PART 1 (COUNCIL DECISION 2002/813/EC)

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

In order to tick one or several possibilities, please use crosses (meaning x or X) into the space provided as (.)

A. General information

1. Details of notification

(a)	Member State of notification	Belgium
(b)	Notification number	B/../../….
(c)	Date of acknowledgement of notification	../../….
(d)	Title of the project	A Phase 2, Randomized, Double-Blind, Multicenter Study to Evaluate the Safety and Immunogenicity of Three Different Potency Levels of V181 (Dengue Quadrivalent Vaccine rDENVΔ30 [live, attenuated]) in Healthy Adults
(e)	Proposed period of release	**From 09/Aug/2022 until 05/May/2023**

2. Notifier

Name of institution or company:

Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc. (hereafter referred to as the Sponsor or MSD)

Legal Registered Address:
One Merck Drive
P.O. Box 100
Whitehouse Station, New Jersey, 08889-0100, U.S.A.

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid	(.)
RNA virus	(x)
DNA virus	(.)

bacterium (.)
fungus (.)
animal
- mammals (.)
- insect (.)
- fish (.)
- other animal (.)

specify phylum, class …

(b) Identity of the GMO (genus and species)
Dengue virus vaccine, Flaviviridae family and Flavivirus genus

(c) Genetic stability – according to Annex IIIa, II, A(10)

Attenuation of the four viral components of V181 (comprising the four serotypes of dengue) is driven by the deletion of 30 nucleotides in the 3' non-coding region (Δ30) of each dengue virus genome. WHO in their guidance on live, attenuated, tetravalent dengue vaccine (Guidelines on the quality, safety and efficacy of dengue tetravalent vaccines [live, attenuated] (World Health Organization, 2013) states that a potential reversion is based on the stability of the attenuating mutation(s), the number of attenuating mutations, and the nature of attenuating mutation. Attenuating mutations that are derived by deletions of segments of RNA are generally more stable against reversion than attenuations based on single nucleotide mutations.

The use of the same Δ30 deletion in all components makes these mutations unlikely to revert to wild type through point mutations or recombination.

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?
Yes (X) No (.)
If yes, insert the country code(s) IL, DE, FI

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?
Yes (.) No (X)
If yes:
- Member State of notification …
- Notification number B/../../…

Please use the following country codes:
Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?
Yes (X) No ( )
If yes:
- Member State of notification

Not applicable. The GMO has not been studied or released in Europe

- Notification number B/../../…

Not applicable. The GMO has not been studied or released in Europe

The same trial will be submitted for countries Australia, Taiwan, South Korea, Israel, US, Canada.

Previous release of the GMO:

V181:

V181 study in the US (continental US and Puerto Rico)

NIH dengue live attenuated tetravalent vaccine (LATV):

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. The NIH dengue monovalent vaccines and different formulations of the LATV were tested in Phase 1 trials in the US and two formulations of the LATV, TV003 and TV005, were studied by the NIH in Thailand and Bangladesh.

TV003 and TV005 are the same GMO and only differ in the potency of the DENV2 serotype included in the formulation: TV003 targets 10^3 plaque forming units of each component whereas TV005 targets 10^3 plaque forming units for DENV1, DENV3, DENV4 components and 10^4 plaque forming units of DENV2 component.

TV003 has been evaluated in 5 different Phase 1 studies in the US:

ClinicalTrials.gov NCT01072786 (Kirkpatrick et al., 2015)

ClinicalTrials.gov NCT01436422 (Kirkpatrick et al., 2015)

ClinicalTrials.gov NCT01506570 (Whitehead et al., 2017)

ClinicalTrials.gov NCT01782300 (Durbin et al., 2016)

ClinicalTrials.gov NCT02021968 (Kirkpatrick D. B. et al., 2016)

TV005 has been evaluated in 5 different Phase 1 studies in the US:

