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Treatment of Respiratory Syncytial Virus Infection: Past, Present and Future

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1. Introduction

Respiratory syncytial virus (RSV) has emerged since its isolation from infected children in 1957 as an important respiratory pathogen (Falsey et al, 2005; Hall et al, 2009; Nair et al, 2010; Ruuskanen et al, 2011). Generally, infection is restricted to the upper respiratory tract and not associated with long-term pathology, but progression to a more severe lower respiratory tract infection is frequent.

In developed countries, there are well-defined high-risk groups in whom infection with RSV is more likely to progress into severe acute lower respiratory tract infection (ALRI) (Simoes, 1999) (Fig. 1). Infants that are born prematurely or close to the RSV season and/or suffering from bronchopulmonay dysplasia or congenital heart disease are at the highest risk to develop severe ALRI because of RSV (Feltes et al, 2007; Hall et al, 1986; Weisman, 2003). Additional high-risk groups include immunosuppressed patients or patients with underlying disorders of cellular immunity, individuals living in institutions and the elderly. In developing countries, host-related risk factors are less well defined although some environmental factors like low socioeconomic status, malnutrition, crowded living conditions and indoor smoke pollution likely attribute also to the development of more severe disease. This seems to be reflected in the fact that although age distribution of RSV infection in children in countries with poor socioeconomic status is similar to that seen in developed countries, older children are more severely affected in developing countries (Law et al, 2002; Simoes, 1999; Weber et al, 1998).

Today the virus is considered as the most important virus causing ALRI and a major cause of hospital admissions and death in young children worldwide (Nair et al, 2010; Rudan et al, 2008; Simoes, 1999). More than 90% of the children are infected at least once by the time they reach the age of two. RSV-associated ALRIs in children under five were recently estimated at around 34 million cases globally, accounting for 22% of all ALRIs, with a mortality rate of approximately 3 to 9% (Nair et al, 2010). In addition, a growing body of evidence suggests that RSV infection results in substantial morbidity among adults with underlying chronic illnesses and the elderly (Falsey et al, 2005). Current data indicate that RSV is the causative agent of approximately 3% of all community-acquired pneumonia cases in adults (reviewed in Ruuskanen et al, 2011). Roughly 2-9% of elderly patients admitted to hospital with pneumonia in the USA have infection associated with this virus (Han et al, 1999) and in this age group, mortality linked to RSV infection is substantial (Thompson et al, 2003). Limited research exists on the economic impact of

RSV-associated ALRI among vulnerable patient populations, but the excess first-year healthcare cost per patient in late-preterm infants was calculated to be roughly $17,000 - $22,000 and $2,000 - $4,000 for inpatient and outpatient RSV ALRI, respectively (Palmer et al, 2010; Stewart et al, 2009); resulting in an annual additional healthcare cost exceeding $650 million among children in the US alone (Paramore et al, 2004).

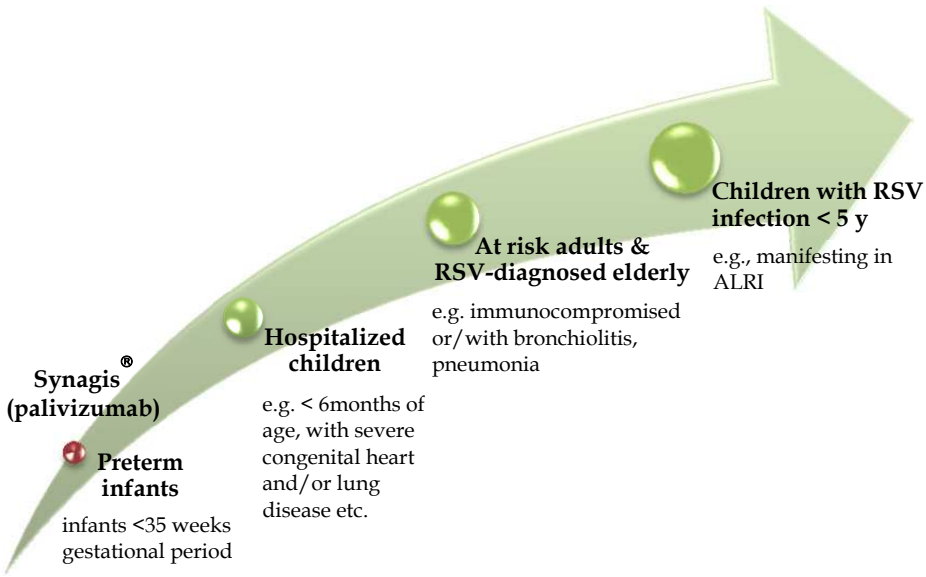

Fig. 1. Stratification of the total respiratory syncytial virus patient population. Groups of RSV patients who may seek specific RSV therapy are indicated by 4 bullets in the arrow, with the relative number of patients in each group increasing in the direction of the arrow. Current therapeutic options are limited to passive immunization of premature infants with palivizumab. Preemies are less than 35 weeks of gestational age and include infants at high risk for developing severe RSV disease. At-risk adults present immunocompromised patients including those with underlying pulmonary or cardiac disease. Elderly patients are more than 65 years of age.

Despite the huge medical and economical burden that is associated with severe RSV infection, no licensed vaccine is available today, and there are only very limited options to treat RSV-associated bronchiolitis (Wainwright, 2009). Chest physiotherapy for acute bronchiolitis in children less than 2 years of age seems not efficacious (Perrotta et al, 2007), although alternative supportive therapy including oxygenation remains a major treatment (Wainwright, 2009; American academy of pediatrics subcommittee on diagnosis and management of bronchiolitis, 2011). Prophylaxis is limited to passive immunization with the humanized monoclonal antibody Synagis®. However, administration of Synagis® is restricted to at-risk infants below the age of two and does not address disease burden in infants with no apparent underlying risk factors. Presently, there is no clear evidence that supports the routine use of ribavirin (Ventre and Randolph, 2007), corticosteroids (Blom et

al, 2007; Ermers et al, 2009; Patel et al, 2004), or bronchodilators (Gadomski and Bhasale, 2006) as mainstays of acute therapy. The usage of ribavirin is limited due to its problematic mode of aerosolic administration, limited efficacy and teratogenicity. Corticosteroids do not seem to improve acute symptoms nor post-bronchiolitic wheezing, while the usage of bronchodilators is not recommended for routine management because of the high-cost, associated adverse events and the questionable efficacy. Clearly, new measures are demanded to decrease the medical burden associated with RSV infection.

2. Pathogenesis and clinical manifestation

RSV infects the upper respiratory tract particularly via the nasopharynx and the eyes, and appears to be spread via large droplets or through fomite contamination. Spread requires either close contact with infected individuals or contact of contaminated hands with nasal or conjunctival mucosa (Collins and Crowe, 2007). The incubation period usually is 3-5 days. RSV infection is associated with a large variety of disease symptoms, many of them related to the age of infection. For instance, neonatal RSV infection is often associated with non-specific symptoms such as failure to thrive, periodic breathing or apnea, and feeding difficulties (Bem et al, 2011), but in older children or adults, nasopharyngeal virus replication leads to rhinitis, cough and sometimes low-grade fever. In cases of severe apnea, mechanical ventilation may be required despite the absence of respiratory failure (Simoes, 1999). Croup also occurs with RSV infection, but especially in susceptible hosts, the virus can spread rapidly to the lower respiratory tract, causing bronchiolitis or pneumonia a few days after the onset of rhinorrhea. Virus likely spreads from the upper to the lower respiratory tract primarily by aspiration of secretions. Some cell-cell fusion may occur, but the rapid kinetics of viral spread suggests that this is not the major route of transmission. The virus is believed to replicate primarily in the superficial layer of the respiratory epithelium and is being shed from the apical surface into the lumen of the respiratory tract (Gardner et al, 1970; Neilson and Yunis, 1990; Zhang et al, 2002). Immunohistochemical sections of airway tissues from infected patients demonstrate a patchy distribution of the infection, with only superficial cells expressing viral antigen. Pathological specimens often demonstrate antigen-positive material in the airway, likely representing sloughing of dead infected cells into the airway. During bronchiolitis, wheeze may be audible on auscultation, and tachypnea and crackles are characteristic (Bem et al, 2011; Simoes, 1999). Air trapping and obstructive atelectasis often result in severe bronchiolitis involving alveolar hypoventilation. As a consequence, RSV-infected infants are at risk for respiratory failure associated with bilateral lung infiltrates, severe bronchospasm, moderate to severe hypoxemia and hypercapnia. In about two thirds of the severe cases bronchiolitis is observed, while in the remainder of cases a restrictive pattern (pneumonia) is observed. Most of the latter patients tend to be younger, have more predisposing underlying disease, and require longer ventilation (Simoes, 1999). In case of interstitial pneumonia, there is widespread inflammation and necrosis of lung parenchyma, and severe lesions of the bronchial and bronchiolar mucosa as well (Aherne et al, 1970). In addition to the acute symptoms, young children may also suffer from delayed sequelae of RSV disease. Recurrent wheezing and airway hyperreactivity have been reported later in childhood (Peebles, 2004; Simoes et al, 2007, 2010), and infant age at the time of initial RSV infection seems to be associated with the potential to develop asthma later in childhood (Sigurs et al, 2005; Wu et al, 2008). In adults, particularly in the elderly, RSV-associated upper or lower respiratory

tract infection may promote exacerbations of asthma and/or chronic obstructive pulmonary disease (Falsey et al, 2005; Falsey, 2007).

