A potential strategy to overcome COVID-19: combination of COVID vaccines with type-1-biased immunomodulation, e.g. by inactivated mycobacteria – a strategy of "double protection"

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PREFACE from January 1st, 2021

After this paper was written in the beginning of November 2020, the situation became much more critical in the second half of December 2020. New variants of SARS-CoV-2 emerged in UK and South Africa, and they seem to be much more aggressive (infectious). It is highly probable that they will spread worldwide earlier or later; if so, the threshold for herd immunity, either by natural infection or by *fully sterilizing* (!) vaccines, will rise from 60 – 70 % to > 85 % or (much?) more - a goal that seems unachievable in most countries.

Moreover, there is now a lot of uncertainty whether the vaccines from the first generation will address these new variants as well as they did with the conventional variants that were around during the first months of the phase III studies. The extremely disappointing results for the AstraZeneca vaccine in the South African substudy (MADHI et al.) may be only the tip of the iceberg of the forthcoming problems with escape variants from the vaccines. Most worrying, the titer of neutralizing antibodies against B.1.351 was zero or below the limits of detection in most of the vaccinated participants even after two full doses of the vaccine. Meanwhile, VoCs and immune evasion from vaccine-generated immunity became the largest concern in context of the vaccine campaigns, and nearly every day one or several papers are published that raise new concerns with regard to immune escape from vaccines, antibody therapies, convalescent plasma, or immunity generated from a former natural infection (in the sense of a true reinfection).

An international study demonstrated meanwhile *in vitro* evolution of SARS-CoV-2 that was initially fully neutralized from convalescent plasma from a given donor, but escaped completely from the neutralizing antibodies after three mutations that happened within 80 days. The changes in the escape variant were found to be similar to those of the new UK and South Africa variants (ANDREANO et al.). Whereas the virus was regarded at first as comparatively stable and mutations were assumed to be unimportant with regard to immune or vaccine responses, it is evident now that escape mutations, both from natural immunity following infection and from vaccine-induced antibodies, may actually occur. And like a proof of concept, the first death following of a *proven* reinfection with another virus variant was reported from Israel in December 2020. On January 11th, a 73 yr old men died in Germany (Freudenstadt area) due to a reinfection (first infection was in April 2020).

The COVID-19 pandemic may never be overcome if the current vaccine is always “one step behind the mutating virus”. Until most of the population is vaccinated, a strain from the next virus generation may be circulating that already escaped from the vaccine that is just in the process of administration. And so may happen with the next and the overnext vaccine and so on.

What will happen if the current vaccines are always “one step behind the virus”, if they always come too late and are thus unable to reduce the worldwide viral load? The larger the wordwide mass of the virus, the higher the risk of mutations or recombinations (as may happen in case of multiple infections at the same time or a few days apart). Immunity generated by natural infections and/or vaccines will then drive the positive selection of critical mutations that will eventually evolve into “true” escape mutations.

There is an urgent need to fight against the virus simultaneously in two different ways (which are, of course, connected with one another by the interplay of the different arms of the immune system): the “classical” way with its neutralizing antibodies and a less well understood specific T cell response, but also a second way that stimulates the trained innate immune response in an unspecific way to elicit heterologous effects by a quick and efficient Th1 cell response against the viral infection that may (i) offer a second line of defense if the vaccine-generated antibody response fails because of escape mutations and (ii) reduce the risk of antibody-dependent enhancement; if the fresh infection is quickly overcome by trained innate immunity, there will be no need for the proliferation of large quantities of badly neutralizing antibodies against a mutated virus that may result, in the worst of all cases, in ADE or other immunopathologies.

Thus, in the new situation we face since the end of December 2020, the need for a “double protection strategy” is more urgent than ever before. It is much too simple and too optimistic to believe that the vaccines from the first generation will bring the COVID-19 pandemic to an end. Of course, if a very optimistic scenario comes actually true, it *may* happen. But this is neither certain, nor is it probable, and one should think beyond that optimistic scenario and prepare for worse (and more likely) scenarios, regardless of whether their probability is 10 % or 90 %. So far, COVID-19 has disappointed many optimistic scenarios, and the immune escape scenarios by VoCs make the situation much more critical than it ever was before.

(End of preface)

Introduction: limitations of the COVID vaccine strategy

At the end of October, a small prospective trial from India was published (JAISWAL et al.) that may become a gamechanger and may offer a clue how the COVID catastrophe may be overcome rather quickly if the strategy works well, provided that the scientific and regulatory (legal) basis for that strategy is created quickly too.

The first reports about phase III trials of “true” COVID vaccines in November 2020 were much more favorable than expected, with risk reductions of about 90 % or more if one starts to count infections one or two weeks after the second dose. However, these favorable results from mRNA-based vaccines could not be replicated in all of the other (non mRNA-based) vaccines (with the exception of Sputnik V). There is a wide range of vaccine efficacy rates (see Fig. 1 in EARLE er al.) that are correlated with antibody responses measured by neutralization or ELISA assays.

However, all of these data are based on the vaccine efficacy in the first ~ two months after vaccination was completed, plus one or two weeks, and it is unknown so far how quickly this high or moderate degree of protection will wane.

Secondly, despite favorable results for older adults from some phase III studies (as fas as older adults were included at all in these early studies), real-life experience showed significant problems with immunosenescence even in the case of top vaccine candidates like Cominarty (YELIN et al., MÜLLER et al.). Vaccine efficacy (YELIN et al.) and neutralizing titres(MÜLLER et al.) decrease with age, even after the full two-dose regimen of Cominarty, and 31.3 % of vaccinated inhabitants of care homes >= 80 years of age had no detectable neutralizing titresin a study from Germany (MÜLLER et al.) – “they were potentially without seroprotection”. This is well in accordance with anecdotal reports about severe and potentially lethal cases of COVID-19 in care facilities after the second dose of Cominarty (pers. comm.). Besides the question of waning immunity over time, there seem to be non-responders in older age groups who have no protection from the beginning.

Moreover, most or all of the vaccines of the first generation are not fully sterilizing, or their sterilizing effect is limited, according to experiments with macaques (see table 1 in: KRAMMER). There are differences in this respect between different vaccines, and mRNA-based vaccines may sterilize better compared to adenovirus-vectored vaccines (e.g. VOGEL et al. vs. VAN DOREMALEN et al.), but this conclusion seems not to be consistent (see table 1 in KRAMMER) what makes the sterilizing effect less predictable. And press reports about the AstraZeneca vaccine suggest *“that the vaccine may prevent people from transmitting the virus”* (CALLAWAY), what would indicate a sufficient degree of sterilization in the naso-/oropharyngeal tract, in contrast to the macaque results.

In the rhesus macaque model of the Oxford/AstraZeneca vaccine, SARS-CoV-2 replicated in the nasopharyngeal tract in vaccinated individuals to the same extent as in unvaccinated individuals (following SARS-CoV-2 inoculation) (VAN DOREMALEN et al.), whereas in case of the Pfizer/Biontech vaccine, nasopharyngeal swabs were negative at day 3 and oropharyngeal swabs showed lower viral load (about two orders of magnitude) at day 1 and 3 following inoculation in vaccinated macaques, compared to unvaccinated individuals (VOGEL et al.). However, the experiments cannot be compared directly to one another because in case of AstraZeneca’s AZD1222, the animals had gotten only one dose of the vaccine, in contrast to two doses in the macaque experiments with mRNA vaccines.

Based on the macaques results with only a single dose, vaccinated people who got the AstraZeneca vaccine may, in the best case, be protected from severe disease and death, but they may acquire the infection, develop mild or even moderate symptoms and be as infectious as unvaccinated people. Such a vaccine may be unable to have any decreasing effect on the R value. And if infected vaccinated people are less likely to become ill, to get

tested and to be isolated, the spread of COVID-19 may even be enhanced by vaccinated people, and the R value may rise, increasing the dangers for unvaccinated people or non-responders to the vaccine. Fortunately, preliminary reports from the phase III trial point to the opposite and don't seem to replicate the macaque results (see above). On the other hand, real-world data from Scotland showed that even Cominarty is unable to prevent household transmission completely, even not after the second dose (SHAH et al.) (though a little bit better after the second dose compared to the first dose). This is in contrast to the macaque data which suggested that Cominarty is not 100 % sterilizing but that naso-/oropharyngeal viral load is so low after two doses that transmission is highly improbable (see figures in VOGEL et al.).

According to animal models with primates and non-primates, nasal administration of different vaccine types elicits a very early local immune response of the nasopharyngeal/respiratory mucosa following SARS-CoV-2 inoculation, which results in sterilizing immunity (see fig. 2 in: KRAMMER). However, none of the current phase III vaccine candidates is administered intranasally.

For sterilizing immunity, one should thus consider intranasal administration; it may be combined with i.m. injection, either simultaneously or (probably better) a few weeks apart. The concept already worked well in a mouse model (RICE et al.). Put simply, it may be that the injected dose may be more relevant for the protection from severe or critical disease for the vaccinated person, whereas the nasally administered dose may be more relevant for the protection of contacts and the fight against the epidemic, though there will be of course interactions between both doses. And it may well be that one needs two injected doses and two intranasal doses (at different point of times) to acquire both self-protective and sterilizing immunity for a longer time (like a full year or so).

However, these are ideas for a second generation of vaccines, and these second generation vaccines are now in a queue since all capacities in pharmaceutical industries and phase II or III trials are blocked by the development, testing, approval and production of the first generation, though there is an urgent need for truly sterilizing vaccines to stop the pandemic and impair the evolution of new variants, including true escape variants.

Moreoever, there are concerns that vaccines may elicit antibody-dependent enhancement (ADE), at least in some people (see ARVIN et al.). ADE in the context of SARS-CoV-2 was already demonstrated *in vitro*, using plasma from severe or critically ill patients (WU et al.). A possible clinical role of ADE is still unclear. WU et al. point out that this doesn't mean that vaccine candidates would necessarily induce ADE or disease severity, but a vaccine that induces high titres of neutralizing antibodies is regarded as safer in this respect because it can eliminate newly invaded virions quickly before ADE occurs, and because neutralizing antibodies mediate ADE only at suboptimal neutralizing concentrations (WU et al.).

