Advances in clinical development for vaccines and therapeutics against respiratory virus infections

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CHAPTER 9

DISCUSSION AND FUTURE PERSPECTIVES

In this dissertation

The work for this thesis has been executed in a turbulent time for the fields of respiratory viral diseases, vaccinology and clinical pharmacology. The research for this thesis initially began with work on clinical trials investigating novel vaccines and adjuvants for respiratory syncytial virus (RSV) and Influenza. Then, half-way through this PhD-project that set out to investigate innovations in clinical development for respiratory viruses, the COVID-19 pandemic broke out.

The COVID-19 pandemic posed an enormous challenge for the entire world including medicine. However, driven by an unprecedented societal demand for treatment and vaccines for COVID-19, the pandemic also led to many innovations in clinical and translational medicine. With limited evidence on possible treatments against SARS-COV-2, existing compounds were first experimentally administered to treat COVID-19. In parallel, new vaccines and drugs were expeditiously brought from bench to bed. The pandemic also illustrated that the traditional linear development paradigm for therapeutic interventions was not suitable to meet the quest for necessary innovation.

This thesis consists of studies investigating vaccines and therapeutics against respiratory viruses. The first sections focus on novel vaccines and adjuvants for RSV and influenza (pre-pandemic). The last section focusses both on a new and repurposed compound against SARS-COV-2, explores novel methods of accelerating vaccine trials during a pandemic and concludes with an overview of several procedures that could expedite (early) clinical development during a pandemic.

RESPIRATORY SYNCYTIAL VIRUS (RSV)

Developing a safe and effective needle-free mucosal RSV vaccine will have many benefits. By boosting local innate and humoral immunity at the site of potential virus entry – the nasal mucosa – this approach may help to protect against infection and possibly prevent subsequent transmission of the virus. Live-attenuated vaccines have historically been safe platforms for intranasal vaccination and have not been associated with vaccine-induced disease enhancement.[1] The genetically modified vaccine candidate RSVΔG was designed using reverse genetics and is characterized by the deletion of the attachment protein (G) of the wild-type virus. The vaccine was expected to be attenuated but still able to replicate by the intact fusion protein (F).[2]

As described in the introduction, RSV-vaccine candidates are tested sequentially in adults (who have had multiple RSV infections throughout their lives), seropositive children and finally in seronegative children. It was hypothesized that high pre-existing levels of neutralizing serum antibodies in adults could pre-emptively neutralize RSVΔG and thereby prevent a successful vaccine immune response.[3] An observational study was performed to characterize the levels and distribution of off-season serum neutralizing antibodies against RSV in healthy adults (*Chapter 2*). This study aimed to identify a threshold titer to be used as eligibility criterion for healthy adult participants in first-in-human studies. The distribution of antibody titers in this population also enables researchers to predict screen failure rates when selecting a threshold titer for clinical trial inclusion. Selecting lower titers of neutralizing antibody may facilitate low-grade replication of the vaccine virus, thereby increasing the chance of successful vaccination with a live-attenuated virus but also observing viral shedding (in adults). However, the prevalence of the antibody titers should be considered when selecting a threshold to avoid screening unrealistically large numbers of subjects. A threshold of 9.6 $\log_2$ neutralizing antibody titer was selected as eligibility criterion for our randomized clinical trial.

Intranasal administration of RSVΔG was shown to be safe and well tolerated in healthy adult volunteers (*Chapter 3*). Minimal signs of viral shedding further confirmed the full attenuated phenotype of RSVΔG. Substantial and prolonged replication in adults is an indicator for under-attenuation in RSV-naïve infants.[1] The safety and viral load results thus paved the way for further investigation in seropositive children. Immunogenicity analysis, however, showed no apparent induction of systemic or mucosal immunity in adults. Previous studies with live-attenuated vaccines have shown that vaccines that are highly attenuated in adults can be immunogenic in seronegative children and even under-attenuated.[4] The immunogenicity data obtained from adults with pre-existing immunity against RSV are therefore not fully predictive for the target pediatric population. The immunogenicity data from this trial also suggest that the pre-established cut-off of 9.6 $\log_2$ for neutralizing antibodies should be reconsidered, such a level of pre-existing humoral immunity may still prevent the full immune response against live-attenuated viruses in healthy adults.

