INFORMATION FOR THE PUBLIC

- ENGLISH -

Pfizer Animal Health

Information for the public

Evaluation of the safety of a Feline Herpes virus, bivalent gene deleted live vaccine, administered as intranasal vaccine to cats.

European Notification number
B/BE/04/~~V1~~ BV1

HG SBB 13.09.04 (1)

The release of genetically modified organisms (GMOs) in the environment is strictly regulated at European level by Directive 2001/18/EC repealing Directive 90/220/EEC and at Belgian level by a new Royal Decreee "regulating the deliberate release and/or marketing of GMOs or products that contain GMOs into the environment" repealing the Royal Decree of 18 December 1998. The regulatory transition procedure is still ongoing for the moment.

To ensure the safe use of GMOs, the provisions of the Royal Decree above stipulate that the release of GMOs for experimental aims is prohibited without prior consent from the competent Minister. The decision is based on a thorough evaluation of the biosafety of the planned release, which is conducted by the Biosafety Advisory Council, composed of different Scientific Committees grouping independent experts from Belgian Universities and governmental institutes.

To acquire the necessary authorization from the competent minister, the Company Pfizer submitted an application dossier to the competent authority. On the basis of the advice of the Biosafety council, the competent minister could grant a permission to the company Pfizer to conduct experiments with Feline Herpes virus, bivalent gene deleted live vaccine as stipulated in the application B/BE/04/V1.

The release will take place at one or more locations in Flanders ~~in the municipalities of Mechelen~~. It is expected to start on August 04 and to be completed on August 05.

* and Brussels in the municipalities of Mechelen, Sint Katelijne Waver and Schaerbeek.

HG SBB 13.09.04 (1)

(1) Correction made by SBB after modification and complementary information received regarding the technical dossier

Table of Contents

General Information

Description of the genetically modified micro-organism (GMM)

Feline Immunodeficiency Virus-Rhinotracheitis Vaccine, Live Feline Herpesvirus Vector, is a gene-deleted, viral vectored vaccine designed to aid in the prevention of infection against FIV in cats and as an aid in the prevention of feline viral rhinotracheitis (FVR) in cats. It contains two, live attenuated FHVs, expressing either the env glycoprotein or gag protein of FIV, hereafter referred to as rFHV-FIVenv or rFHV-FIVgag, respectively. The target species is feline and the route of administration is intranasal. The lyophilized vaccine is intended for use in animals at eight weeks of age or older. The basic vaccination program is a two-dose regime preferentially given at 8 and 12 weeks of age. A booster vaccination is recommended annually.

Type and purpose of the envisaged trial

The safety and efficacy of the test vaccine in cats has been established under controlled conditions. This field safety study will confirm the safety of test vaccine in the target species under field use conditions. This study is required to satisfy the biological licensing requirements in the EU. Privately owned healthy cats will be evaluated in the investigation. All 8-week-old or older cats will be considered irrespective of breed and sex. Pregnant or suspected pregnant queens will be excluded from the investigation. At least 40 animals will be targeted for enrollment in the entire study over multiple sites. This multi-center study will be conducted at approximately 2-20 companion animal practices in Flanders that have a known reputation or interest in feline medicine and the ability to conduct this clinical study according to the protocol. At least 40 total cats need to be enrolled for all sites combined

Cats enrolled at each site will receive vaccinations by a Licensed Veterinarian. A total of two vaccinations will be administered to each cat during the study period. Safety observations of the vaccinated cats will be performed following each vaccination. Two weeks after the second vaccination a final observation will be done by the Veterinarian.

Research/Development Activities

Previous Development Activities

Target Animal Safety

a. Vaccination

Several trials have been conducted in cats ranging from seven to twelve weeks of age at the time of vaccination. In a typical safety or efficacy trial, animals were vaccinated intranasally with either one dose or two doses of vaccine. The latter dosing regime was three to four weeks between vaccinations. The effects of vaccination in these typical trials are summarized in Table 1. In one study (Table 1, rows 1 & 2), response of cats to vaccination was monitored for 31 days and compared to clinical scores of control animals. In efficacy studies (Table 1, rows 3 & 4), the response of cats following primary or booster vaccination was monitored for 14 days. Clinical observations of occasional sneezing or mild, transient serous nasal or ocular discharges were observed in animals given the vaccine. Clinical signs

suggestive of feline viral rhinotracheitis (FVR) were not observed in any of the vaccinated animals. The transient discharges were attributed to the initial replication of the attenuated herpesviruses in the nasal and ocular epithelial tissues. This safety profile is consistent with other modified live intranasal vaccines and the FHV vector (thymidine kinase [TK] deletion without the FIV gene inserts). Overall, the cats

1) tolerated the vaccine,

2) remained active,

3) continued to eat and gain weight,

4) were presented as healthy animals following vaccination.

Table 1. Response of Cats to the rFHV-FIV Vaccine*

										Transient Signs†		
Virus Inoculum ID	**Dose ~**	**Age (Weeks)**	**No. of Cats**	**No. Days Observed**	**No. of Cat Observation Days‡**	**Healthy §**	**Clinical Signs of Disease¶**	**Clinical Fever††**	**Hypothermia ‡‡**	**Sneezing**	**Nasal Serous Discharge**	**Ocular Serous Discharge**
rFHV-FIVenv	Max	7-8	10	31	310	310/310	0/310	1/280 §§	0/310	15/310	42/310	2/310
rFHV-FIVgag	Max	7-8	10	31	310	310/310	0/310	0/280 §§	0/310	8/310	17/310	3/310
rFHV-FIVenv + rFHV-FIVgag¶¶	Min	7-8	35	14	490	490/490	0/490	0/490	0/490	23/490	2/490	8/490
rFHV-FIVenv + rFHV-FIVgag***	Min	11-12	35	14	490	490/490	0/490	0/490	0/490	1/490	31/490	1/490

~ Max = dose used at maximum release titre; Min= dose used at minimum release titre

∗ No mortality and weight loss were observed in any of the cats enrolled in the safety or efficacy studies.