Therefore the situation may become critical when the titre of neutralizing antibodies wanes over time. Since this may happen several months or years following vaccination, such a phenomenon may be undetectable in the first months of phase III trials (before approval), and it might become visible only after longer follow-up when neutralizing antibody titres wane. ADE may also become a problem if new variants of the virus evolve which are only weakly neutralized by antibodies from the vaccine, mimicking the situation of suboptimal neutralizing concentrations as mentioned by WU et al. An example for what may happen is

the variant N439K of the spike protein described by THOMSON et al.: *"Although SARS-CoV-2 is evolving slowly and at present should be controllable by a single vaccine, variation accumulating in the RBM could put this at risk…"* LIU Z et al. described several so-called "escape variants", and HAYASHI et al. showed that the Y435F RBD mutation (originated in minks and then spread to humans) responsed weakly to 4 out of 6 monoclonal antibodies compared to conventional SARS-CoV-2. VILAR and ISOM showed in their study that *"some proteins, such as the Spike or the Nucleocapsid, showed higher degree of variability in specific residues and could present future liabilities in the efficacy of vaccines and therapeutics."* And it is established meanwhile that there are new escape mutants that are able to escape neutralization by existing first-wave anti-SARS-CoV-2 antibodies and re-infect COVID-19 convalescent individuals (e.g., NELSON et al.). Most worrying, this applies to the aggressive South-african 501Y.V2 variant (= B.1.351) (WIBMER et al.), but also to B.1.1.7 from UK (HU J et al.).

Moreover, based on human sera from the 1980s and 1990s, EGUIA et al. demonstrated that the Coronavirus 229E needed 8 – 17 years of evolution to escape from the neutralizing capacity of antibodies generated as a consequence of infection with former versions of the virus. The virus escaped from antibody control by the evolution of the viral spike, especially the RBD. Vaccines will have to be updated periodically.

Meanwhile, immune escape from vaccines, antibodies therapies, convalescent plasma and reinfections because of escape variants are widely accepted as subjects of very great concern, so there is no need any more to discuss these topics in detail in this paper here. Within a few months from the first studies that warned about vaccine escape (as mentioned above), immune evasion and vaccine escape became the dominant topics and it is clear now that the fight against the pandemic will last much longer than originally expected after the first reports about the high efficacy of the vaccines that suggested that a final and definite return to normal life *without COVID fears* can happen between late summer and early winter of 2021, at least in developed countries with access to the top candidates among the vaccines. Immune evasion und vaccine escape made these plans impossible. It may well be that many countries will return to some sort of "normal life" without lockdowns and fully opened schools and kindergardens until then; but this will not work without fears, masks, contact reduction and contact tracing. At best, the situation will be similar then to the time between the first and second wave (summer/early fall 2020), but the fight against the pandemic won't be won, neither on the level of societies nor on individual levels. Testing the titre of neutralizing antibodies against the circulating variants on an individual base will become an important topic then.

ANDREANO et al. studied the evolution of SARS-CoV-2 in the immune population *in vitro* by co-incubating authentic virus with a highly neutralizing plasma from a convalescent patient. The plasma was able to neutralize the virus completely for 7 passages. However, after 3 mutations and 80 days, a variant was generated that was completely resistant to plasma neutralization. The mutations resulted in changes that prevented binding of neutralizing antibodies, and these changes were similar to those observed in the new variants from UK and South Africa, raising the question whether vaccines from the first generation will be able to control such variants.

DEMARCO et al. showed that the damage of respiratory epithelia that results eventually in ARDS is caused by improperly activated macrophages. This dysregulation of the immune

response begins with the activation of macrophages by non-sterilizing antibodies and induction of ACE2 expression. This makes the macrophages susceptible to killing by SARS-CoV-2. Death of macrophages leads to the release of inflammatory mediators and modulates the susceptibility of epithelial cells downstream in the airways to SARS-CoV-2. Thus M1 polarized macrophages damage the surrounding tissue in the presence of non-neutralizing antibodies. COVID vaccines are constructed in a way that they elicit a Th1 response (to avoid a Th2/Th17 response that may trigger immunopathologies). Since no ADE was observed so far in phase I and II studies, DEMARCO recommend to elicit a more balanced immune response by COVID vaccines because it is impossible to avoid the deleterious associations between macrophages and antibodies. As long as sufficient titresof anti-RBD antibodies are generated and present, DEMARCO et al. see no problem for the coexistence with non-neutralizing antibodies.

"However, once immunity starts to wane, a high ratio of non-neutralizing to neutralizing antibodies alongside M1 polarization, may be riskier than RBD-specific vaccination and may suggest that neutralizing antibody titresin vaccinated individuals should be monitored regularly to establish timelines for administration of booster doses." (DEMARCO et al.).

But a similar situation may arise if infection occurs with SARS-CoV-2 variants that are not sensitive to the nAbs from the vaccine, i.e. in case of escape mutations.

One can only hope that antibody-independent effects of COVID vaccines are able to fight successfully against these mutants before they are addressed by new vaccines. The more it is important that one also strengthens antibody-independent ("trained") innate immunity of type I, as will be discussed below, as an alternative way for defense against the virus in cases when the antibody-dependent defense fails e.g. because of escape mutations.

There are press reports about proportionally more serious and critical cases among young (< 50 years) people without comorbidities in Germany during the second wave (October/November), long before the arrival of the aggressive VoCs. One may speculate that this may be the consequence of ADE or similar immunopathologies as a consequence of an asymptomatic and undetected first infection during the first wave which resulted in very low neutralizing antibody titres(since antibody titres some weeks and months after infection are positively correlated with the severity of the disease, with asymptomatic disease at the lower bound). In a study from Mexiko, there was a mortality of 3.9 % (corrected from 4.3 % after update of the original data) among presumed reinfections (MURILLO-ZAMORA et al.), though there was no direct genomic evidence for reinfection instead of reactivation, and one cannot exclude the possibility that some or all cases of secondary disease in that study are due to reactivation of an uncleared primary disease. However, such reports are reminiscent of ADE or similar immunopathologies and warn not to be too optimistic with regard to the efficacy and risks of COVID vaccines.

Finally it is known from natural infections with SARS-CoV-2 that neutralizing antibody titres wane more quickly in men than in women (GRZELAK et al.). If this applies to nAbs titres following vaccination too (what seems plausible), men will be protected to a lower extent and for a shorter time than women, though men need more protection because of their worse prognosis in case they get infected.

It is generally assumed that a type-1-biased immune response following vaccination is needed to elicit a preventive effect and to avoid critical and dangerous immunopathologies in vaccinated individuals in case they get infected (but see DEMARCO et al.). Animal studies and phase I trials examine whether the immune reponse to a vaccine candidate is type-1-biased.

However, with regard to the limitations of the first vaccine generation mentioned above (and maybe later vaccine generations too), it may be helpful to strengthen a favorable (preferentially type-1 biased) immune response by *additional* methods, in addition to the COVID vaccine itself.

For example, it was found that the early expansion of myeloid-derived suppressor cells (MDSC) inhibits SARS-CoV-2-specific T cell responses and corresponds with bad outcomes and mortality (SACCHI A et al.). Though reducing inflammation, these cells inhibit both adaptive and innate anti-viral immune response and prevent virus elimination and patient recovery (SACCHI et al.). This strengthens the need for an early and very strong Th1-biased innate immune response to either prevent or compensate the deleterious suppressive effects of MDSC and TGF beta.

Moreover, AGERER et al. showed the capacity to evade adaptive antibody-independent immune responses via mutations in MHC-I restricted CD8 + cell epitopes. The mutants of SARS-CoV-2 showed diminished or abrogated MHC-I binding; this results in loss of recognition and missing functional response by CD8+ T cells. This may limit the efficacy of subunit vaccines that induce responses only to a limited number of epitopes.

In summary, escape mutations of SARS-CoV-2 may not only involve escape from neutralizing antibody response, but also from adaptive CD8+ cell response. This is very critical because it is generally assumed that T cell reponses to the vaccine may still protect from severe disease when no neutralizing antibodies against the viral variant are present and infection or mild disease cannot be prevented (e.g., MADHI et al. for the failure of AZD1222 against B.1.351).

This strengthens the need for a double protection strategy, based (i) on a specific adaptive immune response by neutralizing antibodies and (CD 8+-based) T cell response on one side, but also (ii) an unspecific strong response of the trained innate immunity from Th1 type.

Moreover, it is suggested that SARS-CoV-2 interferes with innate immunity (e.g. impaired type 1 interferon activity) (HADJADJ et al., DA SILVA ANTUNES et al.), thus strengthening (training) innate immunity may enforce a stronger early antiviral immune response, limiting and terminating the initial infection before any immunopathology may have the chance to evolve. PEYNEAU et al. found *"that innate immune deficiency is present in both non-ICU and ICU COVID-19 patients and associated with disease severity and prognosis."*

Type I interferons are considered as important mediators of innate immunity because of (i) their inherent antiviral activity, (ii) their ability to drive the transcription of genes involved in viral clearance, and (iii) their role in the initiation of innate and adaptive immune responses. Based on longitudinal patient nasopharyngeal samples and airway epithelial organoids, CHEEMARLA et al. found that SARS-CoV-2 initially replicated exponentially with a doubling time of ~6 hours. Virus replication induced interferon stimulated genes (ISGs), but delayed relative to viral replication. The timing and degree of stimulation of ISGs then determines the

extent of viral replication. *"Prior exposure to rhinovirus increased ISG levels at the start of SARS-CoV-2 infection and completely blocked SARS-CoV-2 replication. Conversely, inhibiting ISG induction abrogated interference by rhinovirus and increased SARS-CoV-2 replication rate."* (CHEEMARLA et al.).

Moreover, it was found that low-dose nucleocapsid protein of SARS-CoV-2 suppresses type I interferon signalling and inflammatory cytokines and thus inhibits the early local immune response to the infection that otherwise might be able to eradicate the infection immediately (ZHAO Y et al.). In contrast, high-dose nucleocapsid protein (that may occur after the progression of the infection) upregulates type 1 IFN and inflammatory cytokines and thus contributes to hyperinflammation and immunopathologies. Since nucleocapsid proteins from SARS-CoV-1 and MERS inhibit (early) IFN 1 production by the same pathway (TRIM25 interaction), this mechanism seems to be a common ability of SARS-like coronaviruses that contributed to their evolutionary success.

Training the innate immune system (e.g. by recent BCG injection) elicits early interferon I response by NOD2-dependent epigenetic reprogramming in monocytes (WANNIGAMA and JACQUET), but also their bone marrow progenitors (KALYUZHIN et al.). Cytosolic NOD2 receptors play a key role in BCG-induced trained immunity (KALYUZHIN et al.). "*This actualizes the search for effective immunoactive preparations for prevention of respiratory infections in the pandemic among low molecular weight peptidoglycan fragments of the bacterial cell wall, muramylpeptides (MPs), which are known to be NOD2 agonists"* (KALYUZHIN et al.). KALYUZHIN et al. propose glucosaminylmuramyl dipeptide as a possible candidate (approved for clinical use in Russia and some other post-Soviet countries "for complex treatment and prevention of acute and recurrent respiratory infections.")