ClinicalTrials.gov NCT01072786 (Kirkpatrick et al., 2015

ClinicalTrials.gov NCT01436422(Kirkpatrick et al., 2015

ClinicalTrials.gov NCT02873260

ClinicalTrials.gov NCT02879266

TV003 and TV005 have been evaluated in 2 Phase 2 studies:

ClinicalTrials.gov NCT02332733: Thailand

ClinicalTrials.gov NCT02678455: Bangladesh

Butantan dengue LATV (Butantan-DV) Instituto

Butantan in Brazil used the same dengue viral strains from the NIH to manufacture a dengue LATV analogous to the NIH dengue LATV TV003. Butantan-DV is comprised of the same NIH attenuated viruses, contains the same antigenic composition, is produced using similar methods, and is fundamentally the same as V181.

Butantan-DV was evaluated in 2 trials in Brazil

ClinicalTrials.gov NCT02406729

ClinicalTrials.gov NCT01696422 (Kallas et al., 2020)

7. Summary of the potential environmental impact of the release of the GMOs.

Dengue is a mosquito-borne viral disease that has rapidly spread in all regions of WHO in recent years. Dengue virus is transmitted by female mosquitoes mainly of the species *Aedes aegypti* and, to a lesser extent, *Ae. albopictus*. These mosquitoes are also vectors of chikungunya, yellow fever and Zika viruses. Dengue is widespread throughout the tropics, with local variations in risk influenced by rainfall, temperature, relative humidity and unplanned rapid urbanization. In 2020, dengue affected several countries, with increased number of cases in Bangladesh, Brazil, Cook Islands, Ecuador, India, Indonesia, Maldives, Mauritania, Mayotte (Fr), Nepal, Singapore, Sri Lanka, Sudan, Thailand, Timor-Leste and Yemen. In 2021, dengue continues to affect Brazil, Cook Islands, Colombia, Fiji, Kenya, Paraguay, Peru and Reunion island (https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue).

Dengue virus is only believed to infect humans via direct exposure to blood/blood products or via mosquito bite (i.e.vectorborne disease) (World Health Organization, 2009). 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 (World Health Organization, 2009).

V181 has a similarly limited host range (non-human primates and humans) but studies conducted by the NIH suggest that the attenuated viruses comprising V181 are not transmitted from human to human via mosquitos.

According to the NIH 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 (Troyer et al., 2001). 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 (Troyer et al., 2001).
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 (Troyer et al., 2001); (Blaney Jr et al., 2004); (Blaney Jr et al., 2004); (Rosen et al, 1985).
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) (Whitehead et al., 2003).
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 (Blaney Jr et al., 2004).
In summary, the monovalent components of the tetravalent NIH 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 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 (Poloni et al., 2010); 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. 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.).

In addition, 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.

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 operating procedures (SOPs) for medical waste.

Finally, 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.

In addition, authorized study personnel at the clinical sites will be provided with instructions on how to handle V181 as outlined in section 4.c.

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 unlikely 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.

B. Information relating to the recipient or parental organism from which the GMO is derived

1. Recipient or parental organism characterisation:

(a) Indicate whether the recipient or parental organism is a:

(select one only)

viroid		(.)
RNA virus		(x)
DNA virus		(.)
bacterium		(.)
fungus		(.)
animal		
-	mammals	(.)
-	insect	(.)
-	fish	(.)
-	other animal	(.)
	(specify phylum, class)	…

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	…
(ii)	genus	Flavivirus
(iii)	species	dengue virus (DENV)
(iv)	subspecies	…
(v)	strain	4 Strains (DENV1, DENV2, DENV3, DENV4)
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	Dengue

DENV1: Western Pacific
DENV2: prM-E DENV2 New Guinea C/DENV4 backbone 814669
DENV3: Slemen/78
DENV4: Dominica/81 strain 814669

3. Geographical distribution of the organism

Dengue is widespread throughout the tropics and subtropics where Aedes sp. mosquitos, the vector for dengue transmission, are endemic. Dengue spread if influenced by local s and increases with rainfall, temperature, relative humidity and unplanned rapid urbanization. The virus is not endemic in the EU but travelers visiting the tropics, including territories of EU countries, have been known to return to the EU with infection. Early detection of infectious dengue patients is important to prevent local transmission in areas where the vector is present and active. Dengue is a notifiable disease in the EU and information is collected through the TESSy system (https://www.ecdc.europa.eu/en/dengue-fever/facts).