† The transient signs are expected after intranasal exposure to a live replicating virus.

‡ Cat Observation Days = No. of cats x days observed.

§ Healthy = normal activity with no signs of clinical disease.

¶ Clinical signs include frequent sneezing, fever, conjunctivitis, mucopurulent and copious nasal ocular discharge, depression and anorexia.

†† Body temperature of ≥ 103° F was considered as clinical fever. The body temperature of one cat was 103° F for one day.

‡‡ Body temperature of < 99° F was considered as hypothermia.

§§ Responses were monitored only for 28 days.

¶¶ Cats received a primary vaccination with the two vaccine components combined in a 50/50 mixture.

∗∗∗ Cats received a booster vaccination with the two vaccine components combined in a 50/50 mixture.

b. Reversion to virulence

The method required the backpassage of the vaccine strains through at least five serial passages in cats. Separate groups of 10-12 week old kittens were vaccinated intranasally with one or the other master seed components. Both vaccine components (rFHV-FIVenv and rFHV-FIVgag) were recovered from primary recipient (first passage) cats at a level that allowed intranasal inoculation of cats in subsequent backpassages. Both vaccine components replicated to similar titres in all subsequent passages (Table 2).

Table 2. rFHV-FIV Recovery from Reversion to Virulence Study: Pooled Tissue Suspensions∗

	rFHV-FIVenv			rFHV-FIVgag		
Backpassage No. †	**Kitten ID**	**Necropsy Day**	**Pooled Virus Recov. ($TCID_{50}$/ml)**	**Kitten ID**	**Necropsy Day**	**Pooled Virus Recov. ($TCID_{50}$/ml)**
1	L225	5	$10^{5.58}$	L207	5	$10^{4.83}$
	L321			L253		
	L395			L319		
2	L397	5	$10^{3.92}$	M023	5	$10^{4.92}$
	L399			M025		
	L401			M027		
3	M029	5	$10^{4.75}$	M103	5	$10^{5.10}$
	M075			M127		
	M101			M131		
4	M163	5	$10^{4.26}$	M179	5	$10^{4.30}$
	M167			M183		
	M169			M185		
5	M187	5	$10^{5.41}$	M223	5	$10^{4.41}$
	M189			M225		
	M197			M227		

∗ All kittens were inoculated via the intranasal route.

† Cats in backpassage 1 were inoculated with a titre at max release of rFHV-FIVenv Master Seed Virus (MSV) or rFHV-FIVgag MSV. Cats in backpassages 2 through 5 were inoculated with pooled suspension from previous backpassage.

Kitten ID:+ Kitten identification

The stability of each vaccine component was confirmed via polymerase chain reaction (PCR) and Western blot analysis of the original virus inoculum and viruses recovered from the fifth backpassage. The interpretation of these data was that both strains of the vaccine were able to replicate in the oro-pharynx of the vaccinated cats. Further, the lack of clinical signs suggestive of FVR and unchanged replicative potential over five backpassages confirmed that neither strain was able to revert to a virulent phenotype.

c. Effect of overdosing

Target animal safety studies were conducted by administering varying levels at or just above maximum release titre of rFHV-FIVenv MSV or rFHV-FIVgag MSV in susceptible kittens. Inoculated kittens were observed from 5-31 days. Following inoculation, some of the kittens exhibited occasional sneezing and had mild transient nasal/ocular serous discharges. None of the kittens had significant or copious nasal/ocular discharges during the observation period. It is also evident from the data that none of the kittens had any clinical signs of systemic feline diseases. The daily observations from several target animal safety studies were summarized and shown in Table 3.

Table 3. Safety Profile of rFHV-FIV Master Seeds in Cats Observed During the Post-Inoculation Period*

										Transient Signs†		
Virus Inoculum ID	**Dose ~**	**Age (Weeks)**	**No. of Cats**	**No. Days Observed**	**No. of Cat Observation Days‡**	**Healthy §**	**Clinical Signs of Disease¶**	**Clinical Fever††**	**Hypothermia ‡‡**	**Sneezing**	**Nasal Serous Discharge**	**Ocular Serous Discharge**
rFHV-FIVgag	Max	3-4	10	14	140	140/140	0/140	0/140	3/140	54/140	108/140	13/140
rFHV-FIVenv	Max	7-8	10	31	310	310/310	0/310	1/280§§	0/310	15/310	42/310	2/310
rFHV-FIVenv	Above max	11-12	3	5	15	15/15	0/15	0/15	0/15	2/15	0/15	0/15
rFHV-FIVgag	Above max	11-12	3	5	15	15/15	0/15	0/15	0/15	0/15	0/15	0/15

~ Max= maximum release titre; above max = approximately 0.3 log above maximum release titre

∗ No mortality and weight loss were observed in any of the cats enrolled in the safety studies.

† The transient signs are expected after intranasal exposure to a live replicating virus.

‡ Cat Observation Days = No. of cats x days observed.

§ Healthy = normal activity with no signs of clinical disease.

