SCIENTIFIC DISCUSSION

Invented name:	Virbagen Omega
Active substance:	Recombinant Omega interferon of feline origin
Target species:	Dogs and cats
Therapeutic indication:	Dogs : Reduction of mortality and clinical signs of parvovirosis (enteric form) in dogs from one month of age. Cats : Treatment of cats infected with FeLV and/or FIV, in non-terminal clinical stages, from the age of 9 weeks.
Withdrawal period:	Not applicable
Pharmaceutical form:	Lyophilisate and solvent for suspension for injection
ATCvetcode	QL03AB
Pharmaco-therapeutic group:	Interferons
Marketing Authorisation Holder:	VIRBAC SA L.I.D. 1ère Avenue - 2065 m F-06516 CARROS FRANCE

1. SUMMARY OF THE DOSSIER

In mammals, type-I interferon (IFN) genes form a superfamily consisting of 3 gene families: the alpha interferon (IFN-α), the beta interferon (IFN-β) and the omega interferon (IFN-ω). IFN-ω genes have been identified in man, cattle, sheep, horses, pigs, rabbits, and other mammalian species genomes, but they have not been found in dogs nor reported in rodents.

Type-I IFNs are produced by monocytes/macrophages, dendritic cells and fibroblasts, but are mostly produced by components of the immune system such as leukocytes and T lymphocytes during viral infections. IFN-ω is not expressed in unstimulated cells.

To date, it is known that IFN-ω binds to the same receptors as IFN-α and IFN-β. The type-I IFN receptor is a complex of at least two subunits, but the interaction of different type-I IFNs with these subunits seems to be different. Thus, although all type-I IFNs have qualitatively the same spectrum of biological activity, quantitative differences have been observed: IFNs protect the cell against viral infection, inhibit cell proliferation (including tumour suppressor activities), may play a role in modulating various neuroendocrine and neural functions and stimulating specific cells of the immune system. These effects are linked to the induction of several genes within the target cells following IFN treatment. To date, however, the exact physiological role of IFN-ω remains unknown.

In the literature, information on IFN-ω is very scarce and is almost non-existent in canine therapeutics. Thus, because of the close relationship of IFN-ω with the other type-I IFNs (mainly IFN-α) and because of the now general use of IFN-α/β in human therapeutics, all the available knowledge about IFN-α properties was used to assess this IFN-ω file.

Recombinant feline interferon (rFeIFN) is an IFN-ω, glycoprotein of 170 amino acids, produced by silkworms which were inoculated with a recombinant silkworm nuclear polyhedrosis virus containing the feline IFN-ω gene.

An application to extend the authorisation to cats was submitted subsequent to the authorisation of Virbagen Omega for dogs. No new quality data were provided as the pharmaceutical form, pack sizes and method of manufacture all remain unchanged. New safety and efficacy data for the product in cats was assessed and showed a positive benefit-risk profile, thereby extending the authorisation to cats, from the age of nine weeks.

II. OVERVIEW OF QUALITY

Composition

Virbagen Omega contains Recombinant Omega Interferon of feline origin and is supplied in two presentations; 5MU and 10MU. The product contains common pharmaceutical excipients including sodium chloride, D-sorbitol, gelatin and sodium hydroxide. The product contains no preservative or adjuvant. The diluent supplied (1 ml) is a solution of sodium chloride.

Container

Both the freeze-dried product and the diluent are contained in borosilicate type I glass bottles, with a butyl elastomer seal and an aluminium cap.

Development Pharmaceutics

The development of interferon was carried out by Toray Industries (Japan). The choice of different development pharmaceutical stages was adequately explained, for the active substance and the stabiliser.

Method of manufacture

Freeze-dried pellet:

The method of preparation of the active substance, the freeze-dried vaccine and the solvent were presented in detail. All parameters of the process are monitored carefully to guarantee a consistent quality. Vials and rubbers are sterilised at 121°C for 30 min. The sealed vials are stored at 4°C.

Diluent:

The diluent is sterilised by filtration and filled into the final container, and then terminally sterilised for at least 15 min at 121 °C.

Control of Starting Materials

Conventional pharmaceutical excipients are used and all complied with the relevant Ph.Eur. monographs and certificates of analysis were provided.