3. Disease drivers

Because the pathogenesis of RSV disease is not very well understood, different concepts are prevailing about which are the primary disease drivers. This controversy has major implications for the development of prophylactic or therapeutic strategies for RSV. A first concept places a dominant focus on the host's immune response in causing severe RSV disease (Graham et al, 2002; Openshaw and Tregoning, 2005; Ostler et al, 2002; Pinto et al, 2006). It postulates that RSV disease is a result from an exaggerated Th_2 cellular response with contributions of bystander killing effects caused by cytotoxic T lymphocytes (CTLs) (Aung et al, 2001; Legg et al, 2003). The concept is influenced heavily by the previous experiences with formalin-inactivated RSV vaccines in the 1960s. However, it may be incomplete and may need adjustment. For instance, RSV disease progression is different in different patient groups. While in immunocompetent individuals RSV disease is more characterized by obstruction of the airway, often accompanied by alveolar sparing (Wohl and Chernick, 1978), RSV-related illness in immunocompromised patients presents more as a progressive pneumonia with alveolar infiltrate and fluid, and with less frequent or less degree of wheezing (Englund et al, 1988; Whimbey et al, 1995). In addition, a study evaluating the immune responses in a collection of autopsy specimens from Chilean infants that unfortunately had suffered from a fatal RSV infection, failed to provide evidence for a predicted pathogenic cytotoxic immune response (Welliver et al, 2007). Instead, massive RSV antigen was detected in the lungs accompanied by a lot of apoptotic sloughing of respiratory cells and an absence of cytotoxic T cells. Moreover, deaths were reported at day 4 after onset of disease. This timing essentially eliminates the possibility that an exaggerated immune response would have contributed substantially to the dramatic outcome. Instead of assuming that severe forms of RSV disease are the result of an hyperresponsive immune response, severe disease might be more a consequence of an inadequate response. The study from Welliver et al. (2007) has indicated an important role of the virus to progress RSV disease. In addition, several studies in children and adults have demonstrated a positive correlation between viral load and RSV disease severity. Infant RSV pathogenesis seems to be driven mainly by a rapid and profound viral replication and the ineffectiveness of an adaptive immune response to limit the infection. Moreover, treatment of RSV disease in infants with corticosteroids seems to be ineffectual (Buckingham et al, 2002; Ermers et al, 2009), and a correlation has been demonstrated between the quantity of RSV in the respiratory tract of infants and disease severity (Buckingham et al, 2000; DeVincenzo et al, 2005). A recent study in experimentally infected adults with RSV demonstrated a close correlation between viral load and manifestation of disease symptoms (DeVincenzo et al, 2010). Symptoms appeared near the time of initial virus detection, peaked in severity near the peak of viral load and decreased concomitantly with a decline in viral load. For drug developers and clinicians it is imperative that such aspects of RSV pathology are well studied and understood in all patient populations because they ultimately drive the decision how preventive measures will have the greatest impact on RSV disease and what type of RSV therapeutics will need to be developed. In addition, understanding the disease pathology may also provide insight on whether patients will benefit most from treatment with direct antivirals, immunomodulators or a combination of both.

4. Prevention

Clinicians and health care agencies for many years have been advocating the need for a safe and effective vaccine against RSV (Crowe, 1995; DMID, 2002). Especially infants in their first few months of life are considered the preferred patient population for vaccination (Chang, 2011; Langley and Anderson, 2011; Simoes, 1999). The first clinical trials with a formalin-inactivated vaccine that was immunogenic and showing high rates of seroconversion, were already performed in the 1960s. However, developing a vaccine for very young RSV-naïve infants seems particularly challenging for a number of reasons (Haas, 2009; Murata, 2009; Nokes et al, 2008). Despite the immunogenicity of the formalin-inactivated vaccine, vaccinated children were not protected from subsequent RSV infections. The immature immune system of these infants and the presence of circulating maternal anti-RSV antibodies may attenuate a robust immune response following vaccination (Crowe, 2001; Schmidt, 2007). It is well-recognized now that natural infection and maternally acquired antibodies only induce partial protection against subsequent infections – even in sequential years – and consequently, there have been concerns about the ability of vaccine candidates to induce protective immunity (Langley & Anderson, 2011). Repeated vaccinations may be required because immune protection after natural infection is only limited in time and re-infection with RSV is common in all stages of life. Vaccine strategies therefore need to elicit an immune response which is more robust and prolonged than the immune response against the natural virus. Strain coverage of candidate vaccines may be limited by the genetic variability and the post-translational processing of some of the viral proteins. In addition, RSV-naïve patients who received the formalin-inactivated vaccine, were more likely than placebo recipients to develop severe RSV disease in the lower respiratory tract than when they were naturally infected with RSV later. The mechanism behind this enhanced disease is not completely clear, although failure of the immunogen to elicit an antibody response with effective neutralizing capacity [due to lack of recognition of the immunogen by pattern recognizing receptors like Toll-like receptors (TLRs)] as well as a RSV-specific CD8+ T cell response and induction by the immunogen of an aberrant CD4+ T cell response, is likely to contribute (Delgado et al, 2009; Kapikian et al, 1969; Kim et al, 1969; Murphy and Walsh, 1988). Analysis of lung tissue from two vaccine recipients who later died of RSV infection demonstrated immunopathology not characteristic of naturally occurring RSV lower respiratory tract infection (Neilson and Yunis, 1990). This experience with vaccine-enhanced disease has probably been the main reason why the development of new RSV-vaccines for RSV-naïve infants has been so cautious. The safety profile of vaccine candidates will have to be monitored very closely during development mainly because a RSV vaccine should elicit an appropriate balance between attenuation and immunogenicity and it should not reduce the safety or efficacy of other vaccines that are routinely administered in infants. Effective clearance of the virus may require the induction of a balanced Th_1/Th_2 adaptive immune response, able to promote production of RSV neutralizing antibodies together with an induction of interferon γ-secreting cytotoxic CD8+ T cells (Bueno et al, 2008; Welliver et al, 2007). Therefore, novel vaccine strategies may include addition of TLR-stimulating adjuvants that may help to find a vaccine that elicits an appropriate immune response which is more robust and prolonged than the immune response against the natural infection. To avoid the challenges described above, an alternative vaccine strategy may be to target healthy children between six months and five

years of age or at risk adults in order to promote herd immunity and to eventually indirectly lower the risk of RSV infection in very young infants.

Various strategies have been or are being pursued to develop a safe and effective vaccine against RSV (reviewed in Chang, 2011; Murata, 2009) (Table 1). A major approach is the usage of live-attenuated RSV strains to create a vaccine with the capacity to elicit a protective immune response while avoiding significant disease. Several of such strains, developed using cold passage and/or chemical mutagenesis or reverse genetics, have already been evaluated in clinical trials (Karron et al, 2005; Pringle et al, 1993; Wright et al, 2000, 2006). Vaccination of RSV-naïve infants with cpts248/404, a cold-passaged and mutagenesis-selected live-attenuated RSV strain, resulted in more than 80% infection rate and an approximately 4-fold increase in RSV-specific IgA levels following challenge. The majority of infants that received the vaccine were resistant to infection with a second dose of the vaccine. However, more than 70% of the vaccine recipients developed nasal congestion at the time of peak viral titer, deeming the strain as insufficiently attenuated for use in very young RSV-naïve infants (Wright et al, 2000). This result highlights that finding an appropriate balance between attenuation and immunogenicity is a major challenge for live-attenuated RSV-vaccine candidates. Second-generation live-attenuated RSV strains like rA2cp248/404/1030ΔSH (MEDI-559) and derivatives bearing deletions in the NS2 gene (i.e. rA2cpΔNS2, rA2cp248/404ΔNS2 or rA2cp530/1009ΔNS2) are currently undergoing clinical evaluation in young infants or adults. MEDI-559, now developed by Medimmune, has been demonstrated to elicit an approximately 4-fold increase in anti-RSV antibodies in 44% of previously RSV-naïve patients that received a first vaccine dose (Karron et al, 2005). The results also showed that protective immunity was achieved in a majority of RSV-naïve vaccine recipients. Unfortunately, MEDI-559 shows some genetic and phenotypic instabilities necessitating further improvement of this vaccine candidate (Karron et al, 2005; Murata, 2009).

