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KENMERK CGM/171220-01
ONDERWERP Advies inschaling van werkzaamheden met gg-poliovirussen

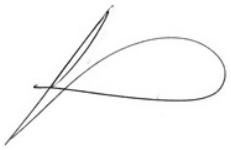
Geachte mevrouw Van Veldhoven,

Naar aanleiding van een adviesvraag betreffende de vergunningaanvraag IG 17-180_2.8-000 getiteld: 'Het gebruik van verzwakte poliovirus type 2 stammen S19/MEF2P1/N18S en S19/S2P1/N18S voor vaccin onderzoek op inperkingsniveau 2' van het Ministerie van Volksgezondheid, Welzijn en Sport, deelt de COGEM u het volgende mee.

Samenvatting:
De COGEM is verzocht te adviseren over de inschaling van werkzaamheden met twee verzwakte genetisch gemodificeerde (gg-) poliovirussen. Het betreft gg-poliovirussen die gebaseerd zijn op de verzwakte Sabin vaccinstam van poliovirus type 3 en de capside eiwitten bevatten van poliovirus type 2.
Polio is een ziekte die voornamelijk kinderen treft onder de vijf jaar. Poliovirus infectie kan in ernstige gevallen leiden tot verlamming en zelfs een dodelijke afloop hebben. Van de 3 typen poliovirus, komt poliovirus type 1 nog voor in Afghanistan, Pakistan en Nigeria. Type 3 is wereldwijd niet meer waargenomen sinds 2012 en poliovirus type 2 is in 2015 officieel uitgeroeid verklaard. Recent is poliovirus type 2 omhoog geschaald naar pathogeniteitsklasse 3. Als gevolg hiervan dienen werkzaamheden met de twee gg-poliovirussen op inperkingsniveau 3 uitgevoerd te worden. Gezien het verzwakte karakter van de gg-poliovirussen verzoekt de vergunningaanvrager om de werkzaamheden op inperkingsniveau 2 uit te mogen voeren.
De gg-poliovirussen bezitten een sterk verminderde capaciteit om te repliceren bij 37°C. Mede hierdoor is de COGEM van oordeel dat de aangebrachte mutaties de gg-poliovirussen verder verzwakt hebben t.o.v. de verzwakte Sabin vaccinstam van poliovirus type 3. De kans op reversie van deze mutaties waarbij een virulenter virus gevormd wordt, acht de COGEM verwaarloosbaar klein. Daarnaast acht de COGEM de mogelijke risico's van deze gg-poliovirussen voor de mens verwaarloosbaar klein, mochten deze gg-poliovirussen in het uitzonderlijke geval onbedoeld in het milieu terecht komen.
Op basis van bovenstaande overwegingen is de COGEM van oordeel dat werkzaamheden met de twee gg-poliovirussen uitgevoerd kunnen worden op ML-II. Op dit inperkingsniveau en met inachtneming van enkele aanvullende voorschriften is de COGEM van mening dat de risico's voor mens en milieu van de voorgenomen werkzaamheden verwaarloosbaar klein zijn.

De door de COGEM gehanteerde overwegingen en het hieruit voortvloeiende advies treft u hierbij aan als bijlage. Op verzoek van de gemandateerde vergunningverlener is het advies in het Engels opgesteld.

Hoogachtend,



Prof. dr. ing. Sybe Schaap
Voorzitter COGEM

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Activities with chimeric genetically-modified polioviruses

COGEM advice CGM/171220-01

1. Introduction

COGEM has been asked to advise on a license application (IG 17-180) from the 'Ministerie van Volkgezondheid, Welzijn en Sport' on activities with two chimeric genetically-modified (GM) polioviruses. These GM polioviruses (S19/MEF2P1/N18S and S19/S2P1/N18S) are derivatives of the attenuated Sabin vaccine strain of poliovirus type 3 in which the capsid proteins coding region P1 has been removed and replaced with the P1 region of MEF-1 or Sabin poliovirus type 2 vaccine strains. Since poliovirus type 2 has been recently classified as a class 3 pathogen, activities with these chimeric polioviruses need to be conducted at ML-III. According to the applicant, activities with these GM polioviruses can be conducted at ML-II, due to the attenuated nature of these chimeric polioviruses, resulting from various mutations present in the 5' non-coding region of the viral genome.

