Environmental Risk Assessment for V181

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1 ENVIRONMENTAL RISK ASSESSMENT ACCORDING TO ANNEX II OF DIRECTIVE 2001/18/EC

Dengue virus is only believed to infect humans via direct exposure to blood/blood products or via mosquito bite {03RK0R}. Dengue viruses are transmitted by Aedes sp. mosquitos which are day-biting mosquitos found commonly in urban environments. The host range of the Aedes Sp. is limited mostly to humans and simians. Among the species that can be infected with dengue viruses, overt symptomatic disease and severe outcomes of infection are limited to humans. Human to human transmission of dengue is mediated primarily by the mosquito vector. Humans are infectious to mosquitoes starting a few days prior to the febrile phase of illness through a few days following defervescence. A mosquito acquiring dengue via a blood meal from an infected human typically become infectious to humans within 10-14 days. The overall burden of dengue can be related to the abundance and density of the mosquito vector as well as the population size and density of the community in which the definitive hosts (i.e. humans) reside {03RK0R}.

Importantly studies conducted by the National Institute of Health (NIH) suggest that the attenuated viruses comprising V181 are not transmitted from human to human via mosquitos. Experimental infection and transmission studies using vaccine virus and mosquito vectors have shown that the peak virus titer of all the V181 live attenuated dengue vaccine viral strains tested thus far in humans were at least ≥100-fold below the viremia level required for transmission to mosquitoes. {04QYLH}. Furthermore, for the vaccine virus to be transmitted from one human to another the following series of events would have to take place: (1) The subject would have to be viremic with a peak virus titer greater than 10^5 PFU/mL; (2) The viremic subject would then have to be bitten by a viable vector mosquito at the peak of viremia; (3) This mosquito would have to live for a 10 – 14 day period following the blood meal to make the mosquito infectious for the vaccine virus; (4) The same mosquito would then have to bite another individual. Therefore, the risk that the DENV vaccine will be transmitted from vaccinated to non-vaccinated populations is very low.

Details of the mosquito transmissibility data are presented below:
The NIH DENV4 component was evaluated for its transmissibility from vaccinees to mosquito, and its capacity to grow within mosquitos. Transmissibility to mosquitos was evaluated in 10 vaccinees who received a 10^5 PFU dose of rDENV4Δ30. *A. albopictus* mosquitoes were fed on vaccinated subjects on days when the subjects were expected to be viremic. Five of the 10 vaccinees had detectable viremia on at least 1 of those days, with titers ranging from 1.0 log10 PFU/mL to 2.3 log10 PFU/mL. Vaccine virus was not detected in any of the 352 mosquitoes that fed on the subjects. Studies in *A aegypti* mosquitos showed that, compared with wild-type virus, rDENV4Δ30 was found to be restricted in its capacity to infect the midgut and to disseminate further. This lack of transmissibility was attributed to both the low level of viremia in subjects and to the restricted capacity of rDENV4Δ30 to disseminate from the midgut of the mosquito to its head {04QYLH}.
The NIH rDENV1Δ30 was tested in a pre-clinical experiment, in which mosquitos were fed blood meals containing serial dilutions of virus suspension of rDENV1Δ30. The rDENV1D30 virus showed low infectivity (>$10^{3.0}$ PFU/mL), which is ≥100-times the mean peak titers of 0.5 to 1.0 log10 PFU/mL observed in clinical trial subjects who received this vaccine. A mosquito

takes only 1 to 2 µL per blood meal, which suggests that transmission of vaccine virus to a mosquito is unlikely {04QYLH} {06DWPN} {06F0GZ} {06F0HD}.
The rDENV2/4Δ30(ME) was inoculated intrathoracically into Tx. splendens, and presence of virus in the head was determined. The MID50 following intrathoracic inoculation of Tx. splendens was determined to be $10^{3.0}$ PFU for rDENV2/4Δ30(ME) {04PXH0}.
While the rDENV3Δ30/31virus vaccine has not been directly tested, DENV3-Sleman/78, the parent virus of the construct, is very poorly transmitted to mosquitoes: Ingestion by *Ae. aegypti* mosquitoes of $10^{4.1}$ PFU of wt DENV3 Sleman/78 infected the midgut of only 4 of 28 (14%) mosquitoes tested and disseminated from the midgut in only 2 of 28 (7%) mosquitoes. The required dose of wild type DENV-3 Sleman/78 is in excess of 10^5 PFU/mL in blood to allow for transmission to *Ae. aegypti* mosquitoes, the natural vector of DENV {06DWPN}.