¶ Clinical signs include frequent sneezing, fever, conjunctivitis, mucopurulent and copious nasal ocular discharge, depression and anorexia.

†† Body temperature of ≥ 103° F was considered as clinical fever. The body temperature of one cat was 103° F for one day.

‡‡ Body temperature of < 99° F was considered as hypothermia. One foster cat had hypothermia for three days.

§§ Temperature responses were monitored only for 28 days.

The data from Table 4 and table 5 show the safety profiles of rFHV-FIV Master Seeds in cats observed during the first five or 14 days of the post-inoculation period, respectively. The mild transient nasal/ocular serous discharges observed in some of the inoculated kittens was considered to be an expected localized immune response to a live attenuated vaccine organism with intranasal application.

Table 4. Safety Profile of rFHV-FIV Master Seeds in Cats Observed During the First Five Days of the Post-Inoculation Period*

										Transient Signs†		
Virus Inoculum ID	**dose ~**	**Age (Weeks)**	**No. of Cats**	**No. Days Observed**	**No. of Cat Observation Days‡**	**Healthy §**	**Clinical Signs of Disease¶**	**Clinical Fever††**	**Hypothermia ‡‡**	**Sneezing**	**Nasal Serous Discharge**	**Ocular Serous Discharge**
rFHV-FIVgag	Max	3-4	10	5	50	50/50	0/50	0/50	0/50	0/50	0/50	0/50
rFHV-FIVenv	Max	7-8	10	5	50	50/50	0/50	0/50	0/50	0/50	0/50	0/50
rFHV-FIVenv	above Max	11-12	3	5	15	15/15	0/15	0/15	0/15	2/15	0/15	0/15
rFHV-FIVgag	above Max	11-12	3	5	15	15/15	0/15	0/15	0/15	0/15	0/15	0/15

~Max= maximum release titre; above max= 0.3 log above maximum release titre

∗ No mortality and weight loss were observed in any of the cats enrolled in the safety studies.

† The transient signs are expected after intranasal exposure to a live replicating virus.

‡ Cat Observation Days = No. of cats x days observed.

§ Healthy = normal activity with no signs of clinical disease.

¶ Clinical signs include frequent sneezing, fever, conjunctivitis, mucopurulent and copious nasal ocular discharge, depression and anorexia.

†† Body temperature of ≥ 103° F was considered as clinical fever.

‡‡ Body temperature of < 99° F was considered as hypothermia.

Table 5. Safety Profile of rFHV-FIV Master Seeds in Cats Observed During the First Fourteen Days of the Post-Inoculation Period*

										Transient Signs†		
Virus Inoculum ID	**Dose ~**	**Age (Weeks)**	**No. of Cats**	**No. Days Observed**	**No. of Cat Observation Days‡**	**Healthy §**	**Clinical Signs of Disease¶**	**Clinical Fever††**	**Hypothermia ‡‡**	**Sneezing**	**Nasal Serous Discharge**	**Ocular Serous Discharge**
rFHV-FIVgag	Max	3-4	10	14	140	137/140	0/50	0/50	3/140	54/140	108/140	13/140
rFHV-FIVenv	Max	7-8	10	14	140	140/140	0/50	0/50	0/50	6/140	16/140	1/140

~ Max= maximum release titre

∗ No mortality and weight loss were observed in any of the cats enrolled in the safety studies.

† The transient signs are expected after intranasal exposure to a live replicating virus.

‡ Cat Observation Days = No. of cats x days observed.

§ Healthy = normal activity with no signs of clinical disease.

¶ Clinical signs include frequent sneezing, fever, conjunctivitis, mucopurulent and copious nasal ocular discharge, depression and anorexia.

†† Body temperature of ≥ 103° F was considered as clinical fever.

‡‡ Body temperature of < 99° F was considered as hypothermia. One foster cat had hypothermia for three days.

d. Shed/Spread Capabilities

Oro-pharyngeal shedding of the vaccine strains from cats has been monitored quantitatively and qualitatively using a tissue culture method that can detect as low as $10^{1.4}$ $TCID_{50}$ per swab. In one set of studies, healthy, FHV negative, nursing, three to five week-old kittens were vaccinated intranasally with a dose in excess of the anticipated release dose of either vaccine component and left with non-vaccinated littermates on their queen for a two-week observation period Despite the expected lack of clinical signs, shedding was observed in all vaccinates (20/20), usually detectable within one to four days post-vaccination and continuing for 9-14 days. By the end of the 14-day studies, 14/20 of the vaccinates were no longer shedding. Despite the close contact and mutual grooming habits of the kittens and their queens, the virus was only detected in 5 out of 10 of the sentinels, and one out of six of the queens. The levels of virus recovery were minimal. The low level of shedding was confirmed by the findings that only 2 out of 10 of the sentinels developed low FHV specific serum responses (1:3 and 1:6), and seroconversion was not detected in the queens (all <1:2). The data indicated that an overdose in very young kittens led to limited shedding of low levels of the vaccine strains following intranasal vaccination. This was consistent with previous findings using the FHV ?TK vaccine vector.