The immunogenicity and safety of a new nanoemulsion-adjuvanted, inactivated mucosal RSV vaccine in mice was reported recently (Lindell et al, 2011). The water-miscible emulsion droplets (<400 nm size) are believed to inactivate viruses by the physical disruption of the viral envelope. Results showed that the immunization with the vaccine induced durable, RSV-specific humoral responses, both systemically and in the lungs. Vaccinated mice exhibited increased protection against subsequent live viral challenge, which was associated with an enhanced Th_1/Th_{17} response. In these studies, vaccinated mice displayed no evidence of Th_2 mediated immunopotentiation, as has been previously described for other inactivated RSV vaccines. One advantage of this approach may be that these types of vaccines can be kept at ambient temperature before administration. Especially in remote areas of developing countries this could represent a significant logistic benefit. NanoBio, the company that is developing this vaccine candidate announced that a phase I clinical trial is planned.

Initial preclinical studies in rodents immunized with subunit vaccines based on RSV F and G protein have resulted in antibody immune responses and lung pathology comparable to those observed with formalin-inactivated RSV (Murphy et al, 1989, 1990). Because these studies have demonstrated that the use of subunit vaccines may have the potential for disease exacerbation, they have since then been considered inappropriate for the very young RSV-naïve pediatric population. Despite the possibility that the usage of adjuvants (e.g; those recognized by specific TLRs) could improve immunogenicity and

Experimental approach	Company/Institution	Vaccine description	Development stage
Inactivated/ attenuated/ genetically engineered virus	AstraZeneca/MedImmune	MEDI-559	Phase II
	NanoBio/NIH	inactivated virus incorporating a nanoemulsion adjuvant	Preclinical
	Merck	Attenuated strains	Preclinical
	Seattle Children's Research Institute	Inactivated mucosal RSV vaacine, nano-emulsion adjuvated	Preclinical
Subunit-based	Novavax/University of Massachusetts	F-targeting VLP-based	Phase I
	Mymetics/MedImmune	Virosome-based	Preclinical
	ImmunoBiosciences	IPN-1	Preclinical
	TI Pharma	T4-214	Preclinical
	Artificial Cell Technologies	Nanofilm carrier	Preclinical
	iBioPharma	RSV subunits produced by non-engineered plants	Preclinical
	LigoCyte	VLP-based	Preclinical
	TechnoVax	VLP-based	Preclinical
	Pevion Biotech	PEV-4 (virosome-based)	Preclinical
	US Government	G-derived immunogenic peptides	Preclinical
	GlaxoSmithKline/ID Biomedical Corporation of Quebec	F protein polypeptides	Preclinical
	Crucell/Janssen	F protein based	Preclinical
	Mucosis BV	Particles expressing viral antigen and containing *Lactococcus lactis* as adjuvant	Preclinical
	Novartis	F protein derived	Preclinical
	University of Groningen	VLP-based vaccine ± pam3csk4	Preclinical
Vector-based	AstraZeneca/MedImmune	MEDI-534	Phase II
	Bavarian Nordic	MVA-BN RSV (vaccinia-based)	Preclinical
	GenVec/NIAID	Adenovector-based	Preclinical
	AlphaVax	Alphavector-based, producing virus-like replicon particles	Preclinical
	Okairon	Adenovector-based	Preclinical
	Crucell/Janssen	Adenovector-based	Preclinical
DNA-based	Inovio Pharmaceuticals/University of Pennsylvania	IL-12 cytokine gene vaccine adjuvant and DNA vaccine	Preclinical

Table 1. Overview of the different companies/institutions currently running active programs in the development of RSV vaccines and the different vaccine approaches applied. Information was obtained from following sources: Thomson Pharma Partnering, ADIS R&D Insight, Citeline Pipeline, and a variety of public domain sources like scientific literature, patents and press releases.

diminish the adverse effects of protein subunit vaccines, a very cautious approach is being followed in the development of such vaccine candidates. Nevertheless, several subunit vaccines have been evaluated already in the clinic (Table 1). Two preparations were evaluated in phase III clinical trials but were finally discontinued. The first, a purified F protein series (PFP-1, 2 and 3) of vaccines underwent the most extensive clinical evaluation in either RSV-seropositive children (Paradiso et al, 1994; Tristram et al, 1993), children with bronchopulmonary dysplasia (Groothuis et al, 1998) or cystic fibrosis (Piedra et al, 1996, 2003), elderly adults (Falsey and Walsh, 1996, 1997) or pregnant women (Munoz et al, 2003). Although in a majority of the pediatric patients a more than 4-fold increase was observed in RSV-neutralizing titre, the rate of re-infection after vaccination was not different between vaccinees and control subjects. Eventually, the lack of efficacy among the different patient groups resulted in the discontinuation of this vaccine series. BBG2Na, a bacterially expressed protein consisting of an amino acid stretch of subgroup A RSV G protein fused to the albumin-binding domain of streptococcal protein G. This vaccine candidate was discontinued because of some unexpected safety observations in the phase III clinical trial and because of anti-RSV serum antibody titers that dropped significantly within four weeks after vaccination in adults (Power et al, 2001). Currently, several other efforts with subunit vaccines that include the use of F and/or G-derived peptides or virus-like particles (VLPs) are undergoing (pre)clinical evaluation, but limited data are available (Table 1). One exception is a prophylactic VLP-based vaccine candidate from Novavax of which the safety, immunogenicity and tolerability is currently being evaluated in a blinded, placebo-controlled, escalating-dose study in 100 healthy adults between 18 and 49 years old. Novavax recently announced that it expects to report the interim top-line data from this trial in October 2011, and they expect to initiate a phase II trial with their vaccine candidate in the beginning of 2012. In addition, data recently presented on a VLP RSV vaccine that consists of a reconstituted viral envelope with or without incorporated the TLR-2 ligand Pam3CSK4, showed that in the presence of adjuvant, virus-specific serum IgG and IgG2a was increased in mice. Moreover, in cotton rats, the vaccine induced virus neutralizing antibodies and did not predispose for enhanced disease (Stegmann et al, 2010). Some of the peptide-derived vaccines have been struggling with immunogenicity and protective efficacy at least in animal models (Singh et al, 2007a, 2007b; Trudel et al, 1991b). Despite an inefficient neutralizing response, protection against RSV was observed in mice with a RSV G-derived peptide vaccine (Bastien et al, 1999; Trudel et al, 1991a).

Already in the 1990s, different live virus vectors including vaccinia- and adenovirus-derived vectors expressing RSV F and/or G protein, have been designed as vector-based RSV vaccines. Some of these vaccine candidates were abandoned because there was no significant immunogenicity nor protective immunity in primates (Collins et al, 1990; Crowe et al, 1993; de Waal et al, 2004), but others are still actively pursued (Table 1). The current candidates seem to be promising in terms of being sufficiently immunogenic and generating protection against RSV in animal challenge studies, but currently limited clinical evaluation of these vaccine candidates is ongoing. MEDI-534, an intranasal recombinant vaccine which expresses the F proteins of RSV and human parainfluenza 3 together with the hemagglutinin-neuraminidase protein of human PIV-3 in a bovine PIV3 virus genomic backbone, is the only exception, and has been shown to be immunogenic and efficacious in RSV challenge studies in primates (Tang et al, 2004). Recently, results of a phase I trial studying viral take, serum antibody response and safety were encouraging

and at the moment, the vaccine is undergoing phase II clinical evaluation in 2-24 month children (van Bleeck et al, 2011). A big advantage of such chimeric vaccine candidates is that they can be developed as a bivalent RSV/PIV vaccine for the pediatric population (Schmidt et al, 2001, 2002; Tang et al, 2003).

Finally, immunization of the host with DNA plasmids that encode for the RSV F or G protein have also been explored, and experiments in rodents have demonstrated that these plasmids are immunogenic and protective to a certain extent (Bembridge et al, 2000a,b; Kumar et al, 2002; Li et al, 1998, 2000). For instance, Inovio Pharmaceuticals recently announced on their company website that they are developing a vaccine that combines an IL-12 cytokine gene vaccine adjuvant and DNA vaccine technology for multiple indications including RSV. The vaccine technology was licensed from the university of Pennsylvania, and is currently undergoing preclinical evaluation (Table 1). The advantages of using this strategy is that the viral proteins can be expressed in the host in their native conformation in the context of the immature immune system of babies, and therefore may overcome the maternal antibody-associated immunosuppression of anti-RSV response (Murata, 2009). However, this technique is far from mature and several technical challenges need to be overcome before this technology can be applied broadly in the clinical setting.

In summary, the development of a RSV vaccine remains a high priority in view of the high disease burden. Considering past experiences with formalin-inactivated vaccine candidates and a limited understanding of the immunopathology of RSV, the way forward to develop a safe and effective vaccine will be a cautious one, and considering the incomplete immune response against natural infection in very young RSV-naïve babies, it may be that two different vaccines need to developed – one for RSV-naïve infants and another for RSV-infected adults. Many different approaches are currently applied to develop a safe and effective vaccine, although only a limited number of vaccine candidates are actually under clinical evaluation. The two most advanced candidate vaccines are MEDI-559 and MEDI-534, currently in phaseI/II of clinical development. However, approval of the first RSV vaccine is not expected before the end of this decade.

5. Immunoprophylaxis

In 1985, two studies reported the protection of cotton rats and primates against subsequent RSV infection by parenteral administration of RSV-neutralizing antibodies (Hemming et al, 1985; Prince et al, 1985). These studies, together with experiences in vaccine trials, initiated the development of a successful strategy of passive immunoprophylaxis against RSV (Table 2).