Based on the analysis of plasma levels of interferons and cytokines and expression of interferon-stimulated genes in mononuclear cells of COVID-19 patients with different severity of disease, KIM et al. demonstrated "*the importance of type I and III interferon reponses during the early phase of infection in controlling COVID-19 progression."*

According to patient data from SPOSITO et al., IFN-III, not IFN-I, plays a key role at mucosal surfaces during life-threatening viral infections. SPOSITO et al. found that IFNs, especially IFN-III, are over-represented in the lower airways in severe patients; in contrast, high levels of IFN-III (and, to as lesser extent, IFN-I) are found in the upper airways of patients with high viral burden, but reduced risk or severity.

Innate lymphoid cells (ILCs) are another part of the innate immune system, and their number is inversely correlated with the severity of disease, clinical and laboratory parameters. *"These results indicate that, by promoting disease tolerance, homeostatic ILCs protect against morbidity and mortality in SARS-CoV-2 infection, and suggest that reduction in the number of ILCs with age and in males accounts for the increased risk of severe COVID-19 in these demographic groups"* (SILVERSTEIN et al.).

Thus there seem to be bilaterial interactions between SARS-CoV-2 and innate immunity: a weakened innate immunity (e.g. low innate lymphoid cell counts), e.g. weakened by ageing, increases susceptibility to severe disease, whereas SARS-CoV-2 itself is able to decrease innate immunity (e.g. by suppression of interferon I response). So it is foreseeable what may happen if a virus that is able to weaken innate immunity on its own infects a person whose

innate immunity is already weak: The early infection cannot be controlled initially, spreads in the respiratory tract and beyond, and then it depends on many complex individual factors (that can hardly be controlled and some of them are not foreseeable) whether the disease will still be able to take a benign course or whether it will progress to immunopathology, severe or lethal disease (as was made visible by SARS-CoV-2-nanoluciferase in a mouse model; see ULLAH et al.).

Thus the only chance to avoid this critical situation is to strengthen both the adaptive immune response (by COVID-specific vaccination) and the innate immune response so that one can be sure that the initial viral infection is controlled in any case (if not by adaptive immunity, then by innate immunity, or *vice versa,* or in cooperation of both arms of the immune system), and that the infection has no chance to progress to a stage when unforeseeable und uncontrollable individual capabilities versus incapabilities, imbalances or dysregulations of the immune reponse (immunopathologies), including autoantibodies (pre-existing or *de novo*) or "overproduction" of "wrong", non-neutralizing antibodies decide about the fate of the disease and the patient.

Inactivated mycobacterium W

In a prospective trial (open label cohort study) from India with 96 front line healthcare workers (HCWs), 32 HCWs received **heat killed Mycobacterium w (= Mw group)** (syn. *Mycobacterium indicus pranii*) as a TLR2 agonist to modulate innate immune response, while 64 HCWs were controls **(JAISWAL et al.).** The trial was performed during the peak of the pandemic in New Delhi.

After 100 days of follow-up for all participants, there was one symptomatic RT-PCR confirmed COVID-19 in the Mw group, and 30 in the control group (3.1 % vs. 46.8 %) (p = 0.0001; protection efficacy: 93.33 %; CI: 53.3 – 99.1 %). Hazard Ratio for developing COVID-19 in the control group was 19.025 (p=0.0038) compared to the Mw group.

Among the 30 cases in the control group, there were 4 hospitalizations. The only infection in the Mw group was mild and the symptoms were present only for 3 days, compared to a median of 12 days (range: 3 – 36 days) in the control group.

The only side effect of the intervention was a self-limiting local injection site reaction in 14 HCWs.

Mycobacterium w is an approved immunomodulatory in India, used for example in leprosy, but also in the context of cancer, genital warts etc. 0.1 ml Mw (Sepsivac, Cadila Pharmaceuticals, India) was given intradermally in each arm.

Subjects in the Mw group underwent two additional random SARS-CoV-2 specific RT-PCR evaluations 4 weeks apart what means that SARS-CoV-2 detection was biased in favor of a higher chance of detection in the Mw group compared to the control group. Moreover, it was reported that the Mw group had a greater number of exposures to COVID-19.

Long-term protective efficacy is still unknown since the study reported about the first 100 days. The authors note that there are differences in the innate immune response generated by mycobacterium w and BCG vaccine, e.g. with regard to the upregulation of natural killer cells. Thus the effects of BCG vaccine on COVID prevalence and outcomes have to be investigated separately.

Moreover, the median age in both groups was 28 years (range: 22 – 56 years), so it remains to be studied whether Mw injection has the same efficacy in older people.

If the results of JAISWAL et al. can be replicated in larger trials, mycobacterium w may offer a benchmark that is hard to beat by "true" COVID vaccines. Besides the high protective effect, there was only one vaccination date (in contrast to two separates dates, a few weeks apart, in most COVID vaccines), and side effects were only local reactions at the injection sites in contrast to the mild systemic illness which has to be expected preferentially on days 1 – 3 after COVID vaccination. Follow-up was 100 days, about 67 % more than the two months demanded by the FDA for COVID vaccines.

And comparing Mw with BCG instead of COVID 19 vaccines, it has to be noted that the mycobacterium w preparation is no live vaccine, but inactivated by heat, resulting in a much more favorable risk profile compared to BCG.

Beside of prophylaxis, a small case series about the treatment of severe COVID patients with mycobacterium w vaccine (0.3 ml/day Immuvac for three consecutive days) is presented by SINGH SEHGAL et al., and the treatment was successful and safe. And there are preliminary reports that Sepsivac reduced death in critically ill patients by more than 50 %:

https://science.thewire.in/health/covid-19-csir-gram-negative-sepsis-sepsivac-clinical-trials/

Most important, Mw must have had a sterilizing effect in JAISWAL et al.; otherwise there would have been much more positive PCR results in the vaccinated group in that trial.

There is a much larger ongoing trial about Mw in India (placebo-controlled RCT) with two doses of Sepsivac, 15 days apart:

NCT04353518 (=CTRI/2020/05/025277)
Link: https://clinicaltrials.gov/ct2/show/NCT04353518

No interim results have been published so far. With regard to the JAISWAL data, it would be very important to have interim results as quickly as possible in order to understand whether the JAISWAL results can be replicated in larger cohorts. In such critical and dangerous times, it would be important to publish interim results of ongoing prophylactic trials quickly (as preprints), whether they are positive or not, so that strategies can be adapted or changed. The concept of interim analysis and early reporting should not be confined to phase III trials of "true" COVID vaccines. Why isn't it practiced in trials with other types of vaccines, e.g. those based on mycobacteria or MMR (see below)?

Other mycobacterial candidates

Besides *Mycobacterium indicus pranii,* there is another interesting candidate: ***Mycobacterium obuense*** **(IMM-101) (KLEEN et al.).** The rationale behind its use in COVID-19 is identical to that for *Mycobacterium indicus pranii;* it is also a TLR2 agonist, and it elicits a type-1 biased immune response. It is already subject of a prophylactic trial in cancer patients with increased risk of COVID exposure in Canada **(NCT04442048, COV-IMMUNO):**

„The treatment regimen with IMM-101 will be one 1.0 mg (= 0.1 mL) dose given on Day 0, followed by a second dose of 0.5 mg (= 0.05 mL) on Day 14 (-2/+5 days), and a third Dose of 0.5 mg (= 0.05 mL) on Day 45 (+/-14 days)"

Link: https://clinicaltrials.gov/ct2/show/NCT04442048

Estimated study completion is planned for March 31st, 2021, and primary completion date is December 31st, 2020.

NCT04453488 (RUTICOVID-19) examines RUTI vaccine in a double-blind placebo-controlled RCT in HCWs in Spain. Each dose of the RUTI® vaccine contains 25 µg of fragmented, purified and liposomed heat-inactivated *Mycobacterium tuberculosis* bacilli. Estimated completion of the study was December 2020.

In contrast to BCG, such inactivated mycobacterial vaccines can be administered even to cancer patients and other risk groups who cannot be infected by a live-attenuated mycobacterial vaccine (KLEEN et al.). Compared to BCG, this is a low-threshold prophylactic measure.

KLEEN et al. propose three different ways how mycobacterial vaccines may help in the current pandemic: *„(i) as prophylaxis, with enhanced innate memory and increased basal systemic type 1 immunity preventing viral establishment; (ii) as a treatment for patients in early stages of disease, with increased local and systemic type 1 inflammation enhancing killing of virally infected host cells; (iii) as an adjuvant for future COVID-19 vaccines."*

Fig. 3 in KLEEN et al. depicts the four different pathways how such a vaccine may act against COVID-19.

Link: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7498663/#B210

MOHAPATRA et al. assume that even contact and infections with environmental non-tuberculous mycobacteria in countries like India may offer sustained nonspecific immunological response in a similar way like supposed for BCG vaccination that may be correlated with reduced disease burden and severity of COVID-19: *"It is possible that people of the TB endemic countries like India despite vast population and growing numbers of COVID19 infection, have acquired some protections from severity and deaths from COVID-19 in comparison to TB non-endemic countries (like Europe and USA). Although it appears that*

such nonspecific immunity may not able to stop COVID 19 infections, but is likely to diminish its impact on severity of COVID-19".

In fact, COVID deaths per 1.000.000 population are much less in countries like India, Pakistan and Bangladesh (November 1st, 2020: India: 89; Bangladesh: 36; Pakistan: 31) compared to world (155.2) or many countries in Europe, North, Central and South America where the burden of TB is much smaller and COVID deaths/1 Mill. about one order of magnitude higher than in India and its neighbor countries. These data support the hypothesis of MOHAPATRA et al. that environmental mycobacteria may elicit some degree of nonspecific immune response which may reduce the risk of critical outcomes. These differences of the "death rates per million population" are even more surprising since about 30 % of all people in South Asia (and 63 % in Bangladesh) are carriers of a special haplotype that is associated with severe disease and inherited from Neanderthals (ZEBERG and PÄÄBO). Now deleterious in the context of COVID-19, it must have presented some advantage to people in South Asia in the past so that it was positively selected, possibly in the context of other infectious diseases. The Neanderthal haplotype is absent from Central Africa and only very rarely found in South America, whereas COVID mortality in South America is nevertheless high. The MOHAPATRA paper may offer an explanation for this conundrum.

