need to be given two or three times and sterilizing immunity cannot be expected. Rather, the goal is to protect against ALRI requiring medical anemion and that goal should be within reach. Apart from St Jude Children Research Hospital's HPIV1 vaccine trials, the NIAID/MedImmune program is currently the only acdve program evaluating HPIV vaccines in children. However, the imerest in HPIV and RSV vaccines seems to be on the rise in industry. Several companies are now pursuing RSV vaccine developmem and several invesrigational vaccines are expected to enter clinical trials within the next few years. This field will certainly benefit from new ideas, and new approaches and competition might encourage a commitmem to develop these vaccines through Phase III to marketing approval. The children most in need, many of them in resource-poor countries and/or without access to supportive care. would certainly deserve them. Although PIV and RSV vaccines will not be on the market within the next 5 years, the hope is to have more than one investigational vaccine in Phase III trials.

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Key issues

- The human parainfluenza viruses (HPIVs) are a common cause of acute upper and lower respiratory illness in infants, young children, the elderly and the immunocompromised.
- HPIV3 is similar to respiratory syncytial virus (RSV) in that it is a common cause of lower respiratory illness, such as bronchiolitiS, in the first year of life.
- HPIV1 and HPIV2 tend to infect later than HPIV3 and are a common cause of croup.
- HPIV1 and HPIV2 vaccines expressing RSV antigens could be used to boost the immune response against RSV.
- Reverse genetics systems (cDNAs) for HPIVs have helped to identify attenuating mutations and to incrementally attenuate HPIVs for use as intranasal vaccines.
- HPIV3 vaccines have passed Phase 1 evaluation and need to be tested in proof-of-concept trials.
- Attenuated HPIV1 and HPIV2 vaccines have entered pediatric trials in Phase I.