Approximately one decade later, the first immunoprophylactic agent – RSV immunoglobulin for intravenous administration (RSV-IVIG) – was approved by the US Food and Drug Administration (FDA). Marketed as RespiGam®, the product was licensed for premature infants and children with/without bronchopulmonary dysplasia, and reduced the RSV hospitalization rate in these patients with 41% (PREVENT study group, 1997). In a second trial, performed in patients with congenital heart disease, only a 31% fewer cases of RSV lower respiratory tract infection were observed in the infused group versus the control group, but specifically in those under 6 months of age, a significant 56% reduction of the number of cases was reported (Simoes et al, 1998). Moreover, administration of RespiGam® was also associated with fewer cases and episodes per child of

otitis media (PREVENT study group, 1997; Simoes et al, 1996). However, the product was difficult to administer and the infusion had a high volume and high protein content. It also had the potential to interfere with other childhood vaccinations because of the presence of antibodies specific for other pathogens. Moreover, it was not recommended for use in children with congenital heart disease because it had been shown that children with cyanotic heart disease are more likely to experience adverse events upon cardiac surgery if they received RespiGam® (Simoes et al, 1998). The drug was taken of the market in 2004.

After the withdrawal from RespiGam®, the use of RSV-IVIG was replaced by the humanized murine monoclonal antibody palivizumab (Synagis®). This drug (approved by the FDA in 1998) is to be used for prophylaxis in those infants most at risk for developing RSV-associated severe ALRI. A multicenter, randomized trial with 15 mg/kg palivizumab given intramuscularly to children every month during the RSV season, resulted in a 55% reduction of laboratory-confirmed RSV hospitalizations (IMpact-RSV study group, 1998; Johnson et al, 1997). Subgroup analysis further demonstrated a significant reduction in hospitalizations of children with (7.9 % vs. 12.8 %, $P = 0.04$) or without (1.8 % vs. 8.1 %, $P < 0.001$) bronchopulmonary dysplasia compared to the control group. In infants born between 32 and 35 weeks of gestational age, the reduction in hospital admissions (80%) was similar between those with and without bronchopulmonary dysplasia. There was a reduction in hospitalization days and the number of intensive care unit (ICU) admissions among individuals that received palivizumab. Palivizumab was not effective in decreasing the incidence of acute otitis media or non-RSV related admissions. In another study, a 45% reduction in hospitalization rate was observed in children with congenital heart disease that received palivizumab versus individuals that received placebo (Feltes et al, 2003). No significant differences were observed in number of deaths, number of ICU admissions, length of stay in ICU, need for mechanical ventilation or length of mechanical ventilation, although it has to be mentioned that the study was not powered to differences in these outcomes. In the clinical trials, the antibody was found safe and well tolerated with less than 3% of antibody recipients showing injection site reactions (IMpact-RSV study group, 1998). Pre- and postlicensure monitoring have not revealed any excess mortality or significant safety problems with palivizumab (Mohan, et al, 2004; Romero, 2003). However, the high cost of the product restricts the use of palivizumab to very young pediatric patients in the developed world, and serious questions are currently being raised about the cost-benefit balance of the product (Hampp et al, 2011; Morris et al, 2009; Smart et al, 2010; Wang et al, 2011). Palivizumab patents will begin to expire from 2015 onwards, and the entrance of biogenerics is expected because of the worldwide market potential. Availability of cheaper biogenerics may represent a game changer, although their potential market impact remains unclear for the moment. For instance, will generic palivizumab ever be cheap enough for use in resource-poor countries?

In an effort to improve immunoprophylactic therapy, next-generation antibodies are being developed and some of them are already progressing through clinical development. A second-generation monoclonal antibody, motavizumab (MedImmune), has completed phase III noninferiority clinical evaluation. Motavizumab is an affinity-matured variant of palivizumab which gives improved protection to the upper respiratory tract and aims to reduce the number of treatment failures that are associated with palivizumab (Wu et al, 2007, 2008). In a randomized, double-blind Phase III trial in at risk infants, the primary endpoint of noninferiority of motavizumab was met by demonstrating a 26% reduction of

hospital admission relative to palivizumab (Carbonell-Estrany et al, 2010). The overall occurance of adverse or serious adverse events did not differ between the 2 groups, but on June 2, 2010, the FDA's Antiviral Drugs Advisory Committee panel voted not to recommend motavizumab for licensure, raising concerns about hypersensitivity issues (allergic skin rash occurring within two days of dosing) as a primary safety alarm during the risk-benefit assessment of motivizumab (Young, 2010). In December last year, MedImmune withdrew its application for licensure of motavizumab and announced that the product will not be further developed for immunoprophylaxis of severe RSV infection. Nevertheless, the antibody has been explored in two randomized, double-blind, placebo-controlled Phase II trials both to evaluate the therapeutic potential in children up to 12 months of age, but no results have been published so far.

ADMA Biologics Inc. has recently evaluated RI-001, a new high-titer RSV-IVIG, in a Phase II trial in immunosuppressed patients with a confirmed upper respiratory RSV infection and at risk for developing severe ALRI. Primary outcome of the study was circulating RI-001 and secondary outcome was the incidence of progression from URTI to LRTI, but no results have been made public.

One other antibody is currently undergoing Phase I clinical evaluation. MEDI-557, a third-generation monoclonal antibody derived from motavizumab, contains a triple mutation known as YTE that extents the half-life of the antibody. A randomised, double-blind phase I study is currently underway in the US to evaluate the safety, tolerability and pharmacokinetics of a single intravenous dose of MEDI-557, although the FDA's decision on motavizumab may impact the development of MEDI-557.

Finally, several antibodies are currently progressed through the preclinical pipeline (Table 2). One of the antibodies, ALX-0171 has recently been shown to reduce RSV viral load in cotton rats, even when administered two days after infection (van Bleeck, 2011). ALX-0171 was also found superior to palivizumab in a plaque-reduction assay of a panel of 51 out of 61 recent clinical RSV isolates. The molecule is a trivalent anti-RSV nanobody consisting of three identical epitopes, based on the smallest functional fragments of heavy chain-only antibodies found in camels and llamas. Ablynx announced on their company website that the program is on track to enter Phase I clinical trials in healthy volunteers during the course of 2011. Evaluation of ALX-0171 as a therapeutic agent may reveal the efficiency of this novel approach.

In summary, many different programs are ongoing to develop new immunoprophylactic agents. Palivizumab today remains the only available agent to prevent severe RSV infection, and although it has been shown to significantly reduce the hospitalization rate in high-risk pediatric patients, more data are needed to demonstrate whether immunoprophylaxis could have a significant impact also on outcomes like death rate, need for mechanical ventilation or reduction of long-term symptomology associated with RSV, like long-term wheezing or asthma development. Moreover, palivizumab treatment is expensive and economic analyses suggest that the use of palivizumab is only cost effective in the highest risk children. For these reasons palivizumab is not broadly available to the general RSV population. The major challenge for the immunoprophylactic agents will be to use them in a cost-effective and judicious manner. The potential appearance of biogenerics or cheaper production processes may present as potential game changers.

Company/Institution	Antibody description	Development stage
AstraZeneca/MedImmune	RespiGam® (RSV-IVIG)	Launched
AstraZeneca/MedImmune	Synagis® (palivizumab)	Launched
ADMA Biologics	RI-001	Phase II
AstraZeneca/MedImmune	MEDI-557	Phase I
Ablynx	ALX-0171®	Preclinical
AstraZeneca/MedImmune	F protein monoclonal antibodies	Preclinical
Symphogen	Polyclonal antibodies against F and G protein	Preclinical
Symphogen	Sym-003 (fully humanized Mab)	Preclinical
Medarex/MedImmune	Mabs using HuMab-Mouse technology	Preclinical
Intracel	HumaRESP (fully humanized Mab)	Preclinical
Kenta Biotech	KBRV-201 (series of human Mabs)	Preclinical
Bioresponse	Polyclonal antibodies against F and G	Preclinical

Table 2. Overview of the different companies/institutions currently active in the development of RSV immunoprophylactic therapy and the different approaches applied.

6. Treatment

Ribavirin (Virazole®) was approved in 1986 in the US for treatment of RSV infection, and is currently the only approved agent for this indication (Ventre & Randolph, 2007). Ribavirin is a nucleoside analog that seems to act by mutagenic incorporation into the viral genome, although the mechanism of action is not fully known. The drug is administered as a small-particle aerosol, 6-18 hrs daily for a period of 3-7 days. Although initial clinical studies in a limited number of subjects indicated a modest beneficial effect on viral load and clinical symptoms, follow-on studies only provided questionable evidence of benefit upon treatment of severe infections (Hall et al, 1983; Ventre & Randolph, 2007). In addition, potential for toxic effects in health care workers (Virazole package insert), and high cost (Glanville et al, 2005) have led to a situation in which ribavirin is not routinely used by most centers to treat RSV in otherwise healthy children. Currently, the American Academy of Pediatrics does not generally recommend ribavirin treatment for RSV infections (American Academy of Pediatrics, 2006).