Starting materials of biological origin

starting material polyclonal antibodies	used for quality control
BM-N cells	culture of recombinant virus
rBNV100	infection of silkworms
silkworms	production of rFeIFN
rFeIFN	active ingredient
artificial food	food for the silkworms
foetal bovine serum	part of culture medium

I. Polyclonal antibodies:

Two kinds of polyclonal antibodies are employed during the quality control performed on the product. One is anti-feline interferon serum (rabbit) used for neutralisation testing, and the other is anti-silkworm-derived substance antibody solution used for testing silkworm-derived material; in this case a silkworm originated substance antigen solution is prepared. The description of the preparation, the specifications and the certificates of analysis were provided. No antibody is employed during the production of rFeIFN.

II. BM-N cells (cells originated from *Bombix mori*):

BM-N cells long-term storage methods were investigated. It was found that the most preferable, and also possible, duration of storage of BM-N cells in a liquid nitrogen tank, without loss of viability, was approximately 9 months. Extraneous agent testing is to be performed according to European specifications.

Stability of recombinant viral DNA: rFeIFN is produced in silkworms using recombinant viral DNA. It is, therefore, extremely important to confirm the stability of recombinant viral DNA encoding rFeIFN. According to the sequence analysis of DNA after the virus replication in BM-N cells, or in silkworms themselves, there was no change in recombinant viral DNA sequence encoding rFeIFN.

Continuous culture of BM-N cells: With respect to the influence of continuous culture of BM-N cells for a long period, on the recombinant virus, rBNV-100 has been replicated using BM-N cells cultured serially for 10 years (from 1989 to 1999). No change in recombinant viral DNA sequence encoding rFeIFN was observed.

III. Recombinant rBNV100:

1) Starting materials:

a) Silkworm nuclear polyhedrosis virus (NPV)

NPV is a double stranded DNA virus, belonging to the Baculoviridae family. A clone is obtained from a wild NPV strain by a plaque assay on BM-N cells,called BmNPV-T3. Its genome has been fully established, thus its purity and sterility is guaranteed. This strain is used in the recombinant DNA technology but it is not included in the final construction.

b) Cells

- LSA-1 cells (ATCC CRL 9462), of feline origin, supplying the gene of interest.
- COS-1 cells (ATCC CRL 1650), derived from monkey and used in the recombinant DNA technology.
- BM-N cells (ATCC CRL8910), originated from *Bombix mori*.

c) Bacteria

- E. coli MC1061, used for amplification to establish the cDNA library.
- E. coli having pFeIFN1, used for preparation of plasmid pUCIFN4.
- E. coli HB101, used for amplification of plasmid pYU871.

d) Plasmids

- pUC18, used to prepare plasmid pUCIFN4.
- M13mp19RFDNA, used to prepare plasmid M13IFN1.
- pBM030 : contains a silkworm polyhedron gene promoter, multi-cloning site, and a terminal codon downstream. It can undergo recombination with silkworm baculovirus genome *in vivo*. It is used to prepare plasmid pBmFeIFN1, the 3 plasmids pBmFeIFN2 and plasmid pYU871.

e) Restriction enzymes

HindIII ; XhoI ; SfaNI ; HincII ; BamHI ; Bg1I ; Bg1II ; Eco0109I ; SmaI.

The rFeIFN gene is isolated from a cell strain, fashioned with the help of restriction enzymes, plasmids, amplified through cells and bacteria, and inserted into a silkworm nuclear polyhedrosis virus (NPV) to produce the recombinant virus rBNV100.

2) Construction of the recombinant virus:

a) To obtain RNA (including interferon omega RNA): feline LSA-1 cells are naturally producing IFN omega. After inducing production of IFN in these cells, all RNA was extracted and purified.

Feline LSA-1 cells, naturally producing IFN omega, were cultured in MEM-L15 medium containing 10% foetal calf serum. After appropriate cell proliferation, TPA (12-O-tetra-decanoylphorbol 13-acetate) was added to induce IFN production. After 20 hours of incubation, these cells are collected. The use of an appropriate extraction method (modified guanidium thiocyanate method) allows extraction and pelleting of RNA. Purification of poly-$(A)^+$ RNA is obtained by HPLC using an oligo (dT) cellulose column.

b) For the creation of feline cDNA library: all the RNA was transcripted into cDNA.