2. Poliovirus

2.1 Poliomyelitis

Poliomyelitis is caused by infection with poliovirus, and mainly affects children below the age of 5. The virus is highly contagious and can affect the central nervous system. Most infected people (90%) do not manifest any symptoms of disease, or display only mild symptoms. Others, however, develop symptoms such as fever, headache, pain in the extremities, nausea and stiffness of the neck. One in 200 infected individuals become paralyzed, which may be lethal when associated with respiratory problems.[1]

Poliovirus spreads primarily via faeces. Infection can occur for example if fecally contaminated water or food is consumed. At the onset of infection, the virus can also be transmitted orally via coughing. Poliovirus mainly replicates in the intestine, and can enter the cervical and mesenteric lymph nodes, resulting in entry of the virus into the circulation. In addition, the virus is resistant to the acid environment of the stomach. In some instances, poliovirus can enter the central nervous system, infect and replicate in motor neurons, consequently resulting in paralysis.[2]

2.2 Genomic structure and attenuation

Polioviruses occur in three serotypes, 1, 2 and 3, and are positive single stranded RNA viruses of the genus *Enterovirus* in the *Picornaviridae* family.[3] A single large open reading frame on the RNA genome is preceded by a 5' non-coding region which contains the Internal Ribosome Entry Site (IRES). Protein translation is initiated from this region, upon which a single polyprotein is produced that is cleaved into more than 15 proteins. Polioviruses have a capsid which is composed of 60 copies of four structural viral proteins VP1, VP2, VP3 and VP4, that are encoded by the capsid proteins region P1. While VP1, VP2 and VP3 are present at the surface of the capsid and

contain antigenic epitopes, VP4 is located in the interior of the capsid.[4] Entry of a target cell by polioviruses is mediated by binding of the virus to CD155 expressed on the cell surface.

The 5' non-coding region forms a highly organized structure containing multiple 'stem loops'. The attenuated phenotype of the Sabin vaccine strains of each of the three poliovirus serotypes, results from mutations in two adjacent stems in the so-called V-domain of the 5' non-coding region, between bases 470-483 and bases 528-538.[5] These mutations lead to attenuation of the poliovirus strains as was shown by reduced neurovirulence and decreased viral growth at physiological temperatures of 37°C.[6] Due to their attenuated nature, these viral strains have been applied for vaccination. Sabin poliovirus type 1, 2 and 3 strains are present in oral poliovirus vaccines (OPV) and have shown to be effective in inducing immunity to all three poliovirus serotypes. The vaccine strains can replicate for a short timeframe in the gut. In addition, they can be shed into the environment, which may facilitate immunization of unvaccinated persons.[7] It has been shown that Sabin poliovirus strains can acquire point mutations in approximately 1 in 2.7 million vaccinations, resulting in a more virulent variant of the virus, also called 'circulating vaccine-derived poliovirus' (cVDPV). Like wild type poliovirus, cVDPV can cause paralysis. Most cases of cVPDV are derived from poliovirus type 2 (~86%).[8]

2.3 Eradication of poliovirus

In 1988 the Global Polio Eradication Initiative (GPEI) was launched by the World Health Assembly (WHA). The GPEI is a public-private partnership led by national governments and five partners: the World Health Organisation (WHO), Rotary International, the US Centers for Disease Control and Prevention (CDC), the United Nations Children's Fund (UNICEF) and the Bill & Melinda Gates Foundation. Its goal is to eradicate polio worldwide. Since its inception, the number of poliovirus incidents has dropped with 99% to 37 reported cases in 2016.[9]