RSV therapy will likely extend to patient groups other than at risk infants over the next decade (e.g. the at risk adults or children suffering from an acute RSV-confirmed upper respiratory tract infection to prevent severe ALRI), not only because therapeutic options with antibodies may expand beyond the very young infants, but also because of ongoing development programs of small-molecules and small interfering RNA (siRNA) agents that may be more suitable for treating patient groups not eligible for prophylactic antibody therapy (Roymans and Koul, 2010).

6.1 Antivirals

6.1.1 N-protein inhibitors

Two investigational agents targeting the nucleocapsid (N) protein, critical to viral replication of RSV, are currently in Phase II clinical evaluation (Table 3). ALN-RSV01 (Alnylam), a siRNA with the potential to be a new class of drugs to treat human disease, and the small molecule RSV-604 (Arrow Therapeutics/Novartis). In a first randomized, placebo-controlled Phase IIa trial, intranasally administered ALN-RSV01 was evaluated in healthy adults challenged with RSV (DeVincenzo et al, 2010). Patients were treated once daily for two days before and three days after RSV inoculation with a nasal spray containing either ALN-RSV01 or placebo, resulting in an approximately 38% decrease in the number of infections detected by quantitative culture of patients that received the investigational agent as compared to patients receiving placebo. In a second randomized, placebo-controlled Phase IIb trial, the safety and efficacy of the product was evaluated in lung transplant patients with a confirmed RSV infection (Zamora et al, 2011). ALN-RSV01 was reported to be well tolerated, with no drug-related serious adverse events or post-inhalation perturbations in lung function. Despite the observation that viral AUC on days 0 to 6

Company/Institution	Therapeutic description	Development stage
Valeant/Shering Plough	Virazole® – Rebetol® (ribavirin)	Launched
Alnylam/Kyowa	ALN-RSV01	Phase II
Alnylam/Kyowa/cubist	ALN-RSV second generation	Phase II
Arrow Therapeutics/Novartis	RSV-604	Phase II
Clarassance	CG-100	Phase II
Microdose Therapeutx	MDT-637 (VP-14637)	Phase I
AstraZeneca/MedImmune	motavizumab	Phase I
Alnylam	Intranasally administered siRNA molecules	Preclinical
Sirnaomics	STP-92 (siRNA delivered through nanoparticle based delivery systems)	Preclinical
Inhibikase	iKT-041 (small molecule RSV inhibitor)	Preclinical
Biota/MedImmune	Second generation small-molecule F inhibitors	Preclinical
Shared Research/University of Tokyo/Todai TLO	siRNA delivered by cationic polyamino acids	Preclinical
Chimerix	Small-molecule nucleoside analogs	Preclinical
AstraZeneca/Trellis Bioscience	Monoclonal antibodies	Preclinical
Mapp Biopharmaceutical	Monoclonal antibodies	Preclinical
AIMM Therapeutics	Monoclonal antibodies	Preclinical

Table 3. Overview of the different companies/institutions currently active in the development of RSV therapeutics and the different approaches applied.

trended lower in the ALN-RSV01 group, no statistically significant antiviral effect could be shown. Interpretation of the viral load data was reported to be confounded by baseline differences between the two groups and by time from symptom onset to first dose (Zamora et al, 2011). However, mean daily symptom scores were lower in individuals that received ALN-RSV01, and the mean cumulative daily total symptom score over a time period of 14 days was significantly lower with ALN-RSV01 (114.7 ± 63.13 vs. 189.3 ± 99.59, P = 0.035). The rate of new or progressive bronchiolitis obliterans syndrome (BOS) was significantly lower (6.3 % vs. 50 %, P = 0.027) in patients who received the study drug relative to the control group, raising the possibility that ALN-RSV01 may be able to lower the risk of late serious clinical sequelae associated with RSV infection like BOS or morbidity and mortality that originate from loss of lung function. Alnylam is also developing second-generation siRNA molecules against RSV. It is expected that a phase IIb pediatric study with the second-generation molecule will start soon.

RSV-604 (Arrow Therapeutics), an oral benzodiazepine with submicromolar potency against RSV A and B subfamilies (Chapman et al, 2007), is being developed in collaboration with Novartis. The safety and efficacy of this compound was tested in a multi-center trial that included RSV-infected adult bone marrow transplant patients. Although initiated in 2005, the study was only completed in the beginning of this year and data from the trial are still expected to be released. In addition, RSV-604 is also undergoing Phase I evaluation with pediatric formulations.

6.1.2 Entry inhibitors

Another interesting strategy to tackle RSV infection is to disturb the virus entry process. Enveloped viruses like RSV, HIV-1 or influenza virus need to attach and fuse with a host cell in order to deposit their genome and to initiate their replication cycle (Lamb and Jardetzky, 2007). In RSV, attachment and fusion are facilitated by the attachment (G) and fusion (F) protein, although it has been shown that the F protein is sufficient to lead to productive viral replication (Karron et al, 1997; Techaarpornkul et al, 2001). It is thought that either G and/or F bind to a specific host cell receptor in order to initiate the entry process, but how exactly this happens remains largely unknown. Binding to glycosaminoglycans containing heparin sulphate has been reported as a potential receptor candidate since addition of heparin blocks virus attachment in vitro (Hallak et al, 2000; Krusat and Streckert, 1997). Recently, evidence was reported strongly suggesting that nucleolin may be a cellular receptor for RSV (Tayyari et al, 2011; van Bleeck et al, 2010). The results were validated via several different strategies. It was shown for instance that nucleolin antibody neutralization and RNAi knock-down of nucleolin was able to inhibit RSV infection, turning nucleolin into a potential target for development of anti-RSV therapeutics (Tayyari et al, 2011).

MBX 300 (NMSO-3), a sulphated sialyl lipid, has been under development by Microbiotix as an attachment inhibitor since it seems to target the G protein (Table 3). The compound has an EC_{50} of approximately 0.2-0.3 μM and appears to be a specific inhibitor for RSV (Douglas, 2004; Kimura et al, 2000). In cotton rats, lung viral titers were reduced significantly when animals were treated intraperitoneally with a daily dose of 100 mg/kg/day (Douglas, 2004). MBX 300 was reported by Microbiotix to display potent oral anti-RSV efficacy and safety in preclinical evaluation, including primates, but no further development activities were announced recently.

The market approval of palivizumab has validated F as a clinically relevant target. Since then, drug research has been focusing on this protein as a target for developing next-generation therapeutics including antibodies and small-molecule inhibitors (Bonfanti and Roymans, 2009; Roymans and Koul, 2010). The availability of structural information on how small-molecules can inhibit the virus-host fusion process has helped in understanding this process better and also helped in identifying potential drug binding pockets in the F protein (Cianci et al, 2004b; Roymans et al, 2010).

RSV-IVIG and palivizumab have also been clinically evaluated as potential therapeutic agents, but the studies have resulted in a mixed outcome. A first randomized, double-blind, placebo-controlled trial with RSV-IVIG in previously healthy children less than 2 years of age hospitalized with a proven RSV lower tract infection, reported some beneficial effect of RSV-IVIG treatment in a subgroup of children with more severe disease, but no evidence was found in the total study cohort for reduced hospitalization or reduced ICU stay when patients were treated with RSV-IVIG compared to placebo recipients (Rodriguez et al, 1997a). A second trial with RSV-IVIG in hospitalized children younger than 2 years at high risk for severe RSV infections did not show any efficacy (Rodriguez et al, 1997b). In addition, two randomized, double-blind, placebo-controlled multicenter trials in previously healthy children less than 2 years of age hospitalized with acute RSV infection demonstrated no difference in disease severity or clinical outcome between patients treated with a single intravenous dose of palivizumab or placebo, albeit that a reduction in tracheal viral load was observed in one study (Malley et al, 1998; Sáez-Llorens et al, 2004).

Despite these mixed outcomes , some programs aim to maximize the therapeutic potential of antibodies to treat RSV (Table 3). Companies like AIMM Therapeutics, Symphogen, Trellis Bioscience or Mapp Biopharmaceutical in collaboration with Vanderbilt University are developing monoclonal antibodies targeting the envelope proteins of RSV. Especially when extracellular (viral) proteins are targeted, therapeutic use of antibodies can be envisioned to limit the infection, provided the antibody is properly delivered in the lung.