The poly-$(A)^+$ RNA is incubated in the presence of oligo-dT, reverse transcriptase, the 4 dnTPs and 2 primers (oligo(dT) primer and oligo(dT)-tailed pcDV1 plasmid primer). After synthesis and purification of the hybrid RNA/DNA strand, a poly-dC tail is created at the 3'-end of the DNA strand through the use of terminal nucleotide transferase. A poly dG sequence is added to serve as a primer on the poly-(dC) tail. DNA polymerase synthetises the second DNA strands to form double-DNA strands. A plasmid linker is coupled to the double-DNA strands and inserted within a plasmid (with DNA ligase), previously opened with a restriction enzyme, achieving the cDNA construction. The cDNA library is thus established after amplification in *E. coli* MC1061 cultures, transfected with these plasmids.

Further details submitted were considered to adequately explain the plasmid construction.

c) With regard to screening of the feline cDNA-containing plasmid: only cDNA coding for IFN is of interest. The screening allows isolation of the appropriate FeIFN cDNA, included in plasmid pFeIFN1.

COS-1 cells were transfected with the plasmids extracted from *E. coli* MC1061 (DEAE-dextran transfection method) and cultured. Each culture was screened for an IFN activity. One IFN-producing culture was selected, the corresponding *E. coli* culture identified, and the plasmids extracted by the alkali extraction method and purified through centrifugation.

COS-1 cells were again transfected with the purified plasmids and screened for an IFN activity. The cells which exhibit the highest activity are identified. The corresponding plasmid is isolated and designated pFeIFN1.

Further details submitted on this part of the process were satisfactory.

d) With regard to the construction of the transfer vector: such a construction is necessary to allow introduction of the cDNA into viruses. After having extracted and purified the pFeIFN1 plasmid, two different methods lead to the construction of 4 different plasmids.

- First method:

The plasmid pFeIFN1 is digested with the restriction enzymes SfaNI and HincII to obtain a 750 bp fragment (SfaNI-DNA_{FeIFN}-HincII) after separation by electrophoresis. Plasmid pBM030 is opened with Bg1II and SmaI and ligated with the above mentioned fragment using T4 DNA ligase. *E. coli* HB 101 was transfected with the plasmid. After cultivation, plasmids are extracted. The appropriate plasmid was selected and called pBmFeIFN1.

- Second method:

The plasmid pFeIFN1 is digested with restrictions enzyme HincII and Bg1I, and bacterial alkaline phosphatase to obtain a 700 bp DNA fragment (HincII-DNA_{FeIFN}-Bg1I) after separation by electrophoresis. The 2 complementary oligomers are associated to provide a double-stranded oligomer. This double-stranded oligomer is then ligated with the FeIFN-encoding DNA fragment at the Bg1I site. Plasmid pBM030 is opened with Bg1II, treated to prevent self-ligation. The double-stranded oligomer and the FeIFN-encoding DNA fragment are then inserted into the plasmid pBM030 using DNA ligase. *E. coli* HB 101 was transfected with the plasmid. After cultivation, plasmids are extracted. Three plasmids were selected and called pBmFeIFN2-1, pBmFeIFN2-2 and pBmFeIFN2-3.

Further details submitted about the above two methods were satisfactory.

e) With regard to the construction of recombinant viruses: co-infection of cells with a wild virus strain and the 4 plasmids providing 4 differents recombinant viruses.

DNA from the Nuclear Polyhedrosis Virus strain (BmNPV-T3 DNA), and the transfer vectors (pBmFeIFN1, pBmFeIFN2-1, pBmFeIFN2-2 and pBmFeIFN2-3) are co-transfected into BM-N cells. Appropriate screening of the cells (cells without polyhedra but with cytopathogenicity) and the culture fluid (with or without antiviral activity) allows discrimination. Cells without polyhedra, but with cytopathogenicity and antiviral activity are sought, because they contain recombinant viruses of interest. The recombinant viruses containing the different plasmids pBmFeIFN1, pBmFeIFN2-1, pBmFeIFN2-2 and pBmFeIFN2-3, are named BmFeIFN1, BmFeIFN2-1, BmFeIFN2-2 and BmFeIFN2-3 respectively.

Each group of infected BM-N cells is added to non-infected BM-N cells and cultured for 5 days at 27°C. Each culture fluid (containing BmFeIFN1, BmFeIFN2-1, BmFeIFN2-2 and BmFeIFN2-3) is collected and stored at -80°C.

f) With regard to the production of FeIFN: the yields of all 4 recombinant virus were comparable.