Poliovirus type 2 has been officially declared to be eradicated in September 2015. Infection with type 3 poliovirus has not been reported since November 2012. In contrast, type 1 poliovirus is still endemic in Afghanistan, Pakistan and Nigeria. The WHO has taken initiative, as part of the 'Polio Eradication & Endgame Strategic Plan 2013-2018',[10] to reduce the use of poliovirus type 2 in laboratories to minimize unintended re-introduction of the virus into the environment.[11]

3. Previous COGEM advice

Because of the severity of poliomyelitis, the relatively low vaccination status of the global population, as well as the worldwide eradication of poliovirus type 2, COGEM has recently advised to classify the virus from a type 2 to type 3 pathogen.[12] As of October 1st, activities with GM viruses that contain genome sequences of poliovirus type 2, need to be performed at ML-III. Bearing in mind the recent incident in The Netherlands concerning inadvertent infection of laboratory personnel with wild type poliovirus type 2, COGEM wants to emphasize that, in line with the eradication programme, strict adherence to biosafety guidelines and containment is essential to avoid inadvertent exposure of laboratory personnel to (GM) polioviruses.

4. Intended work

The applicant has constructed two chimeric GM polioviruses S19/MEF2P1/N18S and S19/S2P1/N18S, which are derivatives of the Sabin poliovirus type 3 vaccine strain. In the genome of these GM polioviruses, a number of mutations have been introduced into the V-domain, resulting in a virus (designated S19) that is highly attenuated. The attenuation of S19 has been demonstrated in *in vitro* experiments with various cell lines. These experiments show that viral growth was effectively reduced at 37°C compared to wild type and the Sabin strain of poliovirus type 3. In addition, intraspinal inoculation of S19 did not demonstrate any neurovirulence *in vivo* in a transgenic mouse model carrying the human receptor for poliovirus, CD155. When S19 was orally administered to cynomolgus macaques, no viral shedding nor seroconversion was observed, in contrast to administration of the Sabin poliovirus type 3 strain.

In addition to the mutations introduced in the V-domain, the GM polioviruses in the current license application also contain a mutation in the sequence coding for protein 2A. This mutation (N18S) was introduced for production purposes to facilitate higher viral titers in Vero cells. Experiments on transgenic mice carrying the human receptor for poliovirus revealed that S19/N18S did not show any neurovirulence upon intraspinal inoculation.

The applicant intends to work with S19/N18S poliovirus type 3, in which the capsid proteins coding region P1 of poliovirus type 3 is substituted by the sequence of P1 from MEF-1 or Sabin poliovirus type 2 vaccine strains. As mentioned above, activities with chimeric GM viruses that contain genome sequences of poliovirus type 2, need to be performed at ML-III. The applicant states that these activities can be safely conducted at ML-II, because of the highly attenuated nature of both GM polioviruses.

5. Considerations and advice

5.1 Attenuation

The applicant intends to use two GM polioviruses, which are derivatives of the Sabin poliovirus type 3 vaccine strain. In the 5' non-coding region of these GM polioviruses, several mutations have been introduced with the aim to weaken the stem loop structure. Both GM polioviruses are replication-competent, but have a reduced thermostability. The capacity of these viruses to replicate at a physiological temperature of 37°C is therefore severely compromised. In addition, experimental studies in cell lines, transgenic mice and cynomolgus macaques have substantiated that S19 viruses are more attenuated than the Sabin poliovirus type 3 vaccine strain, and do not display any neuroviral activity. Based on these data, COGEM is of the opinion that vaccine strains S19/MEF2P1/N18S and S19/S2P1/N18S are more attenuated than the Sabin poliovirus type 3 vaccine strain, which is already attenuated compared to wild type poliovirus type 3.