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (.) No (x) Not known (.)

(b) Indigenous to, or otherwise established in, other EC countries:
(i) Yes (.)

If yes, indicate the type of ecosystem in which it is found:

Atlantic	X	.. (Madeira)
Mediterranean	X	..
Boreal		..
Alpine		..
Continental		..
Macaronesian		..

(ii) No (x)
(iii) Not known (.)

(c) Is it frequently used in the country where the notification is made?
Yes (.) No (x)

(d) Is it frequently kept in the country where the notification is made?
Yes (.) No (x)

4. Natural habitat of the organism

(a) If the organism is a microorganism

water	(.)
soil, free-living	(.)
soil in association with plant-root systems	(.)
in association with plant leaf/stem systems	(.)
other, specify	The host range is restricted to humans, simians and mosquitoes

(b) If the organism is an animal: natural habitat or usual agroecosystem:
Not Applicable

5. (a) Detection techniques
The viral components of V181 are detected using validated RT-PCR assays (details on the assay are provided in Attachment 1. V181 Method TSOP). The assays are designed to distinguish the viral vaccine strains from wild type strains.

(b) Identification techniques
The validated RT-PCR assays are specific for each of the 4 dengue serotypes. The assays can identify which serotype is present in a sample and whether it is a vaccine strain or wild type strain.

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?
Yes (x.) No (.)
If yes, specify
EC Directive 2000/54/EU classifies Dengue virus as human pathogen Risk group 3.

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes (x) No (.) Not known (.)

If yes:

(a) to which of the following organisms:

humans	(x)
animals	(.)
plants	(.)
other	(.)

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC

The host range is restricted to humans, simians and mosquitoes and of those hosts dengue is only known to be pathogenic for humans. Mosquitoes are the vector for transmission from human to human.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

Humans are infective for mosquitoes a few days before and after the febrile period and mosquitoes become infective for humans 8-12 days after infection

…

(b) Generation time in the ecosystem where the release will take place:

Similar to or longer (e.g. slower replication)(c) Way of reproduction: Sexual ..

Asexual x

(c) Factors affecting reproduction:

Dengue viruses are transmitted by Aedes sp. Mosquitos. Temperature can affect reproduction time inside the mosquito.

9. Survivability

(a) ability to form structures enhancing survival or dormancy: Not applicable

(i)	endospores	(.)
(ii)	cysts	(.)
(iii)	sclerotia	(.)
(iv)	asexual spores (fungi)	(.)
(v)	sexual spores (funghi)	(.)
(vi)	eggs	(.)
(vii)	pupae	(.)
(viii)	larvae	(.)
(ix)	other, specify …	

(b) relevant factors affecting survivability:

Dengue is an enveloped virus. These viruses are known to be easily inactivated by routine surface cleaning and disinfection. Their lipid envelope can be easily destroyed by alcohols. Bleach, quaternary ammonium- and phenolic-based disinfectants will inactivate the virus. The virus is stable in dried blood for up to 9 weeks at room temperature.

10. (a) Ways of dissemination

Mosquitoes are the vector for transmission from human to human. There is evidence of the possibility of maternal transmission (from a pregnant mother to her baby). While vertical transmission rates appear low, with the risk of vertical transmission seemingly linked to the timing of the dengue infection during the pregnancy (https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue). Rare case reports of transmission via needlestick in patient care and laboratory accident, blood transfusion, bone marrow transplant or organ transplant, exist.