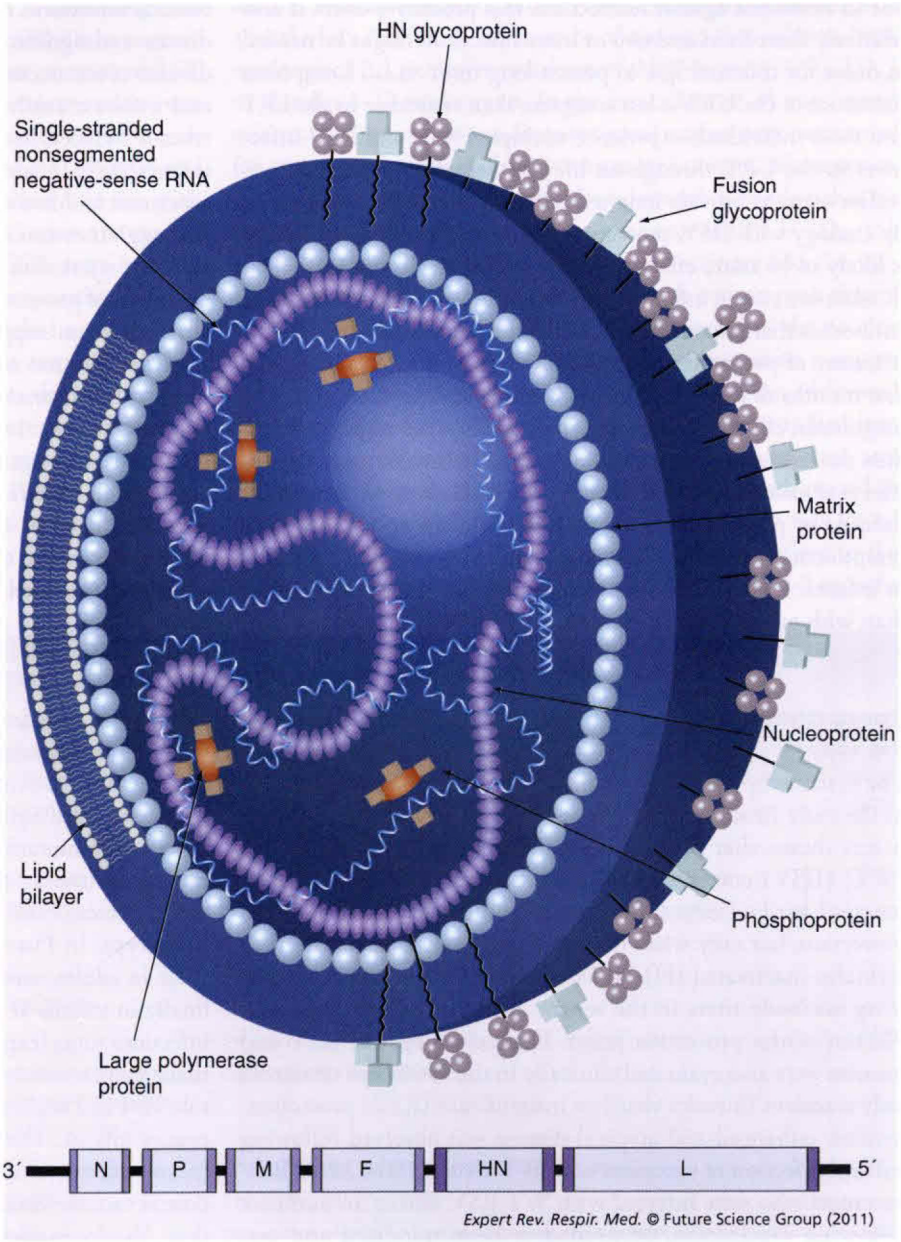

Figure 1. Human parainfluenxa virus virion and genome organization

Parainfluenza virus are enveloped viruses in the paramyxovirus family. Their single-strand negative-sense RNA genomes are approximately 15,500 nucleotides in length and encode six common proteins in the invariant order N-P-M-F-HN-L. The N, P and L proteins form the viral nucleocapsid. The M protein is involved in virion morphogenesis whereas the HN and F proteins mediate adhesion to and fusion with the host cell membrane, respectively. Each gene encodes a single major protein with the exception of the P gene, which, in addition to the P protein, encodes one or more accessory proteins, as indicated.

F: Fusion; HN: Hemagglutinin–neuraminidase; L: Large; M: Matirx; N: Nucleo; **P**: Phospho.

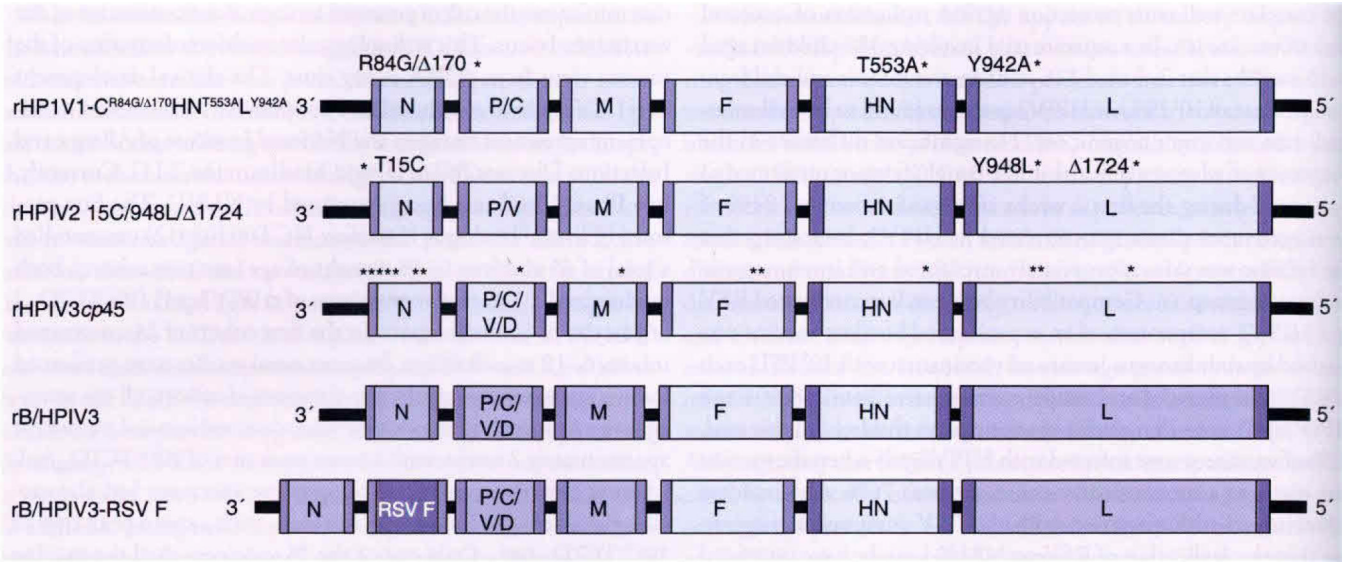

Figure 2. Investigational live-attenuated parainfluenza virus vaccines currently in clinical development