Whereas MOHAPATRA et al. discussed non-tuberculous environmental mycobacteria in tap water in India, a special strain of an environmental mycobacterium was also isolated from a river in Spain, ***Mycobacterium setense manresensis.***

There is now an ongoing prophylactic trial in Spain with the food supplement **Manremyc** in healthcare workers (also known as Nyaditum resae). Manremyc contains heat-inactivated ***Mycobacterium setense manresensis*** and mannitol, and one capsule is administered orally daily for 14 days, followed by an interval of at least 6 months without Manremyc intake (NCT04452773).

Link: https://clinicaltrials.gov/ct2/show/NCT04452773

The original intention of the food supplement Manremyc was to stop progression towards active tuberculosis, or the risk of relapse of active tuberculosis. Since the bacterial strain is occurring naturally in water, including tap water, it is classified as a food supplement (see: EFSA Panel). Thus regulatory restrictions are much lower compared to Sepsivac *(Mycobacterium indicus pranii)* or *Mycobacterium obuense* injections.

However, no interim data are available so far from that trial. As for the mycobacterium w RCT, it would also be important for the Manremyc trial to make interims data available as soon as possible.

However, each Mycobacterium species or subspecies and each BCG vaccine strain has to be investigated separately with regard to their effects on COVID-19. With regard to COVID-19, it is essential to elicit a type-1-biased immune response. The opposite, a type-2-biased response, could be dangerous and may result in an enhancement of disease severity. As KLEEN et al. pointed out, not all BCG vaccines favor a type-1-biased response. This variability may also apply to single mycobacterium species or subspecies, whether inactivated or not. Thus one cannot assume that any BCG vaccine or mycobacterial strain is preventive and

favorable with regard to COVID-19 infection or outcome in the absence of trial results with that individual strain.

ACOSTA et al. discuss different effects of latent TB infections (LTBI) and environmental non-tuberculous mycobacteria (the „mycobacteriome“, including also BCG if it was administered). LTFI (present in a quarter of the human population) triggers M1 macrophage presence and activates innate immune responses („trained immunity“) in a Th1-biased manner with downregulation of Th2 responses, producing *„a bystander effect on acquired immune responses, which could generate a protective environment against heterologous microorganisms, including SARS-CoV-2.“* (ACOSTA et al.). In contrast, non-tuberculous mycobacteria are *„associated with the production of a regulatory environment mediated by transforming growth factor-β (TGF-β) and interleukin-10 (IL-10), induction of T regulatory cells, and inhibition of neutrophil infiltration (…). Such an environment could provide protection against the severe forms of COVID-19, inhibiting the uncontrolled inflammation resulting in the so-called ‚cytokine storm'."* In summary, BCG and LTBI are suggested to affect SARS-CoV-2 multiplication by trained immunity and cross-reactive immune responses, whereas non-tuberculous mycobacteria limit the pathological inflammation that is triggered by the host immune response. According to that model, BCG and LTBI should predominantly affect the early course of the disease, whereas non-tuberculous mycobacteria are expected to attenuate the severity of the disease.

In addition to the trials mentioned above, there are some other registered trials with mycobacterial formulations (but without BCG trials):

ChiCTR2000030016 – inhalation of an inactivated mycobacterial vaccine (mycobacterium vaccae) for treatment of ill patients (China)

NCT04358809 / CTRI/2020/05/025271 – Mycobacterium w in hospitalized patients (but not critically ill) (India)

NCT04347174 / CTRI/2020/04/024846 – Mycobacterium w in critically ill patients (India)

CTRI/2020/05/025350 – Mycobacterium w (Sepsivac) in COVID-19 patients (India)

CTRI/2020/08/027475 – Mycobacterium w (Sepsivac) in mild – moderate COVID-19 (India)

CTRI/2020/09/027741 – Mycobacterium w (Sepsivac) in COVID-19 patients (India)

CTRI/2020/10/028326 – Mycobacterium w in critically ill patients, HCWs and close contacts – assessment of innate memory NK cells in response to MW vaccination

Moreover, there are 25 BCG trials (see table 4 in BAGHERI and MONTAZERI).

Uncertainty about the role of childhood BCG vaccination

There is a lot of controversy whether BCG vaccination in childhood may reduce COVID-related risks. There are ecological studies which point to that, whereas others don't. However, this is no important question with regard to the concept that is discussed here. There is no doubt that childhood BCG vaccination (whether somehow effective or not) is unable to prevent severe COVID or COVID death with certainty. If there is an effect at all, it would be only gradual (small or at best modest). Noone can feel "protected" because he got BCG as a young child.

The only way how childhood BCG vaccination, decades ago, may matter in the context that is discussed here is that one should examine whether people who got BCG vaccine in childhood respond in another way (maybe better?) to a COVID vaccine, or whether COVID vaccine is less efficacious in such a population because the people may have already a sort of "basic protection" elicited by the former BCG vaccination. In the first case, the relative risk reduction following COVID vaccination may be larger in a BCG-vaccinated population (if the BCG vaccination has still a primer effect, decades after vaccination), but in the latter case, the risk reduction may be smaller in BCG-vaccinated populations if there is no such primer effect (perhaps because it waned over time), but still a generally reduced COVID risk due to childhood BCG.

Therefore, a history of childhood or - particularly ! - more recent BCG vaccination may be a confounder that may impact the results of phase III trials of "true" COVID vaccines in one or the other direction, so it should be taken into account in the analyses of phase III trials of COVID vaccines, especially in multinational trials with some countries with universal BCG vaccine strategy and others without BCG strategy.

Beside its role as a possible confounder in vaccine trials, it is not important for the purpose discussed in this paper whether childhood BCG vaccination has an effect on COVID prevalence and outcomes or not. Much more relevant is the effect of *recent* BCG vaccination.

In the first half of August, **AMIRLAK et al.** were the first who reported results of a retrospective BCG trial in HCWs. All staff of the Emirates International Hospital in the United Arab Emirates were offered a booster BCG vaccine in early March 2020. In April, May und June, all the hospital staff were routinely tested (PCR, nasopharyngeal swabs; government-mandated testing). Additional tests were performed in settings of contact with positive patients or in the case of symptoms of staff members.

Among 71 participants who received the booster BCG, no one was positive for COVID-19 until the end of June, compared to 8.6 % (18/209) who did not receive the vaccine ($p = 0.004$). 13 of the 18 positive cases were symptomatic. No local or systemic complications were noted following BCG vaccination. All participants had already received BCG vaccination after birth.

In Greece, older adults who were hospitalized for any reason were offered to participate in a RCT to receive either a single dose of BCG vaccine or placebo at the day of their discharge

(ACTIVATE trial; NCT03296423). Every patient is followed up for 12 months. An interim analysis (April 29th 2020) found a 53 % decrease of the incidence of new infections of any origin and location in the BCG group compared to the placebo group. Among them, the reduction of all respiratory tract infections was 80 %, and patients with coronary heart disease and COPD profited at most. However, this trial started before COVID-19 epidemic and is not specifically about the prevention of COVID-19. Nevertheless, *"this interim analysis clearly enhances the concept that BCG can be protective against COVID-19"* (https://clinicaltrials.gov/ct2/show/NCT04414267), and, as a consequence, a new trial was started (ACTIVATE II, NCT04414267) to examine the effects of a single dose of BCG vaccine in older people (> 50 years) with regard to the prevention of COVID-19.

A retrospective study from Rhode Islands (US) found that among 120 COVID-19 infected patients, those with BCG vaccination in their life history were less likely to require hospital admission (3.7 % vs. 15.8 %, p = 0.019), though the median age was 10 years higher in the BCG group (41 instead of 31 years). After adjusting for demographics and comorbidities, the significance of this result improved even a little more (p = 0.017). One patient in the non-BCG group died (1/38 = 2.6 %), whereas there was no death in the BCG group (WENG C et al.). However, in Israel, BCG vaccination in childhood had no influence on the risk of COVID infection in the age group between 35 and 41 years (HAMIEL et al.). Since there were only 2 cases of severe disease and no death among the 660 infected people of that age group (one case in each group), the results don't allow suggestions with regard to the influence of childhood BCG immunization on the severity of the disease. The cohort was probably too young to analyse such effects. In contrast, in WENG et al. 25 % of all patients were > 50 years and thus differences in the severity of the disease may have become visible more clearly. WENG et al. conclude that prior BCG immunization may decrease the severity of the disease, but not the risk of infection or of symptomatic disease because there were no significant differences with regard to the frequency of COVID-specific symptoms or a summarized unweighted or weighted symptom score between BCG- and non-BCG patients.

But in a multicenter study from Turkey, infected health care workers with a history of BCG vaccination didn't have a smaller risk of hospitalization than those without a BCG scar (in fact, the risk was even higher in the BCG scar group), and the authors suggest a hyper-triggered immune system as explanation (TORUN et al.).

In a small retrospective study of HCWs in a gastroenterology department from a tertiary-care hospital in India (based on PCR and IgG), neither BCG vaccination in childhood nor MMR vaccination was associated with a significant protection from COVID-19 (COVID PCR and/or IgG positive: BCG yes vs. no: 31.8 % vs. 34.5 %; MMR: 38.5 % vs. 27.7 %), whereas there was an insignificant protective trend for the intake of unspecified immune boosters (yes vs. no: 25.7 vs. 35.4 %; OR for no vs. yes: 1.58; CI: 0.65 – 3.82) (KUMAR GOENKA et al.).

Unfortunately, the analysis of the study data according to BCG and MMR status didn't distinguish between symptomatic and asymptomatic disease. If would have been interesting to know whether BCG or MMR had any impact on the occurrence or severity of symptoms.

However, more data are needed with regard to BCG vaccination. First, there may be a difference between the effect on COVID-19 in the case of a recent primary vaccination, of a

recent booster vaccination (like in the study from AMIRLAK et al.), or if one looks at long-term effects of childhood vaccinations. These are three different conditions which have to be investigated apart from one another, and it is especially the latter situation that is regarded as very controversary.

Second, there are several types (strains) of the BCG vaccine which may differ in their anti-COVID effects, as already pointed out by KLEEN et al., including the new type VPM1002. They may have differential effects on type-1- and type-2-biased immune responses.

Third, there is at least a theoretical possibility that BCG vaccination, especially a more recent one, may have deleterious effects in some COVID patients if it further enhances hyperactivity of the immune system and cytokine storms, as suggested by TORUN et al.. But this may depend on the relative impact of that special BCG strain on Th1- vs. Th2-skewed immunity. Therefore results from larger trials are needed in order to exclude such a deleterious effect with certainty.