(b) Factors affecting dissemination

Dengue virus may undergo 2 different cycles of transmission and amplification, sylvatic and urban. In the sylvatic cycle the virus undergoes rounds of infection, amplification, and re-infection between nonhuman primates and arthropod vectors. It is believed that infected arthropod vectors then migrate from jungle environments and initiate the urban cycle in which the cycles of infection, amplification, and re-infection occur between humans and vector species. Dissemination of dengue is directly related to the presence and density of mosquitos that can transmit the virus.

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers)

…, B/../../…

C. **Information relating to the genetic modification**

1. Type of the genetic modification

(i)	insertion of genetic material	(.)
(ii)	deletion of genetic material	(x)
(iii)	base substitution	(.)
(iv)	cell fusion	(.)
(v)	others, specify …	

2. Intended outcome of the genetic modification

Each monovalent virus strain includes a genetic modification of the dengue genome [i.e. deletion of 30 nucleotides in the 3' non-coding region (Δ30)]. rDENV1Δ30, rDENV3Δ30/31, and rDENV4Δ30 are full-length homotypic genomes. The DENV3 component has an additional 31-nucleotide deletion in the 3' non-coding region (Δ30/31). rDENV2/4Δ30(ME) is a chimeric virus with the pre-M and E proteins from DENV2 inserted into an attenuated rDENV4Δ30 backbone where the DENV4 pre-M and E proteins have been deleted. All 4 strains have been fully characterized and their attenuation confirmed through testing in vitro and in vivo studies (Durbin, 2020).

3. (a) Has a vector been used in the process of modification?

Yes (X) No (.)

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?
Yes (.) No (X)

If no, go straight to question 5.

4. If the answer to 3(b) is yes, supply the following information

(a) Type of vector

plasmid	(.)
bacteriophage	(.)
virus	(.)
cosmid	(.)
transposable element	(.)
other, specify	…

(b) Identity of the vector
…

(c) Host range of the vector
…

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype
Yes (.) No (.)

antibiotic resistance	(.)
other, specify	…

Indication of which antibiotic resistance gene is inserted
…

(e) Constituent fragments of the vector
…

(f) Method for introducing the vector into the recipient organism

(i)	transformation	(.)
(ii)	electroporation	(.)
(iii)	macroinjection	(.)
(iv)	microinjection	(.)
(v)	infection	(.)
(vi)	other, specify	…

5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?

(i)	transformation	(x)
(ii)	microinjection	(.)

(iii) microencapsulation (.)
(iv) macroinjection (.)
(v) other, specify …

6. Composition of the insert

(a) Composition of the insert

There is no insert in the rDENV1Δ30, rDENV3Δ30/31, and rDENV4Δ30 components of V181: the vaccine strains include a modification (deletion) of a portion of the dengue virus as the mechanism of attenuation. For the rDENV2/4Δ30(ME) component of V181, the rDENV4Δ30 backbone is utilized and the pre-M and E genes are deleted from the backbone and the homologous pre-M and E genes from DENV2 are inserted in its place (Durbin, 2020).

(b) Source of each constituent part of the insert

In the rDENV2/4Δ30(ME) component of the vaccine the pre-M and E genes are derived from DENV2. All other genes are derived from DENV4.

(c) Intended function of each constituent part of the insert in the GMO

The pre-M and E genes from DENV2 are expressed on the surface of the virus and are responsible for binding to host cell receptor. The E protein also serves as a major immunogen for the vaccine virus.

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify …Integrated into the viral genome

(e) Does the insert contain parts whose product or function are not known?

Yes (.) No (x)
If yes, specify …

D. Information on the organism(s) from which the insert is derived

Applies only to rDENV2/4Δ30(ME)

1. Indicate whether it is a:

viroid (.)
RNA virus (x)
DNA virus (.)
bacterium (.)
fungus (.)
animal
- mammals (.)
- insect (.)
- fish (.)
- other animal (.)
(specify phylum, class) …

other, specify …

2. Complete name

(i)	order and/or higher taxon (for animals)	…
(ii)	family name for plants	
(iii)	genus	Flavivirus
(iv)	species	dengue
(v)	subspecies	serotype 2 (DENV2)
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	dengue

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (x) No (.) Not known (.)
If yes, specify the following:

(b) to which of the following organisms:

humans	(x)
animals	(.)
plants	(.)
other	..