In 2003, a very potent series against RSV of substituted benzimidazoles was reported by Johnson & Johnson (Andries et al, 2003). Exemplified by JNJ-2408068, selected as a candidate for clinical evaluation, the series was putatively shown to target the F protein on the basis of in vitro selection of resistance associated mutations and time-of-addition experiments. JNJ-2408068 has an EC_{50} = 0.16 nM and SI > 625 x 10^3, and was demonstrated to be active against both A and B sybtypes of RSV as well as a panel of clinical isolates. Although the compound reduced lung viral titers in cotton rats to undetectable levels when administered by aerosol shortly prior to or after virus challenge (Wyde et al, 2003), it showed an unfavorable PK profile in terms of tissue retention (Bonfanti et al, 2007). The aminoethylpiperidine moiety was identified as being responsible for the long elimination half-life from several tissues. The subsequent back-up program was focused on the modulation of this basic part of the molecule. The objectives were to reduce the elimination half-life from tissues while keeping the high activity level. This lead optimization program resulted in the discovery of TMC353121 as a clinical candidate (Bonfanti et al, 2008). The in vivo efficacy of this new antiviral was demonstrated in the cotton rat model by different routes of administration: inhalation (1.25 mg/mL solution in the reservoir of the nebulizer) and IV (10 mg/kg dose) lead to >90% inhibition versus control, and oral (40 mg/kg dose) lead to > 80% inhibition versus control. Recent PK-PD modeling of this compound indicated that in order to reach

50% inhibition of the viral load in the cotton rat, a plasma concentration of 200 ng/ml is required, a concentration much higher than the 50% inhibitory concentration observed in vitro in HeLaM cells (0.07 ng/ml)(Rouan et al, 2010). In addition, it was shown in a RSV infectious mouse model that TMC353121 can be used either prophylactically or therapeutically to decrease RSV lung infection and virus-associated lung inflammation and histopathology (Olszewska et al, 2011). Single-dose intravenous administration up to 48 hours after infection reduced the RSV viral load with 1 to 2 $\log_{10}$ at the peak of infection (day 4 post-RSV inoculation in the mouse model), indicating that the window between onset of infection and onset of treatment has a practical utility for RSV infections in the clinical setting (Hegele, 2011). Moreover, investigation of lung histopathology, measurement of inflammatory cells and chemical mediators in bronchoalveolar lavage fluids also demonstrated that host immune and inflammatory responses were attenuated significantly.

A series of benzotriazole benzimidazoles was reported by researchers from Brystol-Myers Squibb as a class of inhibitors that prevent fusion of RSV with the host cell membrane (Meanwell and Krystal, 2007). BMS433771, an azabenzimidazolone derivative, binds to a hydrophobic pocket situated in the central trimeric coiled-coil formed upon refolding of F after initiation of the viral fusion process (Cianci et al, 2004b). The compound was shown to be active against multiple strains and clinical isolates from both A and B subfamilies of RSV with an EC_{50} of approximately 20 nM (Cianci et al, 2004c). In addition, rodent models were used to assess the in vivo activity of BMS-433771 after oral administration. The maximum effect observed was > 1 $\log_{10}$ reduction in viral load at doses of ≥ 5mg/kg in BALB/c mice and ≥ 50 mg/kg in cotton rats (Cianci et al, 2004a).

Wyeth-Ayerst discovered RFI-641, a biphenyl triazine cationic compound that inhibits both A and B subfamilies of RSV with EC_{50} values around 20 nM and with 417 < SI < 2500 (Huntley et al, 2002; Nikitenko et al, 2001). The compound is an analog of CL-309623, a previously identified dendrimer-like stilbene-containing inhibitor with anti-RSV activity (Gazumyan et al, 2000). RFI-641 was shown to inhibit fusion and to interact directly with the RSV F protein (Razinkov et al, 2002). The in vivo potency of RFI-641 was evaluated in different animal model systems (Huntley et al, 2002; Weiss et al, 2003). RFI-641 administered intravenously did not exhibited efficacy in vivo, and therefore, this route of administration was not pursued. However, 1.3 mg/kg intranasally delivered compound 2h prior virus challenge in mice reduced virus lung titers by 1.5 $\log_{10}$, and in cotton rats a dose of 10 mg/kg reduced the lung viral titer by 2.6 to 3.2 $\log_{10}$ when given at a same prophylactic dosing regimen. In the African Green Monkey model, RFI-641-treated monkeys exhibited a 3.4 $\log_{10}$ reduction in viral load. At the 6 mg dose, viral titers were significantly lower on days 4 to 9 (peak viral load) with a 1.8 to 3.4 $\log_{10}$ reduction. In addition, daily therapeutic intranasal administration of RFI-641, initiated 24 h after RSV infection in monkeys, also reduced viral titers.

In 2005, Biota Holdings Ltd. published a patent claiming the discovery of imidazoisoindolone derivatives as a new class of fusion inhibitors of RSV (Bond et al, 2005). BTA9881, was selected as a clinical candidate. The compound is orally bioavailable and has been shown to demonstrate favorable pharmacokinetics in phase I clinical trials (Bond, 2007). However, the clinical development of BTA9881 was stopped because the compound failed to develop an acceptable safety profile. Rights to the compound have been returned by AstraZeneca to Biota, which will attempt to develop more attractive derivatives. In this respect, Biota announced that they discovered a new series of orally bioavailable RSV fusion

inhibitors and they recently published a patent application claiming a new series of fused imidazopyrazinones as RSV fusion inhibitors, with the most potent compounds having a single digit nM in vitro antiviral activity (Mitchell et al, 2011).

Multiple compounds have been in development as a specific RSV fusion inhibitor. However, the attrition rate during pharmaceutical development has been high, and none of the above described compounds are in clinical development today; either as a result of strategic decisions taken by the developing companies or because of unfavorable pharmaceutical properties of the compounds. The only small-molecule fusion inhibitor under clinical evaluation today is MDT-637, developed by MicroDose Therapeutx. MDT-637 was previously reffered to as VP-14637, a bis-tetrazole-benzhydrylphenol derivative, with an EC_{50} = 0.001 μM against RSV in vitro (Douglas et al, 2003). In addition, cotton rats that received as little as 126 μg drug/kg by divided-dose aerosol starting 1 day after viral challenge with either a RSV A or B subtype had significantly lower mean pulmonary RSV titers and reduced histopathology scores than control animals (Wyde et al, 2005). VP-14637 was in Phase I trials prior to a decision from ViroPharma not to develop it further as an aerosol, partly because of excessive organic solvent content of the aerosol, partly because of strategic reasons. In 2009, MicroDose Therapeutx acquired the assets from ViroPharma and they re-formulated MDT-637 as a dry powder for inhalation. Preclinical results have demonstrated that the product can be effectively delivered in both the upper and lower respiratory tract (van Bleeck, 2010), and a Phase I trial assessing the safety, tolerability and pharmacokinetic profile of MDT-637 in 48 healthy adult volunteers is initiated.

6.1.3 RNA-dependant RNA polymerase inhibitors

Two different classes of compounds that target the polymerase complex of RSV have been reported. Screening of a large chemical library resulted in the discovery of benzazepines as inhibitors of RSV (Sudo et al, 2005). YM-53403 was selected as an interesting candidate, and exhibited an EC_{50} = 0.2 μM in a RSV plaque reduction assay. Time-of-addition experiments suggested inhibition to be maximal around 8 h after virus exposure and mutant viruses with single point mutations in the polymerase (L) protein were resistant to inhibition with YM-53403, indicating the compound to be an inhibitor of the RSV L protein. The discovery of a second inhibitor of RSV polymerase through screening of a chemical library with a poly(A) capture assay was reported in 2004 by researchers from Boehringer Ingelheim (Mason et al, 2004). Shortly after, a series of imidazo[4,5-h]isoquinoline-7,9-dione inhibitors were synthesized that target the 5′ capping of viral mRNA transcripts (Liuzzi et al, 2005). The most potent compound, compound D, exhibited an antiviral EC_{50} = 0.021 μM (polymerase IC_{50} = 0.089 μM) with a SI ≤ 400. Lung virus titers in a mouse model were reduced upon intranasal administration of the compounds 3 and 6h post-viral challenge, and then three times daily for 3 days at 0.4-4.1 mk/kg/day. Today none of these compounds are actively developed as a therapeutic.

6.2 Bronchodilators

RSV bronchiolitis resembles asthma in that both conditions cause air trapping and wheezing because of increased airway resistance. Therefore, several drugs that are being used to treat reversible airway smooth muscle constriction in asthma have also been tested in children with RSV infection. However, multiple clinical trials suggest that treatment with β2-

adrenoreceptor agonists like albuterol, only offer a modest short-term improvement (Gadomski and Bhasale, 2010). Racemic epinephrine treatment has been shown to relieve some respiratory distress, but no effect on length of hospitalization could be demonstrated (Langley et al, 2005). Epinephrine stimulates both α- and β-adrenergic receptors, but it is thought that its α-adrenoreceptor stimulation of the sympathetic nervous system could be expected to reduce mucosal edema and to increase airway caliber (Barr et al, 2000). However, a recent study could not demonstrate a difference in effectivity between high volume normal saline alone and nebulized salbutamol-normal saline, epinephrine-normal saline, or 3% saline in mild bronchiolitis (Anil et al, 2010). The reason for the observed discrepancies in the clinical trial results may be that RSV bronchiolitis is caused by a fixed high resistance of the bronchioles due to their small size - potentially further facilitated by sloughed cells and high mucous content - rather than it being caused by reversible smooth muscle constriction, but another reason for the persisting controversy about bronchodilator responsiveness in bronchiolitis could be the lack of sufficiently sensitive methods for assessing lung function in young children (Modl et al, 2000, 2005). A thorough investigation of such factors may help to settle this controversy, and consequently may contribute on how to properly treat RSV infection.