In a second series of studies, kittens at the earliest recommended age of vaccination were used to determine both the amplitude and duration of shedding in an environment where mixing new susceptible sentinels every seven days induced social-stress. This series of experiments was designed to determine the worst-case scenario for vaccine shedding in a stressed kitten. Once again, despite the lack of clinical signs of disease, shedding was observed in all vaccinates (20/20), usually detectable within two to four days post-vaccination. The amount of virus shed was minimal with most detectable amounts at the limit of detection, $10^{1.4}$ $TCID_{50}$/swab. The majority of the animals (16/20) did not shed after **d**ay **p**ost **v**accination 15 (DPV 15), but a few (1-3) animals did show sporadic shedding of low titres of virus after the mixing of new sentinels on DPV 14 or DPV 21. The inability of the low titre shedding to constitute a serious threat of spread in these animals was confirmed by the inability to detect virus or FHV specific seroconversion in the sentinels. The highest probability of spread to a sentinel was seen if co-mingling occurred within the first seven days after vaccination
(2/6 when mixed on day 0 or 7 post vaccination). Again, the shedding was of limited duration and low amplitude, inducing only low titres of FHV neutralizing antibodies in the sentinels (range: 1:2 - 1:8).

Taken together, the shed/spread data demonstrate that there is minimal shedding of the vaccine strains in the oro-pharyngeal secretions following intranasal administration into nursing kittens or target-age kittens exposed to stressful social interactions. Even at its peak, the shedding is ineffective in terms of infecting susceptible cohorts. We consider neonatal kittens and nursing queens to be prime examples of immunoincompetent and immunosuppressed cats. In these animals, the vaccine was confirmed safe.

e. Safety in pregnant cats

FHV is generally associated with upper respiratory disease complexes. Due to the specificity of viral cell tropism, it has not been known to directly affect other body tissues,

systems, or organelles. The only other potential complications (such as generalized infections, abortions, etc.) associated with this virus are due largely to secondary invading pathogens and not the virus directly. Due to the relatively low levels of horizontal transmission from vaccinated cats, the possibility exists that the vaccine virus might contact pregnant queens. The outcome of such an event is currently deemed to be of limited concern to the developing fetus in that the FHV virus does not specifically target the reproductive system. With recent studies conducted in nursing cats and neonatal kittens using our TK gene deleted vaccine, the vaccine virus

1) did not cause pathology in any of the vaccinated kittens and/or in sentinel nursing queens,
2) was not shed at significant enough levels to result in seroconversion in any of exposed sentinel animals.

Thus, horizontal and/or vertical transmission (if any) of this vaccine virus is not anticipated to be a safety concern for neonatal, nursing, immunoincompetent, and/or pregnant cats.

Knowledge and experience obtained in previous development activities

All the experiments described above have demonstrated that the vaccine can be safely administered to cats according the intranasal route. The experiments have shown that the vaccine is safe to use in cats at the youngest age (8 weeks) that will be recommended on the label. It was also demonstrated that the vaccine strains do not revert to the wildtype strain and do not become virulent after five cat passages. It was also demonstrated that during a short period after vaccination the vaccinated cats shed the vaccine strains and that other cats in close contact with the vaccinated cats can become infected. These cats also did not show any clinical signs related to the vaccine strains. All these experiments were carried out under contained conditions in Specific Pathogen Free (SPF) cats. In the planned trial the aim is to investigate the safety of the vaccine in non-SPF cats under field conditions.

Future Activities

The planned trial is the last step in the development of this vaccine. If this trial is successful all the data will be compiled and submitted to the authorities in Europe to apply for a full marketing authorization.

Benefits

In the early days of the development of this vaccine other approaches, e.g. inactivated FIV virus, subunits have been tested, but where not successful. The only successful approach was based on the vaccine described in this application. The vaccine is intended for the vaccination of healthy kittens to aid in the prevention and/or reduction of FIV plasma viral burdens. Subsequent to reduced FIV plasma burdens this product aids in the reduction of clinical signs associated with feline immunodeficiency syndrome in vaccinated cats and aids in the reduction of FIV shed/spread from vaccinated cats. In addition this vaccine aids in the prevention of disease caused by Feline Herpesvirus.

Risks

Estimation of Risk for FHV Vaccine

The assessments of risk calculations from the use of the vaccine are shown in Table 6. The estimation was performed using the approach described by Gay and Orr [1]. Each of the three sections of the assessment was evaluated separately. The "Degree of Certainty Rating II" was selected for the assessment because there was no instance in which the Likelihood rating was Medium or High and the Consequence rating also was Medium or High, which would have made the "Degree of Certainty Rating I" the appropriate scale. Justifications for each of the ratings are summarized in Table 7.

Table 6. Estimation of Risk for Feline Immunodeficiency Virus-Rhinotracheitis Vaccine, Live Feline Herpesvirus Vector

Risk to:	Likelihood of Adverse Event (LL, LM, or LH)†	Degree of Certainty Rating II (C, MC, or U)‡	Consequence Rating (CL, CM, or CH)§	Degree of Certainty Rating II (C, MC, or U)‡	Risk Characterization*	Expected Risk*	Risk Rating*
Animal Health Safety	LL	C	CL	C	LL•C•CL•C	1.000	L
Public Health Safety	LL	C	CL	C	LL•C•CL•C	1.000	L
Environmental Safety	LL	MC	CL	MC	LL•MC•CL•MC	0.5625	L
Overall Range (worst case)	LL	MC	CL	MC	LL•MC•CL•MC	0.5625	L

* Value and risk ratings (Tables 2 and 3, respectively) of the USDA APHIS Risk Analysis for Veterinary Biologics [1], pp. 11-12.
† LL = Likelihood Low; LM = Likelihood Medium; LH = Likelihood High
‡ C = Certain; MC = Moderately Certain; U = Uncertain
§ CL = Consequence Low; Consequence Medium; Consequence High