Four groups of 5 silkworms of the fifth instar are used. On the day after moulting, each group is injected with one of the virus suspension and fed for 4 days at 25°C.

In the case of human IFN-α, it was reported that the base sequence upstream of the initiation codon ATG in the transfer vector greatly affects the production of IFN. In the present situation, however, no difference was seen between the four recombinant viruses, whose modifications range from deletions of 2 to insertion of 5 bp.

g) With regard to the purification of rFeIFN: a 2-step chromatography method is described. When applied to the previously obtained FeIFN, 2 different IFN components were identified. Further details about the purification methods were submitted and found to be satisfactory.

3) Strategy for the production of a single FeIFN:

The production of one single IFN component is based on substitution of one residue via mutagenesis.

a) Obtaining plasmid pUCIFN4: E. coli having pFeIFN1 is cultured and the plamid is extracted and purified by the alkali extraction method. The plasmid is completely digested with XhoI and the DNA fragments approximately 1.2 kb (which contain the genome of interest) are isolated by electro-elution. These DNA fragments are completely digested by SfaNI and HincII and the DNA fragments of approximately 0.75 kb (SfaI-DNAFEFN-HincII, which contains the genome of interest) are isolated in the same way.

   This fragment is ligated with the T4 DNA ligase into plasmid pUC18, previously digested with BamHI and HincII. The new plasmid is called pUCIFN4.

b) Obtaining plasmid M13IFN1 : plasmid pFeIFN1, obtained during the screening process (see above), was used to provide the FeIFN cDNA fragment. Different plasmids (pUCIFN4 and M13IFN1) were constructed to allow mutagenesis.

c) Mutagenesis: performed to allow substitution of Ser residue into Val residue, thus allowing production of one single FeIFN component.

Further clarification was provided, and considered satisfactory, about the method of mutagenesis used.

d) Obtaining plasmid pYU871: after having succeeded in the mutagenesis process, the modified DNA is put into a plasmid, which will be the new transfer vector. The preparation of this plasmid was further explained with a diagram.

e) Obtaining recombinant silkworm polyhedrosis virus: after co-infection of cells with the baculovirus DNA and plasmid pYU871, recombinant and non-recombinant viruses are obtained. Two screening methods (limiting dilution method and plaque method), applied successively, allow isolation of the appropriate recombinant virus, called rBNV100.

Recombinant baculovirus has been constructed by the conventional method (S. Maeda et al.), using silkworm cells. This method utilises viral DNA, but not the virus. Briefly, silkworm cells in culture were co-transfected by the transfer vector containing DNA encoding for FeIFN and wild type virus (BmNPV-T3). Viral DNA undergoes recombination within silkworm cells, resulting in recombinant baculovirus. The method is described in an article by S. Maeda in Cell Technology 4:9, 767 (1985), entitled: Production of protein using silkworm nuclear polyhedrosis virus as vector.

4) Control of genetic stability of the recombinant virus:

The genetic stability was checked and was considered proven as there was no polyhedron-forming capacity. The patterns of breakage after five passages by restriction enzymes are identical to those of the pre-existing rBNV100 DNA and the molecular weight, yield, specific activity and total amino acid sequence of the rFeIFN are identical to those of the pre-existing rBNV100 DNA.

5) Validation of the live material inactivation:

Silkworms infected with rBNV100 produce feline rFeIFN omega. rFeIFN is contained within silkworm body fluid, which obviously also contains the virus. Thus, it is necessary to have evidence of complete viral inactivation to avoid any rBNV100 contamination of rFeIFN.

Two different methods were tested. In conclusion, as the rFeIFN is acid-stable, a six hours acid treatment at pH 1.5 or 2.5 inactivates the virus completely.

Definitions of the various passages are different from those currently used because of the quite specific way of handling the BM-N cells (no seed system). Thus, it should be understood that:

- the Master Viral Bank (MVB) is the mother virus strain.
- the Manufacturing Working Virus Bank (MWVB) is a working seed (MWVB = MVB + 1 passage).
- the Manufacturing Virus Seed (MVS) is the inoculum (cells infected by the baculovirus) directly injected into silkworms (MVS = MWVB + 1 passage).