In summary, the monovalent components of the tetravalent vaccine have multiple barriers to transmission by mosquitos, resulting in an extremely low risk of transmission of vaccine virus between close contacts/other humans via mosquitos. V181 is similar by design to the NIH dengue live attenuated tetravalent vaccine (LATV) TV003 based on using the same starting viral materials and very similar manufacturing processes. Therefore, the same multiple barriers to transmission by mosquitos resulting in an extremely low risk of transmission of vaccine virus between close contacts/other humans via mosquitos, also apply to V181.

Wild-type dengue infection has rarely been reported to result in shedding of the virus in saliva and urine {07X3TC}; while no specific data are available on shedding of V181 in saliva, urine or feces, this is expected to be negligible given the lower viremia level that is induced by V181 compared to the natural dengue infection. Additionally, these routes are not implicated as an environmental source for dengue spread. Even in the event of incidental shedding of genetically modified organism (GMO) in wastewater, the establishment of GMO in such system is not to be expected due to the low volumes released and the destruction of the GMO by wastewater treatment techniques (e.g., temperature, chlorination, etc.).

Finally, exposure of non-host species, if it occurred in the unlikely case of shedding of V181, would not be affected and horizontal gene transfer to bacteria can be excluded. V181 is an RNA virus and is unlikely to contain homologous sequences with bacteria which would allow for such a transfer.

Concluding, the Sponsor considers that this GMO, taking all items into account, i.e. the need of a peak virus titer greater than 10^5 PFU/mL, the need for a mosquito as a vector, the requirement that this vector would have to live for a 10-14 day period and have to bite another individual and the fact that no shedding to the waste water is anticipated that the vaccine does not pose a risk to the environment and can be released for clinical trials.
In addition, authorized personnel at the clinical sites will be provided with instructions on how to handle V181 as outlined in section 1.5.

1.1 Identification of characteristics of V181 which may cause adverse effects

1.1.1 Disease to humans, animals and plants including allergic or toxic effects

Humans:
In the V181 Phase 1 study, V181 was generally well tolerated with the most likely adverse effects (AE) related to V181 being as follows:

Skin and subcutaneous tissue disorders (rash maculo-papular) (36%), injection site reactions (pain, erythema, bruising, swelling or haematoma) 29%, headache (25%), fatigue (25%), myalgia (23%), and malaise (14%).

The AEs from the V181 Phase 1 study are comparable to the clinical studies using NIH dengue LATV and Butantan dengue vaccine (Butantan-DV), vaccines similar to V181based on using the same starting viral materials and similar manufacturing processes. Based on these findings, no significantly different adverse events are expected from V181 in V181-003 study subjects.

Non-human Primates:

Two NIH dengue LATV formulations were evaluated in a safety/toxicology study in rhesus monkeys. One non-clinical pharmacology study with V181 was also performed, including toxicity endpoints in rhesus monkeys. The dengue virus vaccine administered to nonhuman primates as monovalent or tetravalent formulations was well tolerated.

Mosquitos:
Mosquitos serve as arthropod vectors and are not expected to develop disease or toxic effects.

Plants:
No effects on plants are expected.

1.1.2 Effects on the dynamics of populations of species in the receiving environment and the genetic diversity of each of these populations

The biological profile of the V181 viral vaccine candidate including the host range, host specificity, and tissue or cell tropism is expected to be identical to the parental virus except that the viruses comprising V181 replicate less efficiently in human subjects compared to the parental virus strains leading to an attenuated phenotype. The route of transmission is also expected to be the same but with lower viral replication in human subjects and mosquitoes, and the available data suggests that the attenuated viruses would not be transmitted.
Specific stability in the environment of V181 is unknown. Sensitivity to specific agents such as disinfectants of V181 is unknown. However, V181 is an enveloped virus. These viruses are known to be easily inactivated by routine surface cleaning and disinfection (see section 1.5.). Their lipid envelope can be easily destroyed by alcohols and a wide range of commercially available disinfectants tested for inactivation of these viruses are available.