Table 7. Justification for the Risk Ratings*

Risk to:	Likelihood Rating and Reasons†	Consequence Rating and Reasons†
Animal Health Safety	**Low**	**Low**
	no adverse reactions were observed in vaccinated animals at protective dose or field dose	exposure to vaccine produces immune response - potentially less spread of respective wild types
	no evidence for reversion to virulence in backpassage study	extremely low likelihood of the vaccine causing disease
	deletional inactivation of the thymidine kinase gene attenuates the vaccine strains	low pathogenicity of vaccine strains in target animals
	genotype of backpassaged vaccine strains did not change as confirmed via PCR	vaccine strains are genetically stable; low concern for reversion to virulence
	phenotype does not change in vaccine strains recovered from vaccinated animals	vaccine strains are phenotypically stable
	substantial reduction in ability to latently colonize the host	target animal not likely to serve as reservoir
	a lack of mortality or morbidity due to overdosing, even in young kittens	inadvertent overdosing will not harm animal
	possibility of non-target animal exposure, but FHV unable to replicate in non-felid species	vaccine is not able to replicate in non-felid species and will not be able to engender disease
Public Health Safety	**Low**	**Low**
	a low probability that humans would be exposed to high levels of the vaccine	inadvertent ingestion or inhalation will not cause a virus specific clinical response or immune response
	no pathogenicity of FHV or FHV vectors in humans	FHV cannot replicate in humans
	vaccine strains unlikely to be maintained in or shed from human for long periods	low propensity of humans to shed the vaccine strains
Environmental Safety	**Low**	**Low**
	limited duration of shedding following vaccination	limited exposure of the vaccine strains to the environment
	extremely low reversion potential	low risk of vaccine strains reverting to wild types
	host/range restricted to felids	decreased capability to spread throughout the environment
	no evidence for greater survivability of vaccine vs. wild-type strains on surfaces, in kitty litter and in water	the vaccine strains have no advantages over wild types to survive in the environment, thus limiting distribution

* Risk ratings are shown in Table 6.

Overall Risk Assessment

The overall risk rating for the vaccine was determined to be low. Therefore, there should be little concern associated with the release of the vaccine into the environment and there is ample justification for approval to evaluate the vaccine in controlled field studies and for product licensure for sale, distribution, and use in cats.

Containment, control and monitoring measures

Control of GMM and Gene spreading

The probability that the vaccine viruses will persist in the environment is dependent on the degree and duration of shedding from the vaccinated animals, the amount of time the vaccine viruses are exposed to the environment and whether conditions are favorable for the vaccine viruses survival. The vaccine viruses, as well as other indigenous feline herpesviruses, may initially be found in oro-nasal-conjunctival secretions. Exposure can occur from these sources, as well as from a vaccine spill. In the case of a spill, alphaherpesviruses are not particularly suited to long term survival outside of the host, and the genetic manipulations made to the FHV vector would not provide any physical modifications to the viruses that would increase their durability or survivability in the environment. As such, it is considered unlikely that the vaccine strains will survive in the environment any better than the respective wild-type parent.

Assessments of the survivability of the vaccine strains and the wild-type parent were studied using a non-porous surface, kitty litter and water.

Non-porous surface

This type of surface may represent the equivalent of a counter top or floor in a veterinary clinic onto which a spill of vaccine may occur. In order to model the worst-case scenario, a larger volume and quantity of virus was used than will be available in the final product (1 ml as opposed to 0.5 ml; checken greater than the maximum release titre of virus). Further, it was assumed that this type of virus spill would not be detected and cleaned up or disinfected. Briefly, the viruses were set up such that replicate 1-ml aliquots were placed into the wells of sterile 24-well plates]. These plates were placed in a functioning bio-safety laminar flow hood. This meant that over time, (24-48 hours) the material was exposed to air and became desiccated. At each sampling point, the sampled well was adjusted to a total volume of 1 ml (adding sterile water as necessary) and then titrated onto susceptible cells. There was little difference between the viability curves for either of the vaccine strains or the wild-type parent. Viability of all three strains decreased from initial titres with 4.5-6 logs by 240 hours (10 days). These data indicate that a spill will quickly lose its infectivity at typical laboratory temperatures and humiditiy, even in situations where the spill was not cleaned up or disinfected. The data also show that both wild-type FHV and rFHV-FIV have similar rates of decrease in viability.

Kitty litter

Another possible place for a virus spill or contamination is a cat's litter tray. Kitty litter is very absorbent and has a tendency to contain large amounts of lime (thereby increasing its alkalinity). In view of this, it seemed unlikely that the viruses would remain infectious for a long period. To confirm this assumption, samples of the vaccine viruses or wild-type FHV were mixed with a common type of kitty litter. An initial large infectious dose (approximately 10x more than maximum release titre) was added to 100 g of kitty litter, and kept in a sealed tube at room temperature. Each sampling period, a 10-g aliquot was removed and processed for recovery of infectious

virus. Despite large infectious input doses, infectious virus was unable to be recovered 24 hours later. In fact, it was impossible to detect viruses three hours after mixing.

Viability of all three strains decreased within three hours with 1.3-2.0 log, below the limit of detection. These data indicate that if the vaccine is spilled onto kitty litter, the viruses will quickly become undetectable. The data also show that both wild-type FHV and TK-deleted FHV have similar rates of decrease in viability in kitty litter.