DATTA and DATTA found that "early" BCG strains were most effective, whereas "late" strains seem to be ineffective to induce trained immunity because late strains "*are deficient in their ability to produce cell wall methoxymycolic acid which constitute a key group of ligands proficient in inducing trained immunity".* The event rate (a sort of mortality rate) in countries with "late strain" vaccination programs was similar to countries with no BCG program at all (0.031 vs. 0.034), while it was only 0.018 in countries with "early strain" vaccination. Altogether, DATTA and DATTA found a reduction of the event rate by about 40 %, and this applied also to high-risk subgroups like people $\geq$65 years (-36.1 %).

The detailed study of DATTA and DATTA addressed many confounders (like age structure of different populations and the prevalence of relevant comorbidities), but won't put an end to the endless discussions and dozens of papers pro or contra any effect of BCG vaccination in the past. If the protective effect is restricted to "early strains" and to a lesser extent to "mixed strains", but not to "late strains", it may become plausible why ecological studies about childhood BCG vaccination produce so many controversary results.

WHO doesn't recommend BCG vaccination for COVID prevention so far; however, the WHO statement dates from April 12th and the favorable results (ACTIVATE interim analysis, AMIRLAK et al.) were published later. Nevertheless, for the three reasons mentioned above, there is an urgent need for many more data. Interim analyses of larger trials like BRACE or BCG-CORONA would be very helpful.

Based (i) on data from 20 European countries and (ii) a comparison between West and East Germany, SINGH et al. observed a significant negative correlation between COVID incidence and tuberculin immunoreactivity (as a measure of cell-mediated immunity persistence as a result of an exposure to *Mycobacterium spp.,* which may be a BCG vaccine but also a natural infection), and there was also a trend for a negative correlation with COVID mortality.

However, though SINGH et al. reported higher latent TB infection rates in East Germany, based on a single study from 2006 (9.2 % in West Germany compared to 22.5 % in East Germany, when calculated for the year 2020 based on the original data from 2006), and this may correlate well with the lower COVID incidence and mortality (per million population) in East Germany, an own analysis based on the RKI report for October 31st found no significant

difference in the case fatality rates (CFR) of western and eastern German federal countries (mean 2.06 % for 10 western countries; range: 1.35 – 3.0 %; mean 1.93 % for 5 eastern countries; range: 0.81 – 2.98 %; without Berlin).

In contrast, TAKAHASHI H found a worldwide association between latent tuberculosis infection and reduced COVID-19 mortality. According to WHO, about a quarter of the world's population may have latent tuberculosis infection. This is associated with persistent immune responses that are stimulated by *Mycobacterium tuberculosis* antigens in the absence of evidence of clinically active tuberculosis (TAKAHASHI H). The situation of latent tuberculosis infection with regard to COVID 19 may be different from childhood BCG vaccination; in a large ecological study that addressed many confounders, CHIMOYI et al. found no association between BCG vaccination strategy and COVID morbidity and mortality. Their study included 97 countries (73 current BCG vaccination; 13 previous BCG vaccination, 11 never BCG vaccination). Regression models on country level showed no effect of BCG on COVID cases and deaths. There was some initial evidence at time points at the beginning of the epidemic that weakened over time. This may explain positive findings in early studies. Moreover, three studies based on individual data from Sweden, Israel and Germany found no association between BCG vaccination history and COVID hospitalizations (Sweden), PCR positivity in individuals with symptoms suspicious for COVID-19 (Israel) or mortality (Eastern vs. Western Germany). As TAKAHASHI pointed out, *"but that does not exclude the possibility that BCG vaccination later in life may be effective."*

There is a wealth of ecological studies meanwhile *pro* and *contra* any role of early childhood BCG vaccinations with regard to COVID incidence or mortality. As TAKAHASHI showed, there may have been some initial evidence at the beginning of the pandemic that waned as the pandemic progressed and data on infections or deaths became more robust.

It is outside the scope of this paper to discuss this subject*, and there are three reasons not to do so. First, it is very questionable that the protective effect of early childhood BCG vaccination lasts for several decades; it seems to be limited to about 15 years (MOHAPATRA et al.) or even much shorter (< 5 years: SINGH and SINGH). SOHRABI et al. assume *"that the protective efficacy of childhood BCG against all forms of TB may last for 20 years or even longer, but that the non-specific protection is likely much shorter" … "epidemiological data showed that unspecific protective effects might last 3–5 years in human."*

Moreover, ecological studies about BCG vaccination may overlook nonspecific immunological responses due to *environmental non-tuberculous mycobacteria* which may account for positive tuberculin tests in non-BCG-vaccinated people in some countries like India, but elsewhere too (MOHAPATRA et al.). Third, the question whether a new (recent) BCG vaccination offers some degree of protection from COVID-19 is very different from the question whether BCG vaccination decades ago has still some residual effect.

*The rationale behind the idea of BCG vaccination for protection from COVID death but also the controversary results, limitations and confounders of epidemiological and ecological studies are described in detail by GOPALASWAMY et al., whereas the SOHRABI et al. paper is highly recommended to understand the rationale, the molecular and cellular mechanisms behind the concept of trained, unspecific innate immunity following BCG vaccination.

Besides unspecific heterologous effects of BCG vaccination with regard to infections by e.g. viruses or bacteria, there are first hints that there seems to be a more specific effect on SARS-CoV-2: An *in silico* study demonstrated 8 BCG derived peptides with significant sequence homology to either SARS-CoV-2 NSP4 or NSP13 derived peptides (EGGENHUIZEN et al.). In a subsequent *in vitro* co-culture system, "human CD4+ and CD8+ T cells primed with a BCG derived peptide developed enhanced reactivity to its corresponding SARS-CoV-2 derived peptide". The authors recommend to study the concept to use BCG vaccination "to induce cross-reactive SARS-CoV-2 specific T cell responses." (EGGENHUIZEN et al.).

But it is also important to note that BCG vaccination, as primary vaccination or as booster, is problematic or contraindicated in many risk groups for severe COVID-19 (see KLEEN et al.). However, interestingly, there is already a report that BCG vaccination may also be helpful for the treatment of COVID patients, both in those who already got BCG in childhood and those who didn't (PADMANABHAN et al.). In a trial from Mumbai, 60 hospitalized COVID patients with pneumonia and requirement for oxygen therapy were randomized 1 : 1 to receive a single adult dose of intradermal BCG or normal saline (beside standard of care in both groups). Compared to the control group, there was a reduction in oxygen requirements from day 3-4 and improved radiological resolution from day 7-15 in the BCG group. There were 4 ICU admissions and 2 deaths in the control group (concerning altogether 5 patients), but only 1 ICU admission and no death in the BCG group. Whereas specific IgG levels increased in the BCG group, there was no evidence that BCG induced cytokine storms. Only four patients showed localized inflammatory response at the BCG injection site. Of note, a third of the patients were naive for childhood BCG vaccination.

Median age was higher in the BCG group (49 vs. 41.5 years), and there were more obese patients (11 vs. 3) and more males (20 vs. 16) in the BCG group, thus basic risk for worse outcomes was higher in the BCG group.

PADMANABHAN et al. concluded, *"that BCG is a safe, cost-effective treatment that can be introduced as a standard of care in patients with moderate Covid-19 that can reduce requirement of oxygen supplemented beds and disease burden in low resource countries…"*

Though this prospective trial was about treatment of already ill patients with pneumonia and need for oxygen support, it is another hint that recent BCG vaccination may have preventive effects (as already demonstrated in the retrospective preventive trial of AMIRLAK et al.), but also that these effects of a recent BCG dose are independent from presence or absence of childhood BCG vaccination and thus not dependent on a booster situation (in the trial from AMIRLAK et al., all participants had had childhood BCG vaccine).

Apart from BCG, LIN YR et al. found in a small RCT that an inhaled mycobacterial formulation *(Mycobacterium vaccae)* accelerated PCR conversion in oropharyngeal swabs (2.9 vs. 6.8 days in the control group, p = 0.045) in moderate patients and prevented relapse and re-positivity. The study was underpowered (n = 31) to analyse serious clinical outcomes (no death, no conversion to severe or critical disease in both groups). LIU et al. suppose that *M. vaccae* "might be beneficial to the prevention and treatment of COVID-19."

An analysis of (i) available transcriptomic data related to BCG vaccination and viral respiratory infections and (ii) transcriptomic alterations reported in COVID-19 showed *"that BCG vaccination leads to very long-lasting transcriptomic changes which mimic viral*

infections by upregulated antiviral defense response (consistent with the concept of trained immunity)*, and oppose viral infections by downregulated myeloid cell activation mediated immune response"* (SHARMA A). Most important, the study found *"no association of BCG vaccination with systemic inflammation, alleviating the concern that the vaccine may add to inflammatory response and worsen severe COVID-19"* (SHARMA A). The study offers a rationale for lower prevalence and mortality of COVID-19 in countries with childhood BCG vaccination. For the effects of BCG vaccination on trained innate immunity on a cellular and molecular level, see WANNIGAMA and JACQUET.

SOHRABI et al. discuss the option that BCG administration via the respiratory tract (intranasal or as inhalation) may be more efficient with regard to its effects on COVID-19.

Conclusions – the "double protection strategy"

JAISWAL et al. presented the first evidence that a single vaccination with inactivated mycobacteria of a special strain may elicit an immune response which is able (i) to avoid PCR-confirmed COVID-19 infection in most cases of high exposure and (ii) to reduce severity and duration of the disease in the remaining case(s) who got infected in spite of this vaccination.

There is an ongoing large trial about exactly the same subject with the same preparation of mycobacteria (Sepsivac) (**NCT04353518),** but interim results haven't been published so far.

There are two other ongoing trials about inactivated mycobacteria (one injectable and one to be taken orally as a food supplement: *Mycobacterium obuense,* **NCT04442048,** and oral intake of *Mycobacterium setense manresensis,* **NCT04452773)** for prophylaxis**;** so far, no (interim) results are available. The latter would avoid legal restrictions because of its status as a food supplement in the EU.

Indirect evidence in favor of an immune reponse following contact to non-tuberculous mycobacteria comes from countries where many people have contact to such bacteria in drinking water, e.g. India (MOHAPATRA et al.). This may mimic the effect of oral administration like in the case of the food supplement Manremyc (Nyaditum resae).