5.2 Revertants

It has been shown that the mutations in the 5' non-coding region in the genome of the Sabin poliovirus type 3 vaccine strain may revert to the bases that are present in the wild type poliovirus, resulting in a more virulent virus that displays increased replication, shedding and neurovirulence.[13]

The applicant has introduced several additional mutations in the stem loops of the 5' non-coding region, not only changing single bases, but substituting base pairs from G:C to A:U. Besides severely compromising the thermostability of the viruses at 37°C, the applicant states that these multiple mutations prevent the formation of a viral revertant that is less attenuated than the chimeric polioviruses. The applicant comments on the possible occurrence of reversal of the introduced mutations. The S19 strains were developed by the National Institute for Biological Standards and Control (NIBSC). They reported that in more than 400 runs, no reversal of the mutations in the 5' non-coding region of S19 strains was observed. The applicant did state, however, that in three independent laboratories, it has been observed that a single base pair in the 5' non-coding region of a S19 strain reverted to the wild type base pair. This reversal results in the formation of a S18 virus that is still equally attenuated compared to S19. To ensure that a less attenuated virus will not be formed during the intended activities, the applicant plans to limit the activities with the GM polioviruses and Vero cells, on a seed lot basis, to maximally 10 cell passages. In addition, deep sequencing will be performed to determine whether reversal of mutations has occurred.

COGEM is of the opinion that the chance is negligibly small that a more virulent GM poliovirus is formed during the experiments. In addition, COGEM agrees with the strategy to maximize the activities to 10 cell passages and to monitor for reversal of mutations by deep sequencing.

5.3 Spread of the GM polioviruses and risk for the environment

Since S19/MEF2P1/N18S and S19/S2P1/N18S have never been administered to human individuals, there is no data concerning *in vivo* replication of these viruses in humans. As mentioned, the replication potential of S19 in cell lines at physiological temperature of 37°C was shown to be reduced *in vitro*. *In vivo* experiments in transgenic mice demonstrated that the GM polioviruses did not confer any neurovirulence. Moreover, upon oral administration of S19 virus strains to cynomolgus macaques, no replication in the gut was detected, in contrast to the Sabin poliovirus type 3 vaccine strain. Hence, these data collectively show that S19 virus strains are severely hampered in their replication potential at 37°C.

For spread into the environment and human individuals to become inadvertently infected with the GM polioviruses, a breach in containment and escape from laboratories needs to occur. In the unlikely event that this takes place, a number of barriers prevent human individuals from becoming infected with these GM polioviruses, and subsequently shedding of these chimeric viruses. These barriers are 1) a limited survival of the virus outside the host, 2) the severely hampered replication potential of the GM polioviruses at 37°C, 3) the unlikelihood that the chimeric viruses are able to reach the intestine, and 4) a high degree of vaccination against poliovirus in the Dutch population. COGEM deems the chance negligibly small that all these barriers will be overcome and that a scenario will unfold that human individuals become infected with these chimeric viruses, and subsequently shed them into the environment. Moreover, as mentioned above, the chance of formation of a less attenuated virus due to reversal of the introduced mutations, is negligibly small.

A point of concern may be that during activities with the GM polioviruses, recombination or complementation with other polioviruses or type C enteroviruses will occur, resulting in the generation of a virulent GM poliovirus.[14] To avoid this from occurring, COGEM advises that activities with these GM polioviruses need to be performed in cell lines, which are devoid of polioviruses or other type C enteroviruses.

In the unlikely event that the GM polioviruses escape from containment, recombination or complementation with other polioviruses or type C enteroviruses is a theoretical possibility. However, considering the aforementioned barriers that hamper infection and replication of the GM polioviruses in a host cell, it is unlikely that co-infection with other polioviruses or type C enteroviruses will occur. Therefore, COGEM is of the opinion that the chance is negligibly small that a virulent GM poliovirus is generated via recombination or complementation.

6. Conclusion

Considering all of the above, COGEM is of the opinion that activities with the GM polioviruses S19/MEF2P1/N18S and S19/S2P1/N18S can be safely conducted at ML-II, with the following prerequisites:

- Cell lines must be free of poliovirus or other type C enteroviruses;
- Gloves must be worn at all times during the activities;
- Laboratory personnel cannot perform activities with the GM polioviruses if not vaccinated against poliovirus types 1, 2 and 3;
- All open vessel activities must be performed in a Class II Biosafety cabinet

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