Water

An additional place that was considered for contamination was a pet water bowl. Consequently, the viability of the vaccine viruses and the wild-type FHV parent were monitored in deliberately contaminated tap water. Briefly, a sample of tap water was sterilized by filtration (0.22 µm) and then spiked with known amounts of virus. The spiked samples were kept in sealed sterile tubes at room temperature and sampled at least daily to determine the infectious titre. The viability curves for the all of the FHV based viruses in tap water were similar, regardless of sampling time point. Viability of all three strains decreased from initial titres to near the limit of detection by 5 logs by 140 hours (approximately six days). This would indicate that the vaccine viruses are not expected to survive for long periods of time if exposed to the normal environment of the cat, the veterinary office, or the home.

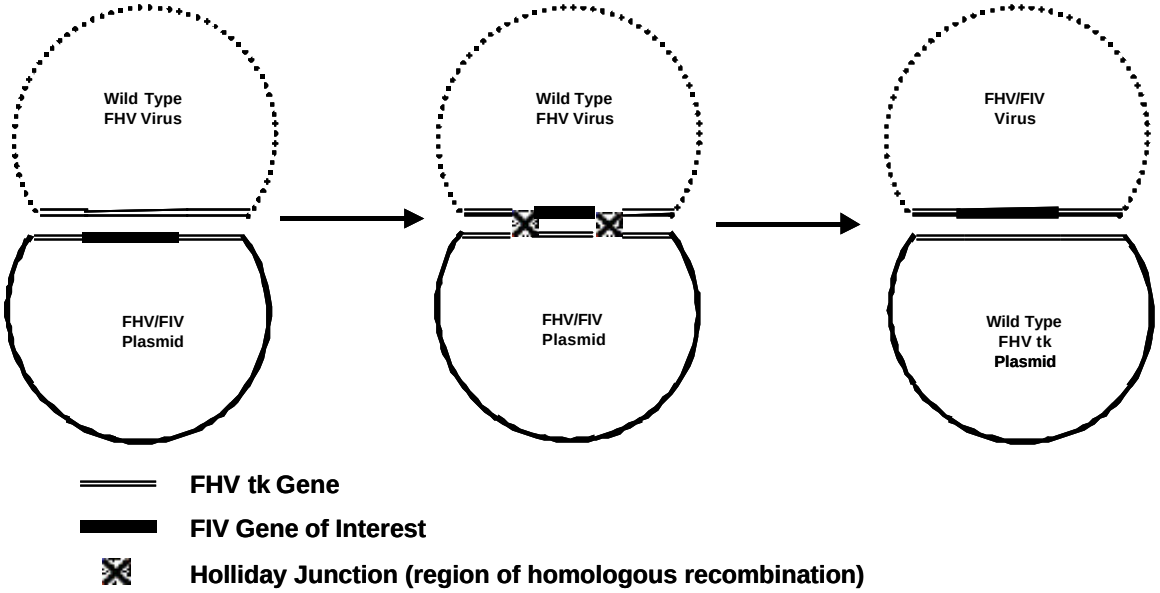
These studies demonstrated that removing a portion of the TK gene from the FHV did not change the environmental stability of the virus from the wild-type FHV parent strain.

Risk of spreading from treated cat to other cats.
In this trial only young kittens will be vaccinated at approximately 8 and 12 weeks of age. At this age the young kittens will be housed indoor and therefore can not infect other outdoor cats. If there are other cats in the same house as the vaccinated cat these cats will be included in the clinical observation during the trial.

Genetic stability of the GMM

The vaccine is comprised of two FHV vectors with a deletion in the TK gene, conferring the TK-negative phenotype (?TK). The attenuated FHV vectors were generated by a process of homologous recombination, in which the flanking TK sequences around the FIV expression cassettes act as homologous sequence crossover sites with the wild-type FHV genome. A schematic representation of these events and the outcome is shown in Figure 1.

Figure 1. Homologous Recombination Between Wild-Type FHV and FHVDTK/FIV Plasmid Vector



To confirm the genetic stability, rFHV-FIVenv and rFHV-FIVgag constructs were passaged sequentially five times in cats (Section b). Viruses recovered from oro-pharyngeal tissues after five passages and original inocula were analyzed by PCR to demonstrate genetic stability. The PCR data indicated that the PCR products generated from primers specific for the TK deletion and the FIVenv or FIVgag gene inserts confirm that the TK deletion and FIV gene inserts remained intact, properly located and stable as compared to the rFHV-FIVenv or rFHV-FIVgag material prior to passage in cats

These data support the conclusion that the virulence-attenuating gene deletions in both vaccine components are genetically stable. There are no known mechanisms for spontaneous generation of a deletion within the genome such as the spontaneous reversion of a point mutation or a single base deletion. However, it is possible for the TK gene to be repaired by homologous recombination with a FHV containing a functional TK. Consequently, the use of the same deletion in both vaccine components prevents the occurrence of recombination with the other vaccine component such that the TK gene would be functionally restored.

Alphaherpesviruses are known to be "recombinogenic" viruses with high rates of recombination both *in vitro* and *in vivo.* In particular, this characteristic is described for herpes simplex virus (HSV) and pseudorabies virus (PRV or Aujeszky's disease virus) in the literature. While little evidence has been documented for recombination between FHV isolates *in vivo,* there is a description of this recombination process *in vitro* if different viruses were used to co-infect the same cells. Recombination *in vivo* between different isolates/strains has been reported for PRV and HSV.

There is a lack of homology between herpesvirus TK genes and host cellular TK genes, and there is limited homology of FHV TK with other known herpesvirus TK genes. The percent homologies between the FHVΔTK3' and FHVΔTK5' backbone virus and other viruses are shown in Table 8 and Table 9, respectively.