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism
Yes (x) No (.) Not known (.)

If yes, give the relevant information under Annex III A, point II(A)(11)(d):
For the rDENV2/4Δ30(ME) chimeric component of V181 the rDENV4Δ30 backbone is utilized and the pre-M and E genes are deleted from the backbone and the homologous pre-M and E genes from DENV2 are inserted in its place. The host range for DENV2 is restricted to humans, simians and mosquitoes and of those hosts DENV2 is only known to be pathogenic for humans.

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?
Yes (x.) No (.)
If yes, specify RG3 as per 2000/54/EU

5. Do the donor and recipient organism exchange genetic material naturally?
Yes (x) No (.) Not known (.)
Recombination between flaviviruses has been observed and so it is possible between DENV2 and DENV4 upon co-infection

E. Information relating to the genetically modified organism

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

(a) is the GMO different from the recipient as far as survivability is concerned?
Yes (.) No (x) Not known (.)
Specify …

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?
Yes (x) No (.) Unknown (.)
Specify The GMO viral strains are attenuated due to reduced viral replication linked to the Δ30 or Δ30/31 deletions in the viral genomes.

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (x) No (.) Not known (.)
Specify The GMO viral strains are attenuated due to reduced viral replication linked to the Δ30 or Δ30/31 deletions in the viral genomes. Viremia is reduced in human subjects and infectivity of mosquitoes and mosquito cell-lines is reduced.

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (x) No (.) Not known (.)
Specify The GMO is attenuated and well tolerated with minimal clinical manifestations in nonclinical and clinical trials .

2. Genetic stability of the genetically modified organism
V181 is a live attenuated dengue tetravalent vaccine. Each monovalent serotype of the vaccine includes a genetic modification of the dengue genome [i.e. deletion of 30 nucleotides in the 3′non-coding region (Δ30)]. Two of the four vaccine constructs have additional modifications. DENV2 is the only serotype that is not a full-length homotypic genome but is instead a chimeric virus with the pre-M and E protein from DENV2 inserted into an attenuated DENV4 backbone and is designated as rDENV2/4Δ30(ME). DENV3 has an additional 31-nucleotide deletion in the 3′ non-coding region and is designated as rDENV3Δ30/31 (Durbin, 2020) . The viruses have been shown to be clinically attenuated by being well tolerated with minimal clinical manifestations in nonclinical and clinical trials. Details are provided in the attached Investigators Brochure (IB).

The attenuation is driven by the deletion of 30 nucleotides in the 3’ non-coding region (Δ30) of the dengue genome. WHO in their guidance on live, attenuated, tetravalent dengue vaccine {Guidelines on the quality, safety and efficacy of dengue tetravalent vaccines (live, attenuated) WHO Technical Report Series, No. 932, WHO Technical Report Series No. 979, 2013} states that a potential reversion is based on the stability of the attenuating mutation(s),

the number of attenuating mutations, and the nature of attenuating mutations. Attenuating mutations that are derived by deletions of segments of RNA are generally more stable against reversion than attenuations based on single nucleotide mutations.

The use of the same Δ30 deletion in all components makes these mutations unlikely to revert to wild type through point mutations or recombination.

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?

Yes (.) No (x) Unknown (.)

(a) to which of the following organisms?

humans (.)
animals (.)
plants (.)
other …

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)

The V181 vaccine viruses are attenuated for pathogenicity in their target populations and demonstrate reduced replication. Recombination between a vaccine virus and a wild type virus, should it happen, should not lead to a more virulent virus than the wild type virus itself.

The probability of non-homologous recombination, such as between V181 and another non-related RNA virus, is substantially lower than homologous recombination between related viruses. 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.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
A PCR method will be used to detect the GMO in blood of vaccinees (Attachment 1).