Because of the presence of the Δ30 deletion in all components reversion back to a wild type (wt) phenotype via a recombination event would require the presence of wt-dengue. Since wt-dengue is not typically present in non-tropical regions such as the USA and Europe, there is

very low probability of co-infection of wt-dengue at the moment of vaccination. Furthermore, even for regions where wt-dengue virus is present, co-infection with wt-Dengue would have to happen within the short time period (up to 3 days) of viremia in vaccinated individuals which is very unlikely.

Positive strand RNA viruses other than picornaviruses, coronaviruses, togaviruses and noroviruses recombine only inefficiently. In different species of arthropod borne flaviviruses differences in observable recombination frequencies in nature are attributable to differences in mechanism of vectoring by ticks and mosquitoes and by differences in both host and vector ecology {05BRX9}. Studies on intra-typic recombination among flaviviruses that occurred on an evolutionary scale in the wild demonstrated that there was little or no recombination in cell culture {04T0H9}, and that substitution of heterologous envelope proteins into a virulent flavivirus backbone resulted in viruses with properties of attenuation matching those of an attenuated vaccine vector] {07WZQN}. Intertypic recombination occurs at a frequency 100-fold lower than in the case of intratypic recombination {07WZLF}.

Virtually all vaccine vectors are either naturally or artificially attenuated for pathogenicity in their target populations and thus demonstrate reduced replication. Recombination between a vaccine vector and a wild type virus, should it happen, should not lead to a construct more virulent than the wild type virus itself{04RHB3}.

The probability of non-homologous recombination, such as between V181 and another non-related RNA virus, is substantially lower than homologous recombination between related virus. The non-homologous recombination mechanism involves a cleavage-joining or joining of RNA fragments, generally occurring without replication or a requirement for the viral RNA polymerase. Non-homologous recombinations are rarely detected principally because they are deleterious. They have been demonstrated as relatively rare event even under forced experimental conditions {054SSX}.

WHO in their 2013 report on the quality, safety and efficacy of dengue tetravalent vaccines states that the potential of recombinants, should they ever emerge, to cause disease or spread would probably be very low. Dual infection laboratory studies between vaccine and wild-type strains are not recommended because the predictive clinical value of such studies would be low {05C0FR}.

In conclusion, negative effects on the dynamics of populations of species in the environment are not expected from the use of V181.

1.1.3 Altered susceptibility to pathogens facilitating the dissemination of infectious diseases and/or creating new reservoirs or vectors

Because of the presence of the Δ30 deletion in all components reversion back to a wild type (wt) phenotype via a recombination event would require the presence of wt-dengue. Since wt-dengue is not typically present in non-tropical regions such as the USA and Europe, there is very low probability of co-infection of wt-dengue at the moment of vaccination. Furthermore, even for regions where wt-dengue virus is present, co-infection with wt-Dengue would have

to happen within the short time period (up to 3 days) of viremia in vaccinated individuals which is very unlikely.

Therefore, it is extremely unlikely that V181 will facilitate the dissemination of infectious disease and/or create a new reservoir or vector.

1.1.4 Compromising prophylactic or therapeutic medical, veterinary, or plant protection treatments

V181 is not expected to affect plants, although studies in plants have not been performed.

1.1.5 Effects on biogeochemistry (biogeochemical cycles), particularly carbon and nitrogen recycling through changes in soil decomposition of organic material

Reports on biogeochemical cycling in soil and plants are not expected.

1.2 Evaluation of the potential consequences of each adverse effect, if it occurs

The potential consequence from each adverse effect discussed in section 1.1 resulting from exposure to V181 is summarized in Table 1.

Table 1 Summary of potential consequences from adverse effects resulting from unintended exposure to V181 in relevant species

Species	Potential consequences from adverse effects
Mosquitos	Unlikely, mosquitos are not susceptible to dengue virus disease.
Non-human Primates	The dengue virus vaccine administered to nonhuman primates as monovalent or tetravalent formulations was well tolerated.
Humans	In the V181 Phase 1 study, V181 was generally well tolerated with the most likely adverse effects related to V181 being as follows: Skin and subcutaneous tissue disorders (rash maculo-papular) (36%), injection site reactions (pain, erythema, bruising, swelling or haematoma) 29%, headache (25%), fatigue (25%), myalgia (23%), and malaise (14%).

In order to reduce the likehood of packages that are damaged or leaking, and thereby prevent accidental exposure to personnel who handle the material during its shipment, the investigational medical product (IMP) is shipped according to the applicable national & international regulations; UN3245.