The lack of immunogenicity signal in adults could also indicate that the selected single dose was too low to induce sufficient immunogenicity. A follow-up dose-selection study could further assess the dose-immune

response relationship. Alternatively, it can be attempted to alter the vaccine concept to improve immunogenicity. Both the genome and the outer surface of the recombinant RSVΔG lack the G-protein. A variant of the vaccine concept would be to complement the outer surface of RSVΔG with G-proteins. The resulting vaccine variant (G-RSVΔG) will be able to attach to the host cell via its G-protein, thereby increasing the initial infection potency.[5] The progeny virions will be identical as RSVΔG (lacking the G-protein) and highly attenuated. Lastly, altering the confirmation state of the F-protein antigen to sustain a pre-confirmation state might also improve immunogenicity of the vaccine candidate.[6]

INFLUENZA VIRUS

Mucosal vaccine candidates could have additional benefits for seasonal influenza vaccination compared to traditional intramuscular vaccination. Live-attenuated intranasal vaccines are currently available but their use in the European Union is limited to the group between 2 and 18 years of age. Elderly are at risk for influenza-related complication and hospitalization and immune response to influenza vaccines weakens at higher age. There is therefore a high need for improved vaccine strategies for this high-risk group. As stated previously, intranasal vaccines have the potential to not only prevent disease but also prevent or reduce transmission of viruses due to eliciting mucosal immune responses. Such vaccine attributes would be especially beneficial for preventing outbreaks among high-risk elderly living in long-term care facility.

An intranasal trivalent virosomal-subunit vaccine adjuvanted with *Escherichia coli* (*E. coli*) heat labile enterotoxin (NasalFlu, Berna Biotech) was withdrawn from the market after epidemiological association with facial nerve paralysis.[7] The role of the *E. coli* enterotoxin adjuvant in the development of facial nerve paralysis has never been fully elucidated, but further use has been abandoned.[8-10] Therefore, the need for alternative safe and potent mucosal adjuvants with sufficient immunogenicity of intranasal influenza vaccines remains. Bacteria like particles (BLP) derived from gram-positive bacteria containing a peptidoglycan outer surface could function as such immunostimulant. BLPs are assumed to activate toll-like receptor (TLR)-2, which subsequently leads to a cascade of events that stimulate innate immune responses and ultimately the potentiation of the adaptive immune response. The randomized control clinical trial described in *Chapter 4* assessed the safety and immunogenicity of BLPs derived from *Lactococcus lactis*, a non-pathogenic gram-positive bacterium combined with inactivated trivalent seasonal influenza vaccine (FluGEM®) in different age groups. Intranasally administered FluGEM showed a favorable safety profile for all explored doses in the age group of 18 to 49 years. Lower doses of FluGEM appeared to elicit higher IgG-titers compared to high doses. The exact immune mechanism of this dose-response relationship remains unknown, but non-linear dose immune response relationships have been described previously for other TLR2 agonists.[11] The immunogenic low dose that was selected for assessment in subjects aged over 65 years (target population) was safe and well tolerated but failed to elicit a strong immune response. The addition of a separate cohort with subjects aged over 65 years in this early clinical study gave valuable insight for the development of this adjuvant for the potential target population. Further research is needed to improve immunogenicity of the BLP-based platform for mucosal vaccination in elderly (for instance dose-optimization or adaptation of a BLP-based delivery system).

SARS-COV-2 AND CLINICAL DEVELOPMENT DURING PANDEMICS

Therapeutics often serve as a first-line defense against an emerging novel pathogen. For the treatment of COVID-19 several antiviral, immunomodulatory and anticoagulant drugs have been approved. Many of these therapies were repurposed compounds or antiviral therapies already in late-stage clinical development for other RNA viruses. Novel compounds with pathogen-specific targets needed to be development in parallel to improve the therapeutic arsenal. One of these novel compounds is ensovibep, a Designated Ankyrin Repeat Protein (DARPin) with cooperative tri-specific binding capability to the SARS-COV-2 Spike-protein. For the clinical development program of ensovibep it was needed to assess the overall feasibility of administering ensovibep in an ambulatory setting. A smaller, open label, first-in-patient study was performed in ambulatory patients with mild-to-moderate COVID-19 (target population) (*Chapter 5*). Administration of ensovibep in an ambulatory setting was well tolerated and no antibody-dependent enhancement of infection was observed. Pharmacokinetic analysis confirmed the relatively long half-life of ensovibep in patients. Interpretation of pharmacodynamic parameters was limited due to the small group size