6.3 Anti-inflammatory agents

After adverse responses to formalin-inactivated RSV vaccination were reported, a believe derived that RSV disease following infection is driven by an exuberant pathogenic immune response (Aung et al, 2001; Kapikian et al, 1969; Kim et al, 1969; Legg et al, 2003). Attempts have been made to develop anti-inflammatory therapy to reduce RSV respiratory distress. Although corticosteroids are commonly used by physicians as anti-inflammatory therapy, a review of more than a dozen clinical trials in both outpatient and hospitalized settings indicated that the drug is of no benefit on its own (Patel et al, 2004). Systemic administration of prednisolone and topical application of fluticasone, budesonide, and deoxiribonuclease I were ineffective (Bulow et al, 1999; Cade et al, 2000; Nasr et al, 2001; Wong et al, 2000). Intravenous dexamethasone had little effect, although when the drug was received by inhalation, patients hospital stay seemed to be reduced (Bentur et al, 2005; Buckingham et al, 2002). Recent data however illustrate that, at least in children, severe forms of RSV disease are related to inadequate rather than hyperresponsive immune reactions (DeVincenzo, 2007; Welliver et al, 2007; Welliver, 2008). Since these compounds only have an effect on the hyper-inflammatory response and not on the virus directly, a more effective treatment might be the combination of an antiviral and an anti-inflammatory compound. A combination of palivizumab and a glucocorticosteroid significantly reduced the RSV viral load in lungs from cotton rats over 3 $\log_{10}$ and reduced pulmonary histopathology significantly (Prince et al, 2000).

A clinical trial reported in 2003 with montelukast, a selective and competitive antagonist of cysteinyl leukotrienes, indicated a possible general reduction of lung symptoms and an improvement in persistent wheezing after RSV-induced bronchiolitis (Bisgaard, 2003). Although cysteinyl leukotrienes seem to contribute to the pathophysiology of RSV-induced bronchiolitis and thus may represent an interesting therapeutic target (Dimova-Yaneva et al, 2004; Oh et al, 2005; Wedde-Beer et al, 2002), the trial results were confounded by the included patient population and conclusions made on improvement of post-bronchiolitis reactive airway disease considering the 4 week therapy window (López-Andreu et al, 2004; Szefler and Simoes, 2003). As the patient population included both very young infants and

children up to 36 months of age, the study effects may have been biased towards effects in the older children; i.e. children that did experience RSV infection in the 1 or 2 previous seasons, and at the time of study, presented with post-RSV reactive airway disease that responds to montelukast (Stein et al, 1999; Szefler and Simoes, 2003). Unfortunately, results from 3 prospective randomized, double-blind, placebo-controlled follow-on trials in children less than 24 months of age were not consistent, leaving a potential benefit of montelukast treatment on post-RSV bronchiolitis symptoms unclear. A first study in 979 patients randomized to placebo or to montelukast treatment at 4 or 8 mg/day for 4 or 20 weeks did not show a significant difference in the percentage of symptom-free days (SFD) over a treatment period of 4 weeks (Bisgaard et al, 2008). In both groups, % SFD was approximately 29%. Post hoc analyses of patients with persistent symptoms (SFD ≤ 30% over weeks 1-2) resulted in slight differences in % SDF over weeks 3 to 24 of 5.7 to 5.9 for montelukast (4 or 8 mg/day, respectively) versus placebo groups. A second study in 58 pediatric patients hospitalized with a first episode of RSV bronchiolitis seemed to confirm the conclusions of the previous study, and concluded that montelukast treatment did not reduce symptoms of cough and wheeze (Proesmans et al, 2009). During the three month trial period, no differences between the treatment and control groups were observed for symptom-free days and nights, and during the one year follow-up, no significant differences were observed in the number of exacerbations or time to first exacerbations, number of unscheduled visits and need to start inhaled steroids. However, a third study performed in 200 children treated with or without 4 mg/day of montelukast for 3 months showed a significantly decreased level of serum eosinophil-derived neurotoxin in treated patients versus placebo controlled patients that remained significantly different for the entire 12-month follow-up period. In addition, cumulative recurrent wheezing episodes at 12 months were significantly lower in the montelukast-treated group (Kim et al, 2010).

Leflunomide (Arava®, Aventis Pharmaceuticals) (Table 4), an immunosuppressive agent approved for treatment of patients with rheumatoid arthritis and currently evaluated in Phase I clinical trials in transplant patients (Williams et al, 2002), has previously been shown to exert a powerful antiviral activity against several viruses like cytomegalovirus and herpes simplex virus (Chong et al, 2006; Knight et al, 2001; Waldman et al, 1999). Recently, it has been demonstrated that the A77 active metabolite of leflunomide dose-dependently inhibits RSV in cell cultures and in vivo (Dunn et al, 2011). Pulmonary viral load in RSV-inoculated cotton rats was reduced > 3 $\log_{10}$ in leflunomide-treated animals versus controls, even when treatment was delayed until day 3 post-challenge.

CG-100 is an intratracheal formulation of recombinant human CC10 (Clara cell 10 kD protein; uteroglobin) under development by Clarassance for the treatment of severe RSV infections and other respiratory indications (Table 4). CC10 protein has several clinical applications in inflammatory, fibrotic, and autoimmune diseases, particularly respiratory diseases. More recently, CC10 has been found to reduce viral titers for influenza and RSV. Two Phase 1/2 clinical trials have been completed with CC10. The first trial was conducted in premature infants with respiratory distress syndrome who were at risk of developing bronchopulmonary dysplasia. These patients are deficient in their own CC10 supply due to extreme prematurity and underdevelopment of the lungs. In this study, CC10 was safe and well-tolerated and demonstrated powerful anti-inflammatory effects in the short term and profound protective effects in keeping infants out of the hospital for respiratory causes over the long term (6 to 12 months after a single dose). The second trial was performed in healthy

adult volunteers with seasonal allergies. CC10 was also safe and well tolerated in these patients. Furthermore, no drug interactions between CC10 and other drugs such as antibiotics, diuretics, decongestants, antihistamines were observed in either trial.

Synairgen and the Jewesh Children Hospital are applying other strategies to tackle RSV infection (Table 4). Synairgen is developing SYN-11, an inhalable interferon beta, in an effort to improve the innate immune respons against RSV. The Jewesh Children Hospital has a program in preclinical development evaluating the efficacy of POPG, palmitoyl-oleoyl-phosphatidylglycerol, one of several lipids in the fluid that lines the air sacs of the lungs. POPG, together with other lipids and proteins in the surfactant fluid, is known to prevent collapse of the air sacs and to contribute to innate immunity. Gilead is developing small-molecule TLR7 agonists with broad-spectrum antiviral activity including RSV. Interferon-alpha production in human peripheral blood mononuclear cells could be induced with a minimum effective concentration of 1 nM.

Company/Institution	Anti-inflammatory/broad-spectrum description	Development stage
Functional Genetics	FGI-101-1A6	Phase I
Respivert/Centocor Ortho Biotech	RV 568	Phase I
Clarassance	CG-100 (Clara cell 10 kD protein or uteroglobin)	Phase I
Synairgen	SYN-11 (inhaled interferon beta)	Preclinical
Jewesh Children Hospital	POPG (palmitoyl-oleoyl-phosphatidylglycerol)	Preclinical
Functional Genetics	FGI-110 (an anti-Nedd4 monoclonal antibody)	Preclinical
Functional Genetics	FGI-103723 (small-molecule caspase 2 inhibitor)	Preclinical
Functional Genetics	FGI-102100 (small-molecule TSG101 inhibitor)	Preclinical
Gilead	Small-molecule TLR7 agonists	Preclinical
Summit	Imino sugars for treatment of CMV, HSV, RSV & VZV	Preclinical
Ohio State University Medical Center	Leflunomide (Arava®, Aventis Pharmaceuticals)	Preclinical
Pathfinder Pharmaceuticals	Compounds restoring host cell defense	Preclinical
Kineta	rOAS-1 (natural human protein for treatment of HCV, RSV & influenza A)	Preclinical
Emory University	JMN3-003 (small-molecule RdRp inhibitor against influenza A, RSV, PIV-3, …	Preclinical

Table 4. Overview of the different companies/institutions currently active in the development of anti-inflammatory compounds against RSV and broad-spectrum antivirals, and the different approaches applied.

In summary, clinical trial results with anti-inflammatory agents are somewhat conflicting but have up to now resulted in modest short-term benefits at best. However, studies with new anti-inflammatory compounds show promise as a potential treatment for RSV disease (Table 4). It will be interesting to see whether anti-inflammatory compounds have the potential as a single drug solution or whether they are most effective in a combination therapy with antiviral compounds.