In the case of an accidental needle-stick injury, the injected dose of V181 will be much lower than the actual subcutaneous dose that is intended to be injected in study subjects.

Table 3 Summary of the likelihood of occurrence of exposure to V181 in relevant species

Species	Likelihood of exposure to V181
Mosquitos	Likelihood of exposure due to shedding from patients is minimal
Non-human Primates	Likelihood of exposure due to shedding from patients is minimal
Humans	Potential dissemination from shedding or direct contact with subjects is unlikely. Potential dissemination from an accidental needle-stick injury is low

1.3 Estimation of the risk posed by each adverse effect

The overall risk of V181 to human health and the environment is considered negligible based on an evaluation of the magnitude of potential adverse effects and likelihood of occurrence. Even in a worst-case scenario, if there was transmission from a treated individual, the data suggest minimal or no untoward effects in the recipient organisms and minimal or no further spread. Based on the totality of the data, it is highly improbable that contact individuals will experience any issues linked to incidental release of V181.

1.4 Management strategies to minimize risk

Management strategies to minimize the risk of adverse effects for non-study subjects and the environment due to treatment with V181 are summarized in Table 4.

To mitigate against the risk of unintentional release, the GMO will be appropriately contained and labelled during transport. Staff handling the GMO and samples that could potentially contain the GMO should be wearing gloves.

In the event of an accidental spill, staff will follow their site standard operating procedure (SOP) for spill response and cleanup. Bleach, quaternary ammonium- and phenolic-based disinfectants will inactivate the virus.

The study subjects will be instructed to not donate blood or fluid products for 6 weeks after vaccination, this further minimizes any possibility that V181 would be transmitted to other humans.

V181 is to be administered by subcutaneous injection in subjects under a clinical trial setting. Each participant will receive a 1 dose (0.5mL) of the GMO/Placebo (Day1). To minimize spread of the GMO post vaccination, the injection site will be covered with an adequate bandage (e.g. any adhesive bandage or gauze and tape) that provides a physical barrier to protect against direct contact. The bandage may be removed when there is no visible fluid leakage at the end of the 30-minute postvaccination observation period. The used, empty vaccine vials and the bandages will be disposed of in standard biomedical waste

container and used syringes will be discarded as medical waste according to the site SOP for medical waste.

Table 4 Recommended measures to reduce the risk of unintentional spread following the intended use of V181

Exposure scenario	Measure
Accidental breakage/spillage of V181 during transport or administration	Medical personnel involved in the administration will wear gloves to minimize exposure. In the event of an accidental spill, staff will follow their site SOP for spill response and cleanup. Bleach, quaternary ammonium- and phenolic-based disinfectants will inactivate the virus. Any unused vials or waste material will be disposed of in compliance with the institutional guidelines for GMO or biohazardous waste, as appropriate.
Accidental needle stick injury by medical personnel	Rare case reports of dengue transmission via needlestick in patient care and laboratory accident, blood transfusion, bone marrow transplant or organ transplant, exist. In case of an accidental needle stick injury, the injected dose of V181 will be much lower than the actual subcutaneous dose that is intended to be injected in study subjects. In the unlike event that the study personnel receive the full dose of V181 via accidental needle stick, the safety profile is expected to be similar to the study participants, which if expected to be favorable. For any affected study personnel, the injection site should be immediately disinfected and covered with an adequate bandage (e.g. any adhesive bandage or gauze and tape) that provides a physical barrier to protect against direct contact. The bandage may be removed when there is no visible fluid leakage at the end of the 30-minute postvaccination observation period. The used, empty vaccine vials and the bandages will be disposed of in standard biomedical waste container and used syringes will be discarded as medical waste according to the site SOP for medical waste. Affected study personnel should be followed for safety according to local procedures for such events.
Direct human contact with V181 shedding Direct contact of animals with V181 shedding	Wild-type dengue infection has rarely been reported to result in shedding of the virus in saliva and urine {07X3TC}. While no specific data are available on shedding of V181 in saliva, urine or feces, these are expected to be negligible given the lower level of viremia induced by V181 compared to wild-type dengue infection. V181 has a very limited host range (non-human primates and humans) and is not transmitted between humans under environmental conditions through arthropod bites (Section 1). Non-human primates as the only other host despite of