Mutations that were introduced into the wild-type virus genome are indicated by asterisks. For HPIV1 and HPIV2, the type and positions of amino acid substitutions are indicated. The *T15C* mutation is a non coding mutation in the leader sequence of HPIV2. For rHPIV3*cp*45 (also referred to as MEDI-560) only the position of mutations is indicated. For B/HPIV3 and B/HPIV3-RSV F, BPIV3 genes are in light purple and HPIV3 genes are in light blue. In B/HPIV3-RSV F (also referred to as MEDI-534), the HRSV (subgroup A F protein is expressed from an additional open reading frame inserted between BPIV3 N and P).
BPIV3: Bovine parainfluenza viruses; *cp*: Cold-passaged; HPIV: Human parainfluenza virus; HRSV: human respiratory syncytial virus; MEDI: MedImmune. LLC; N: Nucleo; P: Phospho.

Table 1

Selected published human parainfluenza virus 3 vaccine trials.

Vaccine	Age range	Number and HPIV3 serostatus of subjects enrolled	Type of study and main outcome	Ref.
HPIV3*cp*45	4–48 months	80 seronegative	Transmission study. No child fulfilled criteria for vaccine virus transmission	[101]
HPIV3*cp*45 ± RSV cpts-248/404	6–18 months	54 seronegative for both HPIV3 and RSV	Phase I study. Safe and immunogenic. Simultaneous RSV/PIV3 vaccination is feasible	[64]
HPIV3*cp*45	6–18 months	380 total 226 seronegative	Phase II study. Safe and immunogenic	[63]
HPIV3*cp*45	18–50 years 6–59 months 1–2 months	20 adults 24 seropositive children 52 seronegative children 49 unscreened infants	Phase I study. Two doses in the youngest cohort. Safe and immunogenic	[47]
HPIV3*cp*45	6 months–10 years	56 seropositive and 58 seronegative	Phase I study. Safe and immunogenic in seronegative children	[62]
BPIV3	2-<6 months 6–36 months	19 unscreened infants 11 seronegative children	Phase I study. Safe and immunogenic. Lower antibody titers against HPIV3 than against BPIV3	[67]
BPIV3	2 months	192 unscreened infants	Phase II study of four doses of vaccine or placebo. Safe and immunogenic. Lower antibody titers against HPIV3 than against BPIV3	[68]
rB/HPIV3-RSV F (MEDI-534)	1–9 years	120 seropositive	Phase I study. Minimal shedding and immunogenicity, as expected for seropositive children	[75]

BPIV: Bovine parainfluenza virus serotype 3; *HPIV3cp45*: Cold-passaged; cpts: Cold-passaged and temperature sensitive; HPIV3: Human parainfluenza virus serotype 3; MEDI: Medlmmune, LLC; RSV: Respiratory syncytial virus.

Table 2

Recent and ongoing clinical trials of human parainfluenza virus vaccines.

Target	Vaccine	Clincaltrials.gov identifier	Sponsor	Patients (n)	Current serostatus enrolling	Current age group (months)	Doses, n (interval)
HPIV3	rHPIV3*cp*45	NCT00308412	NIAID	24	Unscreened	6–12	2 (1–2 months)
	rHPIV3*cp*45	NCT00308412	NIAID	21	Seronegative	6–36	2 (1–2 months)
	rHPIV3*cp*45	NCT01021397 NCT01254175	NIAID	30	Seronegative	6–36	2 (6 months)
	MEDI-560 (rHPIV3*cp*45)	NCT01150799	MedImmune	30	Unscreened	1–12	3 (2 months)
	rB/HPIV3	NCT00366782	NIAID	21	Seronegative	6–36	1
	MEDI-534 (rB/HPIV3-RSVF	NCT00686075	MedImmune	80	Seronegative	6-<24	3 (2 months)
	MEDI-534 (rB/HPIV3-RSVF)	NCT00493285	MedImmune	80	Seronegative	6-<24	3 (2 months)
HPIV1	$C^{R84G/\Delta170}HN^{T553A}L^{Y942A}$	NCT00641017	NIAID	21	Seronegative	6–36	1
	Sendai virus	NCT00186927	St Jude	18	Seropositive	12–59	1
HPIV2	15C/948L/Δ1724	NCT01139437	NIAID	21	Seropositive	15–59	1

cp: Cold-passage; HPIV: Human parainfluenza virus serotype; MEDI: MedImmune, LLC; NIAID: National Institute of Allergy and Infectious Diseases; rB/HPIV3: Recombinant Bovine/Human PIV3; rHPIV: Recombinant HPIV.