"Fresh" BCG vaccination may be an alternative to the administration of inactivated mycobacteria. But whereas there are favorable results in elder people from the ACTIVATE trial from Greece, KLEEN et al. warn that a live attenuated mycobacterial vaccine is contraindicated in many people who need protection from COVID-19 at most because of age or comorbidities. Nevertheless, protective effects of recent BCG vaccination are indirect evidence in favor of a mycobacterium-based concept of "dual COVID protection", a sort of "proof of concept". Whereas no results have been published so far from registered BCG trials with HCWs, the retrospective study from AMIRLAK et al. and the prospective treatment RCT from PADMANABHAN et al. offer first evidence that recent BCG vaccination is efficient too, independent of whether this was the first BCG vaccination or whether the person had already been vaccinated as a child.

However, because of risks, side effects and tolerability, inactivated mycobacteria should be preferred if they elicit the same favorable effects like BCG, and the JAISWAL trial demonstrated very high effectiveness and little (and well tolerable) side effects for the MW preparation (Sepsivac).

The concept is not to *substitute* "real" COVID vaccines by mycobacterial vaccines like MW or oral capsules like Manremyc. Though the JAISWAL trial is very promising, it is improbable that one-time mycobacterium w injection or a similar administration of highly immunogenic mycobacterial strains are able to prevent COVID-19 as well as an *optimal* COVID vaccine. But the question is whether an *optimal* COVID vaccine will be available in the next years at all. An *optimal* vaccine would have to offer nearly 100 % protection and exclude the risk of ADE or similar immunopathologies also under conditions when the protective effect is waning over time, or when there are new mutants of the virus circulating around. This benchmark for a vaccine is extremely high for an infectious disease like COVID-19 with its special, deleterious and self-reinforcing effects on the immune system, and a virus that follows different strategies for immune evasion and vaccine escape.

Thus one can be nearly sure that the COVID vaccines from the first generation won't be "optimal" as defined above, and, most worrying, many or all of them will be sterilizing only modestly. As discussed above, Mycobacterium w seems to be sterilizing, at least in most cases, otherwise there would have been more positive PCR results in JAISWAL et al.

Thus the idea is to generate a sort of "double protection" by combining both COVID vaccine and mycobacterial administration in order to achieve some synergism: to protect people by both an suboptimal COVID vaccine and immunomodulation by inactivated mycobacteria like MW (and maybe others if they prove to be as successful as MW).

If the COVID vaccine itself is unable to elicit an early (mucosal) immune response and sterilizing effect in a vaccinated and exposed/infected individual, maybe the "trained" innate immunity due to mycobacterial immunomodulation may do? The combination of both may offer early sterilization of the COVID infection, i.e. immediate or quick reduction of viral load in the respiratory tract so that the viral infection is no longer able to spread further into the lungs and into the body and to induce the immunopathologies which may result in severe disease, cytokine storms and other serious sequelae including "long covid" in survivors.

Instead of a COVID vaccine alone, the combination of a COVID-vaccine and "trained" innate immunity by administration of inactivated mycobacteria (as a vaccine, oral capsule or inhalation) may improve the short- and long-term efficacy of the COVID vaccine and act synergistically. The trained immunity from the mycobacteria may still offer some degree of protection when the protective effects of the COVID vaccine have already waned. "Trained" innate immunity by mycobacteria may compensate for the deficits of the COVID vaccines.

The "double protection strategy" of both COVID vaccine and mycobacterial vaccine (or oral capsules or inhalation) to strengthen unspecific trained/innate immunity may not only improve the efficacy of the COVID vaccine as such; it may also offer a second line of defense if viral mutants evolve and circulate that are not or only poorly covered by the antibodies from the COVID vaccine, whether this may result in ADE or not. In that case, the unspecific immune reponse which was elicited by the mycobacterial vaccine may still offer protection.

This is an example how double protection by both COVID vaccine and mycobacterial administration may be not only synergistic, but even complementary and possibly life-saving instead of simple and unnecessary redundancy. If the COVID vaccine doesn't work any more e.g. because of infection by an escape mutation, the mycobacterial vaccine may still help.

As shown in the treatment trial of PADMANABHAM et al. (for BCG), the effect of mycobacterial administration can be experienced very quickly. While it will need about two weeks after the first dose of a COVID vaccine to generate (comparatively) low levels of anti-SARS-CoV-2 antibodies, and the full effectiveness of the vaccine cannot be expected before 1 – 2 weeks after the second dose, mycobacteria seem to elicit a quicker response of the immune system; otherwise the results from PADMANABHAM et al. weren't achievable. So if there is a new variant of the virus which is not at all or only badly neutralized by the antibodies from the vaccine, the immune response generated by the mycobacterial preparation (like MW injection) may still be able to eliminate the virus quickly, before the low neutralizing capacity of the vaccine-generated antibodies may trigger ADE or other immunopathologies.

Thus, the combination strategy of COVID vaccine and administration of mycobacteria may prevent the occurence of ADE or similar immunopathologies when new viral variants evolve which are not or only hardly neutralized by the vaccine-generated antibodies.

However, the timeline of their administration is an open question. As quoted above, KLEEN et al. proposed BCG or Mycobacteria as an adjuvant to future COVID vaccines. So the question is whether to administrate the mycobacteria some time *before* the COVID vaccine (as a sort of "primer"), *simultaneously* (on the other arm?), or some *time later,* maybe even repeatedly e.g. in case of oral administration (for Manremyc, there is a recommendation of an interval of at least 6 months before restart of daily intake for 14 days).

The next steps on the way towards this "double protection strategy" should be:

● to make available the interim results of the three ongoing prophylactic studies about different inactivated mycobacteria as mentioned above (MW, *M. obuense,* Manremyc).

Do they replicate the favorite results from JAISWAL et al.?

●● Results from prophylactic BCG trials may also be helpful in the sense of a "proof of concept", though inactivated mycobacteria should be preferred for a "double protection strategy" due to lower risks, less side effects and better tolerability, especially with regard to the risk groups for severe COVID-19. And as JAISWAL et al. pointed out, the mechanisms of immunomodulation by MW are not identical to that of BCG, and KLEEN et al. and DATTA and DATTA point to important differences between different BCG strains. Most important is a type-1-biased immune response.

●● Is oral intake of special inactivated mycobacteria as a food supplement (like Manremyc) as effective as the MW vaccine (if it is, this may eliminate legal restrictions), or is injection as a vaccine mandatory to elicit the wanted immunomodulatory effects?

● to examine the “double protection strategy” in animal models.

It is generally accepted that non-primate and primate models are unable to recapitulate the whole spectrum of human COVID-19 disease with special reference to the later stages of COVID-19 disease, including “true” cytokine storms, severe and critical disease.

However, the rationale behind the “double protection strategy” is to induce an early and quick immune response which results in sterilizing immunity in the upper respiratory tract and lungs immediately after infection occurred. If local mucosal immunity works quickly, the infection cannot expand elsewhere in the body and is quickly overcome. And these early stages of the infection and disease can be recapitulated well in animal models. Thus the “double protection strategy“ is suitable to be tested in animal models, including the time line: mycobacteria as a primer, as an adjuvant (simultaneously) or as a booster (or repeated booster)?

Parallel to research with inactivated mycobacterial preparations, alternative options should be considered and studied which may elicit a similar effect on trained/innate immunity and a type-1-biased immune reponse. However, after the JAISWAL study, the benchmark for that would be rather high, provided that the JAISWAL results can be replicated in the larger trial from India (**NCT04353518).**

Instead of conventional BCG vaccination, one may consider its local administration to the respiratory tract (SOHRABI et al.). However, it would take a long time to study this option from animal models for COVID-19 through all phases of clinical trials, thus the threshold for such a concept would be rather high. But experimental data on non-human primates already showed that *“intranasal or endobronchial administration induces a much more effective protection than any other route”* (SOHRABI et al.), though these trials were not about SARS-CoV-2. But since the natural route for COVID infection is through the respiratory tract, and since *“pulmonary mucosal immunisation was shown to be more efficient than intradermal administration against tuberculosis”,* it is highly plausible that *“intranasal or pulmonary vaccination could be advantageous in developing trained immunity in the cells of the respiratory tract. Efficient training of epithelial cells in healthy individuals may trigger their immune response to effectively curb COVID-19.”* (SOHRABI et al.). Inhalation of mycobacteria *(M. vaccae)* was also practiced in the small RCT from LIN YR et al. mentioned above and accelerated viral clearance in the upper respiratory tract very effectively.

There are sometimes suggestions that **measles vaccine** (or the combined **MMR vaccine**) may elicit a similar effect like the BCG vaccine. The large Crown Coronation trial, which was planned to study the effect of chloroquine prophylaxis in HCWs, was reorganized to study now a single injection of the MMR vaccine in 30.000 HCWs (NCT04333732).

However, it is improbable that MMR vaccine may be as effective as MW was in the JAISWAL trial. If so, there should be no cases of COVID-19 in young children in countries where childhood MMR vaccine is mandatory. At the best case, the lower COVID-19 prevalence and

the lower infectiousness of young (pre-school) children may be attributed partly to the MMR vaccine, but even if this is true, this is much less than what was achieved in the JAISWAL trial. And after more than 13 months of the pandemic, it would have become obvious if recent MMR vaccination offers full or nearly full protection from COVID-19. This is evidently not the case.

ASHFORD et al. suggested that the low infection rates and mild disease in children > 1 years in US may be due to childhood vaccinations, especially MMR, since the first MMR dose is recommended at 12-15 months by the CDC. However, data from Germany (where the first MMR dose is recommended in a similar age and vaccination rates are very high) put this into question, as far as the COVID infection rates are concerned:

Incidence per 100.000, Germany, RKI, age groups: 0 – 6 years, April 1st, 2021; cumulated incidence 2020 – March 2021 (source: survstat@rki 2.0):

Age: 0 years	1345
1 year	1418
2 years	1482
3 years	1643
4 years	1839
5 years	2032
6 years	2087

Thus COVID incidence increases with age even in young children. At the age of 2, one can be sure that most of all children have got both MMR doses in Germany. So if there is an effect of MMR on COVID incidence in children at all, one would expect a decreasing incidence starting at the age of 1, and, more pronounced, the age of 2. But the opposite is true (but see **supplement**).

However, these data don't exclude the possibility that the high proportion of asymptomatic or mild disease in children is a consequence of MMR vaccination, and it would be interesting to analyse the vaccination history of the rare cases of more severe grades of disease in preschool or school children. Nevertheless, the effects of MMR vaccination on COVID incidence and outcomes may be different in young children compared to (re-)vaccination of adults; thus there is a need to study that subject separately in adults (like in the CROWN CORONATION trial).