6.4 Broad-spectrum antivirals

Targeting of a host cell factor is an antiviral strategy that has received considerable interest the last couple of years (Table 4). This approach is expected to increase the barrier for spontaneous viral escape from inhibition, since the loss of a host factor is less likely compensated by viral mutations than the high-affinity binding of a pathogen-directed antiviral with its viral target site. Because of some overlap in the host cell pathways used by different viruses to successfully replicate, this strategy offers potential to discover agents with broad-spectrum antiviral activity, and to move beyond the 'one-bug-one-drug' paradigm. A potential downside of the strategy is the higher potential for undesirable side effects induced by these drugs, although short-term treatment of acute infections like RSV may render drug-induced side effects tolerable to some extent.

In 2007, several micromolar hits were reported from a RNase L target-based high-throughput screen with the two most active compounds exhibiting EC_{50}s of 26 and 22 μM (Thakur et al, 2007). RNase L is a principal mediator of innate immunity to viral infections in vertebrates, and is required for a complete interferon antiviral response against certain RNA viruses (Malathi et al, 2007). Compounds were shown to bind to the 2-5A binding domain of RNase L, thereby inducing RNase L dimerization and activation. Interestingly, broad-spectrum antiviral activity against different types of RNA viruses was demonstrated, including human parainfluenza virus 3 (hPIV-3), but unfortunately, activity against RSV was not tested in this study.

In the same year, a paper appeared that reported the potential of arbidol as a broad-spectrum antiviral. The compound was found antivirally active in cellular infection assays of influenza A, RSV, human rhinovirus type 14, coxsackie virus B3 and adenovirus type 7, with EC_{50}s ranging from 2.7 to 13.8 μg/ml (Shi et al, 2007). Orally administered arbidol at 50 or 100 mg/kg/day starting at 24h before virus exposure and continuing for 6 days, reduced mean lung influenza A titers significantly as well as mortality.

Recently, JMN3-003 has been optimized from a class of compounds that was identified from a high-throughput screening campaign that aimed for discovering compounds with acceptable antiviral activity and selectivity index against a number of viruses (Krumm et al, 2011). JMN3-003 indeed demonstrates activity against members of both the ortho- and paramyxoviridae like influenza, RSV and hPIV-3, with EC_{50}-values ranging from 0.01 to 0.08 μM. The compound was found metabolically stable after incubation with S9 hepatocyte subcellular fraction, and viral escape attempts failed to induce resistance after prolonged exposure to JMN3-003. Extensive experimentation to elucidate the mechanism of action of the compound revealed that it blocks a host factor that is required for viral RNA-dependent RNA polymerase activity.

In addition to the examples above, many similar programs, targeting host factors like caspases (Kinch and Goldblatt, 2009a), TSG101 (Kinch and Goldblatt, 2009b), EGFR (jung,

2010; Monick et al, 2005), or kinases (Kalman, 2008) are currently in different stages of preclinical development (Table 4). The most advanced programs today are in Phase I clinical development. For instance, RV 568 is a small-molecule, narrow-spectrum phosphotransferase inhibitor under development by RespiVert (Centocor Ortho Biotech) as an inhaled therapy for lung and airway disorders such as asthma, chronic obstructive pulmonary disease, and allergic rhinitis. Moreover, efficacy studies in BALB/c mice infected with RSV, demonstrated a 83% and 67% inhibition of lung viral titer on days 3 and 5, respectively, when animals were treated with 2 μg/20 μl intratracheally, once daily for days -1 till 5 post infection (Anderson et al, 2011). In the meantime, a randomised, single-blind, placebo-controlled, phase I trial to investigate the effects of intranasal RV 568 on inflammation caused by infection of RSV has been completed but no results have been reported.

FGI-101-1A6 is a fully human monoclonal antibody that targets a host protein, TSG101, which is uniquely exposed on the surface of virus-infected cells. FGI-101-1A6 targets and eliminates infected cells via induction of normal host defense mechanisms. Functional Genetics Inc. has demonstrated that FGI-101-1A6 can selectively identify and eliminate cells that have been infected with many virus types, including RSV. They are currently recruiting for a randomized-placebo controlled Phase I trial to show the safety and tolerability of intravenously administered FGI-101-1A6. In addition to this, Functional Genetics focuses also on the development of antibodies against additional targets. For instance, FGI 110, an anti-Nedd4 monoclonal antibody is in preclinical development for HIV-1, RSV and influenza (Table 4).

Despite the fact that all programs are in the early (pre)clinical stages of development, this area seems to be very dynamic in terms of new initiatives to develop broad-spectrum antiviral therapies, and much more of these programs are expected to initiate clinical evaluation in the next few years.

6.5 Antibiotics

Bronchiolitis occurs most often during the first year of life and has most commonly a viral cause. RSV is the most common aethiological agent causing bronchiolitis, although it is recognized now that other viruses like human metapneumovirus, parainfluenza virus, adenovirus or rhinovirus may contribute significantly to the number of cases (Hall et al, 2009; Nair et al, 2010; Rudan et al, 2008; Simoes, 1999; Williams, 2004). A recent updated review of five randomized controlled trials comparing antibiotics to placebo in a total of 543 children less than 2 years of age diagnosed with bronchiolitis found minimal evidence to support the use of antibiotics to treat bronchiolitis (Spurling et al, 2011). The 2011 update included four randomized controlled trials investigating the use of macrolides for bronchiolitis. Macrolides are thought to have anti-inflammatory activities as well as antibiotic activity (Culic et al, 2001), and so are thought to have potential benefit for treating bronchiolitis. Of the five studies included in the review, one smaller study including 21 children found that clarithromycin treatment may reduce hospital admission (8% clarithromycin versus 44% placebo, P = 0.081), but this study was associated with a potential higher risk of performance, detection and reporting bias (Kabir et al, 2009). One other study found mixed results for the effects of antibiotics on wheeze, but did not identify any difference for other symptom measures (Mazumder et al, 2009). Four included studies did not find any difference between antibiotics and placebo for their primary outcomes of length

of illness (Field et al, 1966) or lengt of hospital stay (Kabir et al, 2009; Kneyber et al, 2008; Mazumder et al, 2009). Despite these results, antibiotics are commonly used in 34% to 99% of hospitalized infants, even in those that do not require mechanical ventilation (Kabir et al, 2003; Vogel et al, 2003). The use of antibiotics is often associated with adverse reactions and community acquired bacterial resistance (Brook, 1998). On the other hand, severe bronchiolitis is commonly associated with bacterial co-infection (Bezerra et al, 2011). For these reasons, it is currently advised that antibiotics are used cautiously when treating bronchiolitis. Although no anti-inflammatory effects may be expected from the use of certain antibiotics, their use may be justified in cases when there is concern about secondary bacterial co-infections, particularly in very sick infants that require intensive care admission.

7. Conclusions

Despite the huge medical burden which is associated with RSV infection in different patient populations worldwide, no effective vaccine is available today. Several different approaches to design new vaccine candidates, i.e. live attenuated vaccines, subunit-based vaccines, or vector- or DNA-based vaccines, are being applied, but because of host-related challenges and limitations associated to each of the vaccine approaches, it will probably take another couple of years before the first vaccines will be approved. Likely, different vaccines may be required for RSV-naïve and experienced patients. Despite the many therapy failures with palivizumab, the use of it will remain the main therapy in the near future in the very young infants to provide protection against severe ALRI development due to RSV. However, new antibodies for RSV prophylaxis with improved efficacy and a more amenable route of administration or dosing regimen over palivizumab are currently being developed. The therapeutic potential of such antibodies is also being evaluated. Cost will nevertheless remain an important determinant for the generalisability of antibody treatment, especially in underdeveloped countries and adult patients. The potential appearance of biogenerics in this area may represent a potential game changer. In the meantime, several programs are ongoing to develop specific RSV and broadly-active antivirals. While several RSV viral targets have been clinically validated, attrition rate has been high during compound development, and as a consequence, first approval of such compounds will likely not happen in the near future. The area of broadly-active antiviral development has gained much interest the last couple of years, but the programs are still in early stages of pharmaceutical development, and because of the higher potential for drug-related adverse effects due to the targeting of host factors, it remains to be seen how high the attrition rate will be. Therefore, the first approval of a new RSV therapeutic agents will likely only take place in 2015-2016 at best.

8. References

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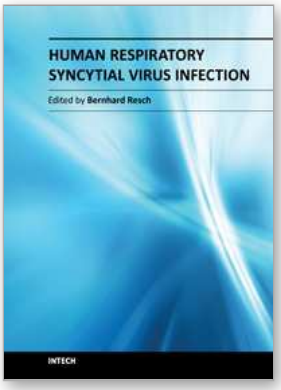

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In this online Open Access book on "Human RSV Infections", several distinguished authors contribute their experience in respiratory syncytial virology. A major focus lies on the fascinating pathophysiology of RSV and represents recent and actual work on different mechanisms involved in the complex pathogenesis of the virus. The second section elucidates epidemiologic and diagnostic aspects of RSV infection covering a more clinical view of RSV disease. At last, treatment modalities including the search for a vaccine that is still not in sight are discussed and conclude this book, thus building up a circle that runs from experimental models of RSV related lung disease over clinical aspects of disease to the latest news of therapeutic and prophylactic approaches to human RSV infection.

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