(b) Techniques used to identify the GMO
Sequencing of Master Virus Seed will confirm identity of the GMO

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)

Vaccination of human subjects in a clinical trial to show safety and immunogenicity with the vaccine candidate to protect against dengue virus and to generate data to support registration of the vaccine.

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?

Yes (x) No (.)

If yes, specify …

The virus is not endemic to the EU but travelers visiting the tropics have been known to return to the EU with infection. Dengue is widespread throughout the tropics and subtropics, with local variations in risk influenced by rainfall, temperature, relative humidity and unplanned rapid urbanization. The major vector, Aedes aegypti, is not endemic to Europe. Aedes albopictus is established in southern Europe but uncommon in the countries where the proposed study will take place (https://www.ecdc.europa.eu/en/disease-vectors/surveillance-and-disease-data/mosquito-maps)

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):
The GMO will be administered subcutaneously within an international Phase II clinical trial, at 4 sites in Belgium:

Site 1:
Principal Investigator: Prof. Dr. Isabel Leroux-Roels
UZ Gent – CEVAC – Center For Vaccinology
Corneel Heymanslaan 10
B-9000 Gent

Site 2:
Principal Investigator: Prof. Dr. Charlotte Martin
CHU Saint-Pierre
Rue Haute 32
B-1000 Brussels

Site 3:
Principal Investigator: Prof. Dr. Patrick Soentjens
Instituut voor Tropische Geneeskunde – Department Clinical Services
Nationalestraat 155
B-2000 Antwerpen

Site 4:
Principal Investigator: Dr. Erik Buntinx
ANIMA Research Center
Alkerstraat 28
B-3570 Alken

(b) Size of the site (m^2): N/A
Expected patients included per site:

- UZ Gent – CEVAC – Center For Vaccinology: 60 subjects
- CHU Saint-Pierre: 25 subjects

- Instituut voor Tropische Geneeskunde – Department Clinical Services: 60 subjects
- ANIMA Research Center: 40 subjects

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
NA

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
The GMO is to be administered subcutaneous in a clinical trial setting. The release into the environment would only be expected to occur by incidental shedding from participants. See also section G.

4. Method and amount of release

(a) Quantities of GMOs to be released:
The clinical vector or placebo will be administered as a 0.5-mL subcutaneous injection at Visit 1 (Day 1). Each of the sites listed above intents to include 43 patients in the study. A total amount of 185 patients is planned for Belgium. Participants will be assigned randomly in a 2:4:4:1 ratio to the V181 High Potency Level Group, V181 Mid Potency Level Group, V181 Low Potency Level Group, and placebo. The V181 study vaccine potency levels to be evaluated in this trial are $10^{4.0}$ to $10^{4.95}$ pfu/serotype for the V181 High Potency Level Group, $10^{2.8}$ to $10^{3.8}$ pfu/serotype for the V181 Mid Potency Level Group, and $10^{2.0}$ to $10^{2.5}$ pfu/serotype for the V181 Low Potency Level Group. Final potency levels, within those specified ranges, are to be determined.

At clinical sites, partial or empty vials will be properly discarded as biohazardous waste. Clinical supplies that are affected by a temperature excursion and determined to be unacceptable for future use will be returned to the Sponsor or discarded per local guidance. At last patient last visit but no later than the end of the study, the site personnel will return all unused clinical supplies to the sponsor or discard the clinical supplies per local guidance. Where local discard and destruction is appropriate, the investigator is responsible for ensuring that a local discard/destruction procedure is documented.

(b) Duration of the operation:
The Sponsor estimates that the study will require approximately 20 months from the time the first participant signs the informed consent until the last participant's last study-related telephone call or visit.
Each participant will participate in the study for approximately 12 months from the time the participant provides documented informed consent through the final contact.

(c) Methods and procedures to avoid and/or minimize the spread of the GMOs beyond the site of the release
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 SOP for spill response and cleanup. Bleach, quaternary ammonium- and phenolic-based disinfectants will inactivate the virus.