But it seems unrealistic to expect that the Crown Coronation Trial may show a degree of protection by the MMR vaccine comparable to that of MW in the JAISWAL trial. If there is some effect of MMR, the combination with a suboptimal COVID vaccine, before (as a sort of primer) or some time after COVID vaccination (as a sort of booster) may still be interesting, but this is probably less efficient than the idea of a "double protection strategy" based on inactivated mycobacteria if one takes the JAISWAL trial as a benchmark.

So far, LARENAS-LINNEMANN and RODRIGUEZ-MONROY reported about 36 COVID patients (6 > 55 years) who got infected within a few months after MMR vaccination, and all of them had a mild course of COVID-19. But the evidence from that small study is very weak. It may be a starting point for large studies of that kind.

PAWLOWSKI et al. found similar effects of HIB, MMR and inactivated polio vaccines in a study from US (Mayo Clinic). Vaccination with one (or several) of these vaccines during the last year was associated with point estimates of the OR between 0.53 and 0.57 with regard to positive PCR testing results. In the 2 year interval, HIB and Polio performed better than MMR (0.51, 0.51 and 0.69), and the same applied to the 5 year interval (0.61 vs. 0.62 vs. 0.76). Based on the point estimates, the effect of MMR seems to be less long-lasting. The varicella vaccine, a live vaccine like MMR, performed only a little worse than MMR:

Vaccine	**Age group**	**Inactivated vs. live vaccine**	**OR 1 year**	**OR 2 years**	**OR 5 years**
HIB	**most <9**	**inact.**	**0.53**	**0.51**	**0.61**
MMR	**most <9**	**live**	**0.56**	**0.69**	**0.76**
Polio	**most <9**	**inact.**	**0.57**	**0.51**	**0.62**
Varicella	**most <9**	**live**	**0.62**	**0.63**	**0.80**

However, since most vaccinations with these four vaccines were performed in children, the study doesn't allow any conclusions how (re-)vaccination with these vaccines in adult or elder people may modify their COVID risks.

Whereas it is supposed that protective effects against COVID-19 infection or worse outcomes are due to the measles component of the vaccine, GOLD et al. showed an inverse correlation ($r = -0.71$, $p < 0.001$) between mumps antibody titres (from MMR vaccination = MMR II group) in recovered patients and the severity of their former COVID-19 disease.* This association did not apply to patients with mumps titres due to former mumps disease. No associations were found with measles und rubella antibody titres, both following vaccination or natural infection.

*"within the MMR Ⅱ group, mumps titresof 134 to 300 arbitrary units (AU)/ml (*n* = 8) were found only in those who were functionally immune or asymptomatic; all with mild symptoms had mumps titresbelow 134 AU/ml (*n* = 17); all with moderate symptoms had mumps titresbelow 75 AU/ml (*n* = 11); all who had been hospitalized and had required oxygen had mumps titresbelow 32 AU/ml (*n* = 5)." (GOLD et al.).

However, a possible protective effect of vaccines like MMR is not directly antibody-mediated, since the antibodies generated by these vaccines (and many others: BCG, Pneumococcal, Rotavirus, Diphtheria, Tetanus, Pertussis, Hepatitis B, Haemophilus influenzae, Hepatitis B, Meningococcal) are not cross-reactive and unable to neutralize SARS-CoV-2 (KANDEIL A et al.). Thus the mumps antibody titres in the study from GOLD et al. have to be regarded only as a proxy for other pathways and capabilities of the immune system (like trained immunity) which were elicited by MMR vaccination, but not by natural disease. ANBARASU et al. suggest induction of interferons and activation of killer cells as innate immune responses following MMR vaccination. PAWLOWSKI et al. assume that *"interferon signalling indirectly mediated through MMR vaccine could potentially contribute to cross-protection towards SARS-CoV-2."*

Whereas there is a lot of discussion whether recent MMR vaccination may have some protective effect or not, an interim analysis of the Crown Coronation Trial may resolve this question much better than any theoretical discussion, small case-control study or ecological study can do.

Beside vaccines, **beta- or alpha-glucanes** are also suggested as immunomodulators which may "train" innate immunity (DE MARCO CASTRO, GELLER and YAN, MURPHY et al., JAWHARA, KAR and JOOSTEN) and may also contribute to the efficacy of vaccines (JIN Y et al., MORENO-MENDIETA et al., HUANG H et al., VETVICKA V et al.). Beta-glucanes "*act as a training agent which results in amplified immune responses when these trained immune cells are exposed to a secondary stimulus*" (JAWHARA). It was reported that training of human monocytes with beta-glucan enhanced the capacity of the immune response to eliminate fungi, bacteria, viruses and even parasites (JAWHARA). The phenomenon of trained innate immunity exposure to beta-glucans is associated with a variety of epigenetic mechanisms.

However, if the JAISWAL results can be replicated in the larger ongoing trial, it will be hard to touch this high benchmark. But since Mycobacterium w suspension is not available outside India, there is an urgent need to look for alternatives that are not so sensitive to legal and regulatory restrictions.

Unfortunately, there is no clinical trial about glucans registered in the WHO trial register about COVID-19 trials (date: 2020, November 24th; search: "glucan", "glucans", "yeast", "Saccharomyces", "cerevisiae", "shiitake", "Lentinus").

Eventually, even the **conundrum of Ivermectin (IVM) prophylaxis** may be possibly explained by innate immunity? Ivermectin prophylaxis of COVID-19 is highly effective, with a risk reduction for infection or symptomatic disease of about 90 % (see https://ivmmeta.com/) (based on a meta-analysis of 9 trials with 3663 participants; without differences in effect size between meta-analysed RCTs and Non-RCTs; accessed January 12th, 2021) and probably an even higher risk reduction with regard to worse outcomes like severe disease and death.

This protective effect is observed without reduction of the effect size if IVM is administered only once per month (ALAM et al.). This effectiveness cannot be explained by the direct antiviral effects (which are supposed to be suboptimal in common dose regimens, according to *in vitro* data), since the half life of IVM is only 16-18 hours in plasma, followed by wide tissue distribution, but even this ranges only from 4 up to 12 days (ALAM et al.). This makes it very hard to explain prophylactic antiviral activity of a single dose of IVM for a full month. ALAM et al. note that IVM has also immunomodulatory effects.

In fact, it was found to be very successful in the treatment of rosacea. However, rosacea is supposed to be caused by a dysregulation of the innate immune system (ALI et al., GUPTA et al., STEIN GOLD et al.). Successful treatment of rosacea by IVM may suggest that IVM helps to restore the regular function of the innate immune system. If so, this may also explain its prophylactic effect against COVID-19 far beyond its presence in the body that may be associated with direct antiviral action.

In that sense, one may ask whether it acts on the innate immune system in a similar way like mycobacterium w or other mycobacterial antigens, like some BCG strains or beta-glucans? This is only a hypothesis but it may explain the prophylaxis conundrum of IVM. If so, IVM could become part of the double protection strategy in those (tropical) countries where IVM is already recommended or indicated for its antiparasitic action.

In fact, the long-term monitoring of the effects of COVID vaccinations may help to understand about IVM if one compares the vaccine efficacy in comparable (e.g. neighbouring) countries with different antiparasitic IVM strategies (e.g. high uptake of IVM prophylaxis in one country compared to no recommendation in the neighbor country). If IVM strengthens the trained innate immune response (in a way that should be Th1-skewed) in the sense of the "double protection strategy", then (i) COVID vaccine efficacy should be higher in countries (or regions or individuals) with IVM prophylaxis, (ii) COVID vaccine efficacy should last longer (e.g. longer time until booster is needed), (iii) protection should still be present when new COVID strains arrive that evade the direct immune response of the COVID vaccine.

However, this is only a hypothesis and more research is needed to understand the immunomodulatory actions of IVM, their associations with the innate immune system, and whether IVM may be a component for the double protection strategy – a second line of defense if vaccine efficacy fails, and if such an effect is not only due its limited antiviral capacities, but especially because of its immunomodulatory effects that seem to impact innate immunity.

Another interesting finding from a study from Ethiopia is that intestinal parasitic infections (helminthic or protozoan) are associated with a lower risk for severe disease (aOR for severe disease in case of any intestinal parasitic infection: 0.41; CI: 0.22 – 0.77; protozoa: 0.45; CI: 0.21 – 0.98; helminths: 0.37; CI: 0.17 – 0.80) (GEBRECHERKOS et al.). Parasite-driven immunomodulatory responses may mute hyperinflammation via their influence on T helper cell responses. However, the parasite-driven response is Th2-skewed and counterbalances the hyperinflammation caused by Th1 response. This seems to counteract the need for a Th1-skewed immune response in case of COVID-19 infection. However, the needed Th1-skewed response is stage-specific: In the early stage of COVID-19 infection, immediately after virus inoculation, a Th1 response is needed in order to eradicate the infection so that the infection doesn't progress to a later stage at all. That's the reason why COVID-specific vaccines or unspecific vaccines like Sepsivac have to elicit a Th1-skewed immune response. But if the infection cannot be eradicated and if it progresses, the Th1 response supports hyperinflammation, and in that stage, the immune response should become more Th2-skewed to stop hyperinflammation and to counterbalance the Th1 response. This may explain why intestinal parasitic infection that promote a Th2-skewed immune reponse reduce the risk of severe disease. On the other hand, parasitic infections are probably unable to reduce the risk of COVID infection, but this was not subject of the study of GEBRECHERKOS et al.

ZIMMERMANN and CURTIS gave an exhaustive overview about the many reasons that may explain why children are less affected by COVID-19, both with regard to incidence and severity / prognosis / mortality. Among other factors, they point out that children have a

stronger innate immune response (the first-line defense against SARS-CoV-2) with a higher number of NK cells. *"Another important factor is 'trained immunity' which involves epigenetic reprogramming of innate immune cells (including NK cells) after exposure to certain stimuli, including infections and vaccinations, leading to 'memory'.These trained cells react faster and more strongly to subsequent pathogen challenge providing enhanced protection."* (ZIMMERMANN and CURTIS). However, the new data from children show that there is no clear evidence that they have a lower susceptibility to SARS-CoV-2, particularly with regard to VoCs like B.1.1.7. Nevertheless, a higher proportion of asymptomatic or paucisymptomatic disease may be responsible for a lower risk of detection and thus underreporting.