Table 8. Percent Homologies of FHVΔTK3' With Other Herpesvirus TK Genes*

	FHVΔTK3'	**Marek 1**	**PRV**	**Canine Herpesvirus**	**Bovine Herpesvirus2**	**Equine Herpesvirus**	**FHV wt**
FHVΔTK3'	100	17	25	23	19	19	37
Marek 1		100	40	45	36	44	45
PRV			100	46	56	58	47
Canine HV†				100	43	47	54
Bovine HV2					100	45	42
Equine HV						100	53
FHV wt							100

* Thymidine kinase sequences were searched for and retrieved from public databases. The Align X module of Vector NTI 7, which uses the Clustal W algorithm, was utilized to perform a multiple alignment comparing these sequences with the known sequence of the 3' end of the FHVΔTK region which remains in the FHV/FIV viruses. The multiple alignment data was then used to generate similarity values.

† HV = herpesvirus

Table 9. Percent Homologies of FHVΔTK5' With Other Herpesvirus TK Genes*

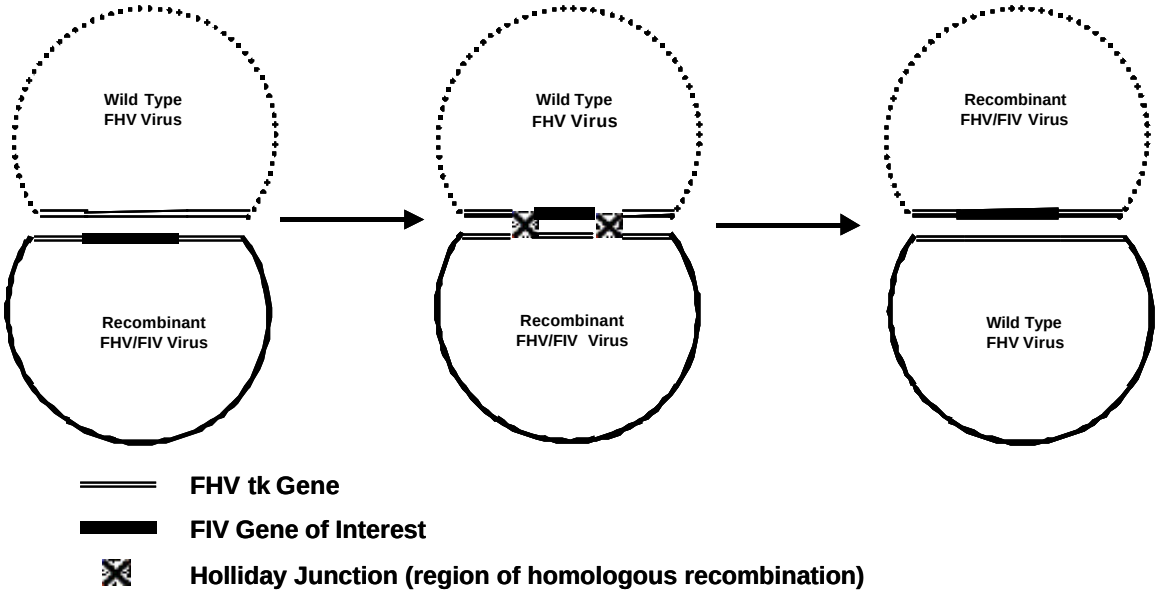
	FHVΔTK5'	**Marek 1**	**PRV**	**Canine Herpesvirus**	**Bovine Herpesvirus2**	**Equine Herpesvirus**	**FHV wt**
FHVΔTK5'	100	19	20	23	20	22	39
Marek 1		100	38	45	37	42	44
PRV			100	46	56	58	48
Canine HV†				100	44	46	54
Bovine HV2					100	45	41
Equine HV						100	54
FHV wt							100

* Thymidine kinase sequences were searched for and retrieved from public databases. The Align X module of Vector NTI 7, which uses the Clustal W algorithm, was utilized to perform a multiple alignment comparing these sequences with the known sequence of the 5' end of the FHVΔTK region which remains in the FHV/FIV viruses. The multiple alignment data was then used to generate similarity values.

† HV = herpesvirus

The low degree of homology between various herpesvirus TK genes and cellular TK genes makes it unlikely that the FHV ?TK gene can be restored by other herpesviruses or cellular TK genes. This means that the primary method of restoring the virulent TK phenotype would be the unlikely co-infection of the same cell with an intact, TK-positive FHV and a vaccine vector. In this hypothetical case, homologous recombination may occur to restore the TK-positive phenotype of the vaccine strain. However, in doing so, this would generate a TK-negative phenotype in the wild-type donor (refer to Figure 2 for a diagrammatical representation of homologous recombination).

Figure 2. Homologous Recombination Between Wild-Type FHV and FHVΔTK/FIV Virus



The net result would be silent, as the unlikely restoration of the TK-positive phenotype would result in a reciprocal TK-negative phenotype due to a virus with the ?TK deletion containing the FIV antigen expression cassette.

These data above show that the ?TK deletion and accompanying FIV inserts are stable. The two virus components cannot restore virulence in each other. While it is theoretically possible for a co-infection with a wild-type FHV to restore virulence via homologous recombination, the net result is that the wild-type virus will gain the ?TK genotype (and therefore TK-negative phenotype) along with the FIV expression cassette (Figure 2).