5. Short description of average environmental conditions (weather, temperature, etc.)
The treatment of clinical trial patients will be performed in a hospital or an outpatient clinical setting in an independent room under ambient indoor conditions.

6. Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release.

As noted in Section 6, V181 is similar by design to the NIH dengue LATV and Butantan-DV based on using the same starting viral materials and similar manufacturing processes. These vaccines have identical consensus sequences except for minor differences and are produced by a similar manufacturing process. Thus, results from NIH studies with NIH LATV and Butantan studies with Butantan-DV are considered applicable for Merck-manufactured V181. The attached IB provides details of the non-clinical and clinical program conducted to date.

The absence of a clinically apparent dengue-like illness with V181 is likely a result of the high degree of attenuation of the NIH vaccine viruses. Peak viremia titers are more than ≥100-fold lower than those observed with symptomatic wild type dengue infection (Whitehead et al., 2003), (Durbin et al., 2013).

V181, NIH LATV and Butantan-DV were well tolerated by study participants in Phase 1 and Phase 2 studies with these vaccines. As outlined in the attached IB, the only adverse effects that occurred significantly more frequently in vaccinees compared with placebo recipients was a mild transient rash and an occasional mild transient leukopenia. The low level viral replication of the attenuated strains and lack of infectivity for mosquitoes mean that the risk of dissemination is negligible since that is the key mechanism for dengue spread.

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

The vaccine strains result from a direct modification of the 4 viruses which leads to reduced replication and reduced infectivity for mosquitoes compared to wild type dengue viruses. Shedding from vaccinated people or infection of mosquitoes is anticipated to be very limited which makes it highly unlikely that V181 reaches the environment at large. This is again because the mosquitos that are hosts for dengue are not endemic in the countries where the trial will take place and, even if they are sporadically present, the low level of viremia induced by the vaccine does not cause infectivity in mosquitos.

1. Name of target organism (if applicable)

(i)	order and/or higher taxon (for animals)	…
(ii)	family name for plants	…
(iii)	genus	Aedes
(iv)	species	Aedes species (ex. Aegypti; albopictus)
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…

(ix) common name mosquitoes

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
None. The vaccine viruses have been shown to not efficiently infect mosquitoes or mosquito cells

3. Any other potentially significant interactions with other organisms in the environment
No

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (x) Not known (.)
Give details
Increased competitiveness or invasiveness is unlikely due to the attenuated nature of the vaccine and low level viral replication.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
The low level viral replication of the attenuated strains and lack of infectivity for mosquitoes mean the risk of dissemination is negligible since that is the key mechanism for dengue spread.

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO

None

(i) order and/or higher taxon (for animals) …
(ii) family name for plants …
(iii) genus …
(iv) species …
(v) subspecies …
(vi) strain …
(vii) cultivar/breeding line …
(viii) pathovar …
(ix) common name …

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
Negligible due to low infectivity of mosquitoes.

(b) from other organisms to the GMO:
Very low – would require coinfection and recombination which is very unlikely given short period and very low level of vaccine viremia observed. Recombination between a vaccine virus and a wild type virus, should it happen, should not lead to a more virulent virus than the wild type virus itself (Condit et al., 2016).

(c) likely consequences of gene transfer:
none

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):
Not applicable

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
Not applicable

H. Information relating to monitoring

No environmental monitoring is planned

1. Methods for monitoring the GMOs

2. Methods for monitoring ecosystem effects
…

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
…

4. Size of the monitoring area (m^2)
… m^2

5. Duration of the monitoring
…

6. Frequency of the monitoring
…

I. Information on post-release and waste treatment

1. Post-release treatment of the site
None

2. Post-release treatment of the GMOs
None

3. (a) Type and amount of waste generated
Empty vials, medical waste, vaccination site bandage, used PPE

3. (b) Treatment of waste
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

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread

   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.