and non-controlled design. However, the magnitude of the decline in viral load was comparable to monoclonal antibodies that received emergency authorization for COVID-19.[12,13] There was no apparent difference between high or low doses for pharmacodynamic outcomes. Outcomes of this study facilitated the next-stage clinical development of ensovibep. It was later shown that ensovibep, like many other antivirals administered later in the disease course, did not improve clinical outcomes in hospitalized COVID-19 patients compared to patients receiving standard care (including remdesivir).[14-16] However, preliminary top-line data from the ambulatory population suggest a possible reduction of COVID-19 hospitalization and death in patients treated with ensovibep (press communication), highlighting the need for early treatment initiation of antivirals in respiratory virus infections.[16] Further data from late-stage clinical development is required by regulators for the market authorization of ensovibep.

Off-label use of 4-aminoquinolines (chloroquine and hydroxychloroquine) for the treatment and prophylaxis of COVID-19 occurred on a large scale in the early phase of the COVID-19 pandemic.[15] The *in vitro* antiviral activity against SARS-COV-2, its well-characterized safety profile from auto-immune and malaria indications and widespread availability made these compounds candidates for repurposing for the treatment and prophylaxis of COVID-19. In addition, it was hypothesized that the immunomodulatory effects of hydroxychloroquine could also treat or prevent adverse immune reactions (such as cytokine storms) that occurred in critically ill COVID-19 patients. The exact mechanism of hydroxycholoroquine's immunomodulation is not completely understood. In short, hydroxychloroquine is believed to have multiple effects on both innate and adaptive immunity, including endosomal TLR signalling, inhibition of T cell activation, and altered differentiation of memory B cells. However, *in vitro* experiments assessing the immunomodulatory effects often used hydroxychloroquine concentrations that far exceded clinical concentrations observed in patients.[17-20] To better assess and quantify the immunomodulatory properties of clinically relevant doses of hydroxychloroquine, we conducted a study (*Chapter 6*) that combined both *in vitro* and *ex vivo* experiments on human peripheral blood mononuclear cells (PBMCs). For the *ex vivo* part of this study, a randomized clinical trial was performed in healthy volunteers that received a 5-day treatment course of hydroxychloroquine with a cumulative dose of 2400 mg. This was the dose that was recommended

(off-label) regimen by national guidelines for the treatment of moderate-to-severe COVID-19 at the time of the study. The *in vitro* part of this study showed that hydroxychloroquine had strong dose-dependent inhibitive effects on TLR responses and to a lesser extent inhibited B-cell proliferation but had no effects on T cell activation. Strong immunosuppressive effects were observed at high (>1000ng/mL) concentrations of hydroxychloroquine. Such concentrations, and thus immune effects, were unlikely attained in PBMCs from our clinical study that used a 5-day course of hydroxychloroquine with peak plasma levels of 100-150 ng/mL. The discrepancy between *in vivo* and *in vitro* experiments suggests that the dose-regimen used for off-label treatment of COVID-19 resulted in insufficient drug exposure of hydroxychloroquine to reach clinically relevant concentrations. A slow clinical onset (3-6 months) of immunomodulatory therapeutic effects of hydroxychloroquine is observed in patients with auto-immune conditions that use comparable daily dosing regimen.[21,22] This can in part be attributed to hydroxychloroquine's high volume of distribution, possibly explained by sequestration to lysosomes.[21] Steady-state concentrations are only reached after months while the drug is likely to further accumulate intracellularly. One of the limitations of this study was that we did not measure the intracellular concentration of hydroxychloroquine. This study exemplifies that a *reverse translation approach* may provide mechanistic insights that further oppose the use of hydroxychloroquine for COVID-19 based on functional immunological effects. It corroborates and explains the clinical evidence that there is no role for (short-term use of) hydroxychloroquine for prevention or treatment of COVID-19.[23]