The information provided in the previous paragraphs demonstrates that there is little homology between the TK genes of feline herpesviruses and other non-herpesvirus sources of TK genes. In fact, there is limited homology between the TK genes of the different members of the alphaherpesviridae (Table 8 and Table 9). One possible source of homologous template for successful recombination with the rFHV-FIV vaccine viruses is the homologous TK gene from the wild type FHV (Figure 2). However, this scenario is unlikely to occur given that in order for recombination to be successful, the viruses must at least co-infect the same cell, most likely at the same time and can only occur in feline cells because FHV only grows in feline cells. Even ignoring other factors that might favor recombination (e.g., the need to have relatively equal amounts of wild-type FHV and rFHV virus), the net result of recombination between a TK-negative FHV-FIV vaccine and FHV wild-type would be that the wild-type FHV becomes a TK-negative FHV-FIV and the vaccine virus becomes a normal

FHV. Thus, in acting as a donor of the TK gene, the wild-type parent will become TK-negative due to inclusion of the FIV gene, even as it replaces the FIV gene of the vaccine strain with the wild-type TK gene. Thus, the calculated risk of such an unlikely event is extremely low, because the stoichiometry of the reaction is such that neither phenotype of virus (TK-positive or TK-negative/FIV) will increase or decrease at the expense or benefit of the other.

Destruction of GMM containing material

The empty vials and applicator will be collected at each study site by the Sponsor and will be destroyed internally at the Sponsors R&D site according the internal procedures for recombinant products.

Training requirements

The Veterinarian that will vaccinate the cats will be trained how to vaccinate the cats intranasal. He/she will also be trained how to handle the recombinant vaccine and to decontaminate the environment of vaccination during any spill of the vaccine.

Emergency situations

Despite the negligible risk related to the use of Pfizer FIV an emergency plan is established. In case of accidental injection to humans we recommend to seek medical advice immediately and show the package insert or the label to the physician. In the case of accidental breaking of a vial the contaminated surface should be disinfected with bleach.

In case of an unexpected event, 3 operating phases are implemented:

-Alert phase
Any observation which can not be related to the normal post vaccinal adverse reactions (and transient lethary) must be reported to the investigator veterinary surgeon and to the monitor of the trial.
The concerned animal will be kept indoors by its owner.

-Investigation phase
Appropriate samples are collected and sent to the laboratory for virus isolation and identification.
Treatment of the animal is immediately prescribed by the veterinary surgeon.

-Action phase
The diagnosis is known before the end of the trial and the event is not related to the vaccine:
The investigator starts treating the concerned animal

The diagnosis is known before the end of the trial and the event is related to the vaccine:
The recruitment of cats for the trial is stopped. Owners of cats which have already been vaccinated with Pfizer FIV vaccine are asked to keep their cats indoors for a 1 month follow up.
The cause of the event is not known by the end of the trial

If the cause of the unexpected event is not established at the end of the trial, an adverse reaction related to the vaccine cannot be eliminated. The follow up of all the animals included in the trial will be extended for 1 month after the trial.

Other containment, control and monitoring measures

Not applicable.

Responsibilities of the notifier

The consent that could be given to the notifier by the competent Minister stipulates that the notifier takes complete civilian liability regarding the damage that could be caused by the deliberate release to the health of human animals and environment.

Inspection by the public authorities

Inspectors are in charge of inspecting the trials for compliance with the conditions specified in the consent and to investigate potential breaches of the consent. In case where mismanagement or fraud is identified specific sanctions will be imposed.

Activity report

At the end of the trial an activity report prepared by the notifier needs to be delivered to the competent authority. This activity report includes at least the following data:
The site and period of release
The precise nature of the actually released GMMs
The aims of the trial
The measures that were taken to prevent unwanted release of transgenic material
If applicable the measures that were taken to protect the subject (patient/animal) during administration of the GMM-containing study drug.
If applicable the measures that were taken to protect the relatives of the treated patients
The measures that were taken to protect the workers who had to manipulate the GMM containing material
The method used for the destruction of the unused or contaminated material
The results obtained during the trial
An overview of the monitoring of patient/animal for GMM shedding
An overview of the monitoring of GMM or recombinant DNA in the environment.

Reference

[1] Gay CG, Orr RL. Risk analysis for veterinary biologics. Animal and Plant Health Inspection Service, USDA. 4 February 1994.

Glossary

Products of DNA using genetic modification technology
Products of are produced by genetic modification in which DNA coding for the required product is introduced, usually by means of a plasmid or a viral vector, into a suitable micro-organism or cell line, in which that DNA is expressed and translated into protein. The desired product is then recovered by extraction and purification. The cell or micro-organism before harbouring the vector is referred to as the host cell, and

the stable association of the two used in the manufacturing process is referred to as the host-vector system.

Vector/Recipient
A replicative competent micro-organism (bateria or virus) into the genetic sequence(s) of interest will be inserted.

Vector vaccine (according to the Ph. Eur. Monograph 0062)
Vector vaccines are liquid or freeze-dried preparations of one or more types of live micro-organisms (bacteria or viruses) that are non-pathogenic or have low pathogenicity for the target species and in which have been inserted one or more genes encoding antigens that stimulate an immune response protective against other microorganisms.

Genetic modified live vector vaccines
Genetic modified live vector vaccines are preparations of one of more types of live bacteria or viruses. One or more DNA/RNA sequences encoding foreign antigens have been inserted into these organisms. These organisms generally have a stable non or low pathogenic phenotype for the species the vaccines is intended for.

Contact

If you have any comment on the public dossier or our activities or wish to obtain additional information on the deliberate release, please contact us at the following address.

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