Direct contact of mosquitos with V181 shedding	humans are unlikely to have direct contact with V181 shedding, due to their natural habitat. To minimize spread of the GMO post vaccination, the injection site will be covered with an adequate bandage (e.g. any adhesive bandage or gauze and tape) that provides a physical barrier to protect against direct contact. The bandage may be removed when there is no visible fluid leakage at the end of the 30-minute postvaccination observation period. The used, empty vaccine vials and the bandages will be disposed of in standard biomedical waste container and used syringes will be discarded as medical waste according to the site standard SOP for medical waste. Due to existing rare reports of dengue transmission via blood transfusion, bone marrow transplant or organ transplant, the study subjects will be instructed to not donate blood or fluid products for 6 weeks after vaccination, this further minimizes any possibility that V181 would be transmitted to other humans.
Transmission of V181 to non-subjects through mosquitos	The monovalent components of the tetravalent vaccine have multiple barriers to transmission by mosquitos, resulting in an extremely low risk of transmission of vaccine virus between close contacts/other humans via mosquitos. Therefore, no further specific measures have been implemented regarding transmission of V181 to non- study subjects through mosquitos.
Unintended use / Misuse	Doses of V181 to be delivered to the sites for injections are well controlled and are handled as GMO per local regulations. Only medical personnel trained to handle V181 have access to the drug. If eye contact occurs, eyes will be flushed with tepid tap water for 5 minutes. If skin contact occurs, area will be washed with ordinary soap and tap water. Detailed instructions on accidental breakage/spillage have been developed and will accompany each shipment of V181. Any unused vaccine or waste material should be disposed of in compliance with the institutional guidelines for genetically modified organisms or biohazardous waste, as appropriate. If breakage/spillage were to occur, bleach, quaternary ammonium- and phenolic-based disinfectants will inactivate the virus.

(i) Methods and procedures for controlling V181 in case of unexpected spread

The risk of unexpected spread is negligible, and procedures for controlling V181 are not necessary.

Nevertheless, as V181 is an investigational medicinal product it will be handled during shipment and at site as per relevant clinical trial regulations, local law. The IMP will only be

released by a Qualified Person after all requirements for quality and safety have been met. All applicable information will be provided to the appropriate competent authority for approval. The IMP will only be handled at site by qualified, trained, and delegated staff. All handling instruction will be collected in a pharmacy manual which will be shared with the applicable site staff; each delegated staff will be trained. Also, the site staff will receive clear directions for documenting the control of the IMP from the time of receipt at site until the final accountability log completion. Full reconciliation will be executed by the Sponsor to verify each vial from arrival at site to administration and/or destruction.

(ii) Methods for decontamination of the areas affected

To mitigate against the risk of unintentional release, the GMO will be appropriately contained and labelled during transport. Staff handling the GMO and samples that could potentially contain the GMO should be wearing gloves. If breakage/spillage were to occur, bleach, quaternary ammonium- and phenolic-based disinfectants are proven to reduce viral infection potential after only a few minutes.

As no exposure and thus no spread is expected, disposal and sanitation plans for plants, animals, and soils are not required.

(iii) Methods for the isolation of the area affected by the spread

As no exposure and spread is expected, isolation methods are not necessary. In the theoretical case of a direct exposure of humans or animals to V181 shedding no transmission is expected. Also, transmission of V181 to other humans or animals through mosquitos is not expected as outlined in Table 4. Therefore, no methods for isolation are required.

(iv) Plans for protecting human health and the environment in case of the occurrence of an undesirable effect

Hygiene measures taken during administration of V181 prevent V181 exposure to humans and the environment. Staff handling the GMO and samples that could potentially contain the GMO should be wearing gloves. If breakage/spillage were to occur, bleach, quaternary ammonium- and phenolic-based disinfectants are proven to reduce viral infection potential after only a few minutes.

1.5 Determination of the overall risk of V181

The overall risk of V181 to human health and the environment is considered negligible based on an evaluation of the magnitude of potential adverse effects and likelihood of occurrence. Management strategies demonstrate that exposure of V181 to people and animals can mostly be prevented and that transmission through mosquitos, the almost exclusive mode of transmission, could not be observed. Vaccination of subjects will take place under controlled conditions and in a clinical trial setting, in order to prevent release into the environment by accident. Based on the totality of the data, it is highly improbable that non-treated individuals or non-human primates will experience any issues linked to incidental release of V181.