Next to innovation in drug and vaccine development, a pandemic also demands innovation in research methodology, organization and regulations. Large scale deployment of COVID-19 vaccines depended on the evaluation of pivotal phase III field trials. In these trials, thousands of participants are vaccinated in endemic countries to eventually compare cases in the control group to the active group in order to evaluate the efficacy of the vaccine. However, if infection rates drop, for instance due to governmental measures to prevent spread of the disease (such as social distancing, quarantine, promoting hygiene etc.), it will take longer to establish vaccine efficacy or the study can become underpowered. In *Chapter 7*, we therefore explored a more agile approach to conducting vaccine field trials, namely by identifying local surges of infection spread

in a population, so-called hot-spots. *In silico* experiments were performed by modelling a pandemic outbreak and simulating vaccine trials using the proposed *hot-spot* identification approach versus a traditional vaccine trial. Our experiments showed that the key endpoints (such as achieving a certain number of cases) can be reached more efficiently using the *hot-spot* approach and duration of vaccine trials can be reduced accordingly. The model parameters can be adjusted to fit disease characteristics for any future pandemic threat. This chapter also highlights the need to prepare clinical trial infrastructure for future pandemics. Several organizational requirements are provided to improve vaccine trial conduct for a more rapid and agile response to a pandemic.

During a pandemic it is important to select the most promising compounds as early as possible and stop clinical development for less promising candidates to avoid wasting valuable time and resources. Early phase clinical trials play a vital role in this. Next to safety and tolerability assessment in healthy individuals, these trials can also provide preliminary insight in the intended effects and should utilize a cyclical translational approach (e.g. forward and backward translation). Next to a rational 'content-driven' approach, pandemic drug development also requires acceleration of the 'administrative' part of the drug developmental trajectory. *Chapter 8* identifies five organizational and regulatory bottlenecks specific for early-stage vaccine clinical development and provides recommendations to expedite clinical development in a pandemic setting.

Medical ethics review committees and competent authorities should utilize accelerated review programs for pandemic clinical development. Fortunately, many committees and authorities have quickly adapted fast-track procedures for COVID-19 clinical trials which reduced time from submission of study-protocol to first-dose significantly.[24] Several new vaccines platforms such as reverse engineered live-attenuated, chimeric and recombinant vector vaccines are classified as genetically modified organisms (GMO). Clinical trials that investigate GMO vaccines need to additionally comply with European GMO-legislation relating to biosafety and possible introduction of the product into the environment. Time before approval for use of GMOs in clinical trials varies greatly. It takes several months to years depending on the country reviewing the application. In the Netherlands special exemptions for COVID-19 clinical research were implemented for GMO-based vaccines.[25]

Recruitment and screening of potential participants are costly and time-consuming activities for (early-phase) clinical trials. Conditional approval of study protocols may allow the investigator to identify eligible participants for clinical trials. Alternatively, a pool of healthy and willing participants may be identified, screened for eligibility and kept on stand-by before the trial commences. At CHDR the *beReady* protocol was designed to identify and (pre-)screen healthy potential participants based on the common standard eligibility criteria for COVID-19 clinical trials. Participants that were found to be potentially eligible for study participation were pooled in a database and kept on stand-by, participants were subsequently invited to partake in COVID-19 clinical trials. This approach reduced recruitment time and screen failures substantially. Validation of laboratory assays may be a rate-limiting step for clinical trial start-up. Harmonization and centralization of laboratory is needed to enable better comparison of results between various vaccine trials. The Coalition for Epidemic Preparedness Innovations (CEPI) has since established a centralized laboratory network that support COVID-19 vaccine development and the World Health Organization has issued international standards for immune assays.[26,27]

Developers are advised to seek regulatory advice early on to improve early-phase clinical trial design. Clinical trials can then be tailored to address key questions needed for market licensing. The Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have set-up mandated task forces of (in)formal consultation and advice.[28]

Future perspectives

The COVID-19 pandemic has led to several breakthroughs for the treatment and prevention of respiratory virus disease. A real gamechanger was the utilization of mRNA and adenovector-based platforms, that were developed decades earlier. These platforms differ from previous vaccine technologies in that it uses the recipient's cell own translational system to generate antigens, much like natural occurring virus infections. In addition, it is relatively simple to adapt these vaccines to include new antigens against novel mutations of concern. As these novel vaccine technologies have come into use, we are only starting to understand their potential. Longevity of immune responses may be further improved, and heterologous prime-boost regimens could utilize a potential synergistic effect between mRNA and adeno

vector-based vaccines. The current successes of these platforms may be a starting point for the development of novel vaccines for other viruses or pathogens.