Also PIERCE et al. proposed that a more robust early innate immune response to SARS-CoV-2 may protect children against severe disease. Compared to adults, children displayed higher expression of genes associated with interferon signaling, NLRP3 inflammasome and other *innate* pathways in a small comparative study, and nasal fluids of children contained higher levels of IFN-alpha 2, IFN-gamma, IP-10, IL-8, IL-1beta than nasal fluids of adults (PIERCE et al.). *"These findings suggest that a more robust innate immune response in children compared to adults contributes to favorable clinical outcomes"* (PIERCE et al.).

The idea behind the second (non-specific) arm of the "double protection strategy" is thus to rejuvenate the innate immune system that it regains (or improves) some of its capabilities that are more pronounced in children than in older adults and that may contribute to the much lower risks and much better outcomes of COVID-19 in children.

An interesting phenomenon was found in the AZD1222 substudies from AstraZeneca (VOYSEY et al., Table S4): the antibody titres that were generated by two standard doses of the vaccine were much higher in South Africa compared to UK, and the effect was even more pronounced in elder participants (49287 vs. 19860 units). AZD1222 seems to be more immunogenic in South Africa than in UK. It would be interesting to see whether this can be replicated with other vaccines. If so, this could be another result of differences in trained innate immunity.

With regard to the limitations of the upcoming vaccines, including possible residual risks like ADE (less probable) or escape mutations (both from neutralizing antibodies and from CD8+ T cells) (very probable), it's worth to investigate not only one, but **different strategies** which may be able (i) to improve the protective effects of the vaccines, (ii) to strengthen their early (!) sterilizing effects, (iii) to reduce the risks of ADE and other immunopathologies which may occur if early sterilization was not successful, and (iv) to develop a strategy to strengthen a type-1-biased innate immune response following COVID infection, resulting in quick eradication of the infection, thereby avoiding the immunopathologies which are responsible for the severe and fatal outcomes of COVID infections.

Moreoever, it is necessary to take into account sex differences in the immune response to COVID-19. The balance between different arms of the immune system (like T cell response, innate immune activation) is sex-dependent (TAKAHASHI T et al.). Strategies to change this balance, e.g. by strengthening the Th1-biased innate immune response, may work differently in men and women. Any study that touches or involves this field should **absolutely perform sex-specific subgroup analyses,** whether it is powered for that or not. It is not enough only to adjust for sex. **One needs to see separate results for men and women.**

Eventually, there is a possibility that the “double protection strategy” – COVID-specific neutralizing antibodies and T-cell immunity on one side and Th1-polarized trained innate immunity on the other side – may be achieved by a vaccine that combines both aspects.

COUNOUPAS et al. combined BCG with a stabilized trimeric form of the SARS-CoV-2 spike antigen. The vaccine (BCG:CoVac) promoted both (i) the rapid development of virus-specific IgG antibodies in sera of vaccinated mice and (ii) a “Th-1 biased response in terms of IgG antibody subclass and cytokine release by vaccine-specific CD4+ and CD8+ T cells”. Though a single injection generated neutralizing antibody titres greater than in sera from infected individuals, the antibody response can be further increased by a booster with a heterologous vaccine combination (spike protein plus alum) without the BCG component. Such a vaccine may integrate the “double protection strategy” within a single vaccine dose or heterologous vaccine primer-booster regimen.

“Trained-immunity-based vaccines, which can stimulate both non-specific and specific immune responses, might provide a broader protection far beyond the conventional vaccines currently used.” (KAR and JOOSTEN, based on STABELL-BENN and CURTIS, 4th Innate Immune Memory Meeting). However, as long as such an “universal” vaccine that fully addresses both adaptive and innate memory is not available, one needs to consider to combine two separate strategies, a COVID-specific vaccine and an unspecific vaccine or a non-vaccine like e.g. beta-glucans to train innate immune memory, but the resulting immune response needs to be biased towards a Th1-response.

However, a serious concern with regard to the concept of a double protection strategy (with strengthening the early innate immune response as one arm of the strategy) was found by GUO et al.. They observed that emerging SARS-CoV-2 variants evolved to resist the antiviral IFN-I and IFN-III response. GUO et al. confirmed the evasion of innate immunity for B.1, B.1.1.7 and B.1.351 isolates. This weakens or eliminates the interferon pathway, i.e. the early interferon reponse, of the trained innate immune response. However, other pathways of the trained innate immunity may still be effective, thus the consequences of the evasion from the interferon pathway remain unclear.

With Sepsivac as a benchmark for a highly effective method to strengthen the trained immune response, it would be important to repeat the JAISWAL trial in a population that is already dominated by the critical variants, e.g. B.1.1.7 or B.1.351 in order to find out whether Sepsivac still works as well as it did in India in 2020. Such a trial could quickly answer the question whether the concept of the double protection strategy is still working under the new conditions (of the new variants), or whether this concept has no future in the times of VoCs. Moreover, ongoing BCG trials for COVID prevention should analyse their data along the timeline of local SARS-CoV-2 variants. It would be important to know whether the efficacy of BCG (or MMR) prevention was reduced since the time when VoCs became dominant.

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Supplement

Age	2020 (complete)	weeks 49-53/20	weeks 1-2/21	weeks 3-4/21	weeks 5-6/21	weeks 7-8/21	weeks 9-10/21	Weeks 11-12/21
0	831	290	102	67	58	60	87	142
1	697	272	108	95	80	94	135	230
2	743	300	100	93	83	90	149	253
3	829	359	100	90	86	100	175	290
4	924	387	107	98	85	103	191	326
5	1047	423	110	95	89	106	203	360
6	1103	437	116	99	85	99	191	336

Incidence (per 100.000) according to one-year age groups in Germany; 0 – 6 years; source: survstat@rki, RKI. Accessed April 1st, 2021.

Comment:

The first MMR vaccination is usually given in Germany around the first birthday, shortly before or shortly thereafter. The second dose is usually given during the second year of life. When a child turns two, it usually has gotten both doses. However, the first dose is much more important than the second dose with regard to the immune response. If MMR vaccination affects COVID-19, the incidence is expected to be lower in 1-year old children compared to <1 year old children, and this effect should be a little (but not very much) stronger in 2-year old children. In older children, the increasing numbers of contacts (e.g., in kindergardens) are expected to (over-)compensate for a favorable effect of MMR vaccinations.

Taking that into account, the incidence data from 2020 are well compatible with a small effect of vaccinations like MMR on COVID incidence. At the age of 1 and 2, the incidence is lower than at the age of 0, when the child has no MMR vaccination (or, at most, one dose during the last weeks of that year). With increasing age from 3 years on, COVID incidence grows, but this is well in accordance with the rise of contacts e.g. in kindergardens (that were only partially closed in Germany in 2020).

If one looks separately on December 2020, children aged 1 year had still the lowest incidence. In the beginning of 2021 (weeks 1 and 2), there was no large difference in the incidence between 0 and 4 years. However, there is an anomaly around and after christmas in many countries because less testing and/or delays in reporting, thus these data should be considered with caution. Since week 3/2021, when the data become more reliable, the situation changed drastically compared to 2020: Now the children aged 0 have the lowest incidence, and the incidence shows a trend to increase with age even in the earliest years.

The pattern of rising incidence was still irregular in weeks 3-8/21, but became very stable for age groups 0 – 5 from weeks 9-10/21. The steady increase of the incidence may be explained with an increase of contacts, e.g. an increasing proportion of children who visit a child care facility/kindergarden. Nevertheless, the results point against a protective effect of MMR vaccination with regard to infection or even symptomatic disease (because symptomatic children have a higher chance to be tested compared to asymptomatic children) in 2021.

Since weeks 3-4/21, and more pronounced a few weeks later, the situation becomes opposite to 2020. The incidence data from 2021 evidently contradict the suggestion that MMR vaccination may impact COVID incidence (or incidence of symptomatic disease) in children, whereas the data from 2020 still pointed to such an association, though only to a small to moderate extent. The difference between 2020 and 2021 cannot be explained by changes in child care in these age groups (0 – 2 years), whereas older age groups may show stronger impacts of openings and closings of kindergardens.

One may hypothesize that this change between 2020 and 2021 may be associated with the expansion of the B.1.1.7 variant in Germany since the beginning of 2021, and its rise to dominance in February and March. As mentioned above, GUO et al. discovered that B.1.1.7 evaded from innate immunity by weakening the early innate interferon reponse. MMR vaccinations are supposed to train innate immunity. The lower incidence of 1 and 2 year old children compared to children < 1 year in 2020 in Germany may be the consequence of better trained innate immunity in 1 and 2 year old children due to MMR vaccinations that started around the first birthday. If B.1.1.7 evades from innate immunity, the training of innate immunity (e.g., by MMR vaccination) may not matter any more.

This is an early, still very hypothetic hint that the data from GUO et al. have real-life consequences in a way that training of innate immunity (e.g., by MMR vaccination) may not be helpful any more against new variants like B.1.1.7 and B.1.351. This underlines the necessity, as already mentioned above, that long-term trials about methods to strengthen trained innate immunity (like BCG, MMR etc.) need to be analysed along the timeline with respect to the occurrence and prevalence of VoCs; different time intervals should become subject of sub-analyses in order to find out whether the efficacy of the intervention (e.g. BCG or MMR) changed after the arrival of new variants, and their rise to dominance.

In summary, if new variants escape from early trained innate immunity, they weaken the concept of the double protection strategy. In the worst case, a concept that may have worked well with the virus variants that circulated in 2020, may become ineffective.

However, one should still bear in mind that children of the age groups mentioned above are affected usually only by mild or paucisymptomatic disease. It may still be that innate immunity and its training by vaccinations like MMR contribute to this mild form of COVID-19. If so, B.1.1.7 seems to escape from direct eradication by the trained innate immunity; however, it is still controlled well enough not to cause a lot of harm and serious disease. Children may be more often symptomatic now (because of B.1.1.7) and thus infected children may have a higher chance of getting a positive test result. Nevertheless, there are no data from Germany that point to an increase of deaths or severe cases in very young children since the rise of B.1.1.7. That said, training innate immunity and the double protection strategy may still work in a situation when such variants are dominant with

respect to the severity of the disease. It may still result in milder disease. Nevertheless, if the new variants suppress the innate interferon reponse in the upper respiratory tract, it is obvious that the probability must increase that an infection may become manifest following inoculation (i.e. not directly eradicated), and that it may become more symptomatic (due to higher viral loads), resulting in a higher chance of being tested and receiving a positive test result.

With regard to the many prophylactic trials about trained innate immunity, e.g. BCG and MMR, it is important not only to consider infections or positive tests along the timeline, but also to consider the severity of the disease and possible changes in disease severity along the timeline, taking into account the changes in the viral landscape in the geographical areas where the trials were performed.