2. Methods for removal of the GMO(s) of the areas potentially affected
   N/A

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
   N/A

4. Plans for protecting human health and the environment in the event of an undesirable 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. Management strategies demonstrate that exposure of V181 to people and animals can be prevented altogether. Administration of the vaccine would take place under controlled conditions in order to prevent release into the environment by accident. Shedding from vaccinated people or infection of mosquitoes is anticipated to be very limited which makes it highly unlikely that V181 reaches the environment at large.

B. References

Blaney Jr, J. E. (2004). Genetically Modified, Live Attenuated Dengue Virus Type 3 Vaccine Candidates. *American Journal of Tropical Medicine and Hygiene*, 811-821.

Blaney Jr, J. E. (2004). Vaccine candidates derived from a novel infectious cDNA clone of an American genotype dengue virus type 2. *BMC Infectious Diseases*.

Condit, R. C. (2016). Unique safety issues associated with virus-vectored vaccines: Potential for and theoritical consequences of recombination with wild type virus strains. *Elsevier*, 6610-6616.

Durbin, A. P. (2013). A Single Dose of Any of Four Different Live Attentuated Tetravalent Dengue Vaccines Is Safe and Immunogenic in Flavivirus-naive Adults: A Randomized, Double-blind Clinical Trial. *JID*, 957-965.

Durbin, A. P. (2016). A 12-Month–Interval Dosing Study in Adults Indicates That a Single Dose of the National Institute of Allergy and Infectious Diseases Tetravalent Dengue Vaccine Induces a Robust Neutralizing Antibody Response. *The Journal of Infectious Diseaes* , 832-835.

Durbin, A. P. (2020). Historical discourse on the development of the live attentuated tetravalent dengue vaccine candidate TV003/TV005. *Current Opinion in Virology*, 79-87.

Kallas, E. G. (2020). Safety and immunogenicity of the tetravalent, live-attentuated dengue vaccine Butantan-DV in adults in Brazil: a two-step, double-blind, randomised placebo-controlled phase 2 trial. *Lancet Infectious Diseases*, 3020-30023.

Kirkpatrick, B. D. (2015). Robust and Balanced Immune Responses to All 4 Dengue Virus Serotypes Following Administration of a Single Dose of a Live Attenuated Tetravalent

Dengue Vaccine to Healthy, Flavivirus-Naive Adults. *The Joural of Infectious Diseases*, 702-710.

Kirkpatrick, B. D. (2016). The live attenuated dengue vaccine TV003 elicits complete protection against dengue in a human challenge model. *Science Translation Medicine*, 330-336.

Poloni, T. R. (2010). Detection of dengue virus in saliva and urine by real time RT-PCR. *Virology Journal*.

Rosen, L. (1985). Comparative Susceptibility Of Mosquito Species And Straints to Oral and Parenteral Infection With Dengue and Japanese Encephalitis Viruses. *American Journal of Tropical Medicine and Hgyiene*, 603-615.

Troyer, J. M. (2001). A Live Attentuated Recombinant Dengue-4 virus Candidate With Restricted Capactiy for Dissemination in Mosquitoes and Lack Of Transmission From Vaccinees to Mosquitoes. *American Journal Tropical Medicine and Hygiene* , 414-419.

Whitehead, S. S. (2003). Substitution of the Structural Genes of Dengue Virus Type 4 with those of Type 2 Results in Chimeric Vaccine Candidates which are Attentuated for Mosquitoes, Mice, and Rhesus Monkeys. *Elsevier*, 4307-4316.

Whitehead, S. S. (2017). In a randomized trial, the live attentuated tetravelent dengue vaccine TV003 is well-tolerated and highly immunogenic in subjects with flavivirus exposure prior to vaccination. *PLOS Neglected Tropical Diseases*, 1-19.

World Health Organization. (2013). *WHO Expert Committee on Biological Standardization.* Geneva: World Health Organization.

World Health Organization. (2009). *Guidelines For Diagnosis, Treatment, Prevention and Control* . Geneva: World Health Organization.