There has also been a renewed interest for needle-free mucosal vaccination because locally elicited immunity could help to prevent infection, reduce transmission of respiratory viruses and reduce vaccine hesitancy due to injection-related fears.[29,30] Evoking potent systemic and long-lasting mucosal immune response will be difficult to achieve in all age groups, as was illustrated in *Chapters 3 and 4* in this thesis. Novel delivery technologies and safe adjuvants are needed to improve immunogenicity of mucosal vaccines. Development of intranasal vaccines may be the next step to prevent not only disease but also spread of respiratory viruses.

For RSV there may be a breakthrough imminent with several vaccines candidates in late-stage clinical development.[31,32] Immunogenicity and efficacy data from controlled human challenge studies show promising results for these compounds.[33,34] The majority of these late-stage vaccines utilize the prefusion configuration of the fusion protein (F) as main antigen.[35] All current vaccines in late-stage development use vaccination strategies that target either the elderly or maternal population (to subsequently immunize newborns).[35]

Advances have also been made in the development of a universal influenza vaccine, which is considered a holy grail in vaccinology. With many new pan-influenza vaccine candidates attempting to direct an immune response to more conserved regions on the hemagglutinin protein. A universal vaccine could also serve as an important defense against pandemic influenza. Similar initiatives are ongoing to develop pan-corona vaccines.

Major challenges for the development of therapeutic drugs against respiratory viruses will remain. The clinical benefits of current antiviral therapies are limited, especially during late-stage infection. In addition, the risk will remain that drug-resistant virus strains emerge under therapeutic pressure. Ideally, new antiviral therapies should have broad effectivity against multiple strains of viruses, exhibit more potent pharmacodynamic effects such as viral load reduction and decreased duration of shedding with drug formulations that enable outpatient use (e.g. oral or inhalation formulations). Easy-to-use formulations that can be used at home, early in the disease course, could improve efficacy and clinical benefit. Use of antiviral combination preparations could also improve efficacy and reduce the development of antiviral resistance.

Demonstrating pharmacodynamic effects in early clinical trials that involve healthy volunteers is often difficult for compounds targeting infectious diseases. These compounds generally bind to targets that are only present on the pathogen itself or depend on host-pathogen interaction and are of course not present in non-infected healthy volunteers. Controlled human infection models (also known as *human challenge studies)* can provide researchers with the unique opportunity to assess pharmacodynamics or vaccine efficacy relatively early in the development trajectory. That is, if there is a sound scientific and ethical justification for exposing healthy volunteers to a pathogen. Although human challenge studies cannot replace large phase 3 studies, they can provide important insight about the potential of a new therapy or vaccine before field trials are initiated. Information on dose-(immune)response relations from challenge study will also improve outcomes in subsequent phase 3 trials. Even more importantly, the unique circumstances created by controlled human infection models could solve knowledge gaps in the pathogenesis of respiratory viruses and may help to identify new correlates of protection. In recent years human challenges have been frequently used for the clinical development of vaccines and drugs against RSV, a self-limiting disease in healthy adults, and may also play a role in the development of other seasonal respiratory viruses. It is a prerequisite that virus challenge stocks used in these studies are updated to represent the most prevalent and clinically relevant virus strains.

Lastly, the COVID-19 pandemic showed that equitable access to new therapies and vaccine can be significantly improved. Sharing of intellectual property, technology and know-how could enable more diverse geographical spread of production facilities and help to better distribute and give access to vaccines and medicines. In addition, investing in supply (cold) chain facilities is needed for low-to-middle income countries to ensure equitable access to healthcare products.

Conclusion

This thesis exemplifies several innovative approaches for the clinical development of vaccines and therapeutics against respiratory viruses. However, there still there remains an urgent need for further innovation to prevent and treat respiratory viruses. Due to the mutagenic capability of these viruses and natural selection we will need to adapt to keep up with the viruses. We are essentially aiming at a moving target.

The COVID-19 pandemic has shown that the traditional development paradigm for vaccines and therapeutics can be altered. This paradigm change was the key to success to dampen a pandemic threat. It is inevitable that new pandemic threats will emerge. Globalization, overpopulation, intensive life-stock farming and climate change is likely to increase the risk of new epidemics. We should reflect and learn lessons from the response to COVID-19 to be better prepared for the next pandemic. Pandemic preparedness and investing in further innovation could prevent future emerging infectious diseases from becoming the next global health disaster.

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