So, the Master Seed Virus was obtained by amplification of rBNV100 virus through 2 passages on BM-N cells after a 5-days incubation period. After the second passage, the mixture is centrifuged and the supernatant is collected. The supernatant constitutes the Master Seed Virus.

The recombinant virus seed lot is controlled at three steps:

- MVB: Master Virus Bank,
- MWVB: Manufacturing Working Virus Bank,
- MVS: Manufacturing Virus Seed.

Master Virus Bank	
Tests	**Norms**
Identity	
Total amino acid sequence	Identical to that of pre-existing rBNV100
Specific activity of rFeIFN	$0.17 \leq$ S.A. ≤ 0.27 MU/µg
Molecular weight of rFeIFN	$25 \leq$ M.W. ≤ 30 kDa
Restriction enzyme digestion pattern of viral DNA	Patterns of breakage by restriction enzymes identical at the pre-existing rBNV100 DNA
RFeIFN producing activity	Potency in the range 7 – 15 MU/ml
Nucleotide sequence of rFeIFN encoding DNA of virus	Identical to that of reference sequence (GENBANK S62636)
Titration	None
Viral purity	
Deficiency of polyhedron body production	No polyhedron-forming capacity

Manufacturing Working Virus Bank	
Tests	**Norms**
Identity	
Specific activity of rFeIFN	$0.17 \leq$ S.A. ≤ 0.27 MU/µg
Molecular weight of rFeIFN	$25 \leq$ M.W. ≤ 30 kDa
Restriction enzyme digestion pattern of viral DNA	Patterns of breakage by restriction enzymes identical at the pre-existing rBNV100 DNA
rFeIFN producing activity	Potency in the range 7 – 15 MU/ml
Nucleotide sequence of rFeIFN encoding DNA of virus	Identical to that of reference sequence (GENBANK S62636)
Titration	None
Viral purity	
Deficiency of polyhedron body production	No polyhedron-forming capacity
Absence of extraneous agents	Absence of extraneous agents
Absence of bacterial and fungal contamination	Absence of bacterial and fungal contamination
Absence of mycoplasma contamination	Absence of mycoplasma contamination

Manufacturing Virus Seed	
Tests	**Norms**
Identity rFeIFN producing activity Nucleotide sequence of rFeIFN encoding DNA of virus	 Potency in the range 7 – 15 MU/ml Identical to that of reference sequence (GENBANK S62636)
Titration	None
Viral purity Deficiency of polyhedron body production Absence of extraneous agents Absence of bacterial and fungal contamination Absence of mycoplasma contamination	 No polyhedron-forming capacity Absence of extraneous agents Absence of bacterial and fungal contamination Absence of mycoplasma contamination

The absence of extraneous agents directly on the inoculum (which is both baculovirus and cells) was shown, thus guaranteeing the absence of contamination to a satisfactory level.

Various extraneous agents were searched for, including all relevant canine agents, as well as feline viruses and Bovine Viral Diarrhoea Virus.

It was clarified that the results of a more general test to detect all retroviruses was available, whatever their origin, through the detection of reverse transcriptase activity in the samples to be tested. This test is a Fluorescent Product enhanced Reverse Transcriptase test (PERT). The results of the test were negative, which confirmed the absence of any retrovirus (and consequently also of the feline sarcoma virus [FSV]) in both Manufacturing Working Virus bank (MWVB) and Manufacturing Virus Seed (MVS). Negative retroviral reverse transcriptase activity confirms the absence of any retrovirus, including FSV virus, in MWVB and MVS.

IV. SPF silkworms:

Silkworm eggs are obtained from pebrine-free moths. They are fed with artificial food only. Silkworms in the fourth instar are used and are considered to be free of Viral flacherie, Nuclear polyhedrosis, Cytoplasmic polyhedrosis, Densonucleosis, Yellow muscardine, Aspergillosis and Pebrine.

Among these diseases, pebrine can be the most problematic while rearing silkworms. Mother moths of silkworms are tested for pebrine and if the test is negative, their eggs are used for the production of rFeIFN. A description of the pebrine test was provided.

Measures to ensure the healthy status of the silkworm flock, including the surveillance of the silkworms and measures regarding their food and the environment in which they are reared were employed. Any disease in silkworm rearing quite rapidly destroys the entire population, with macroscopical changes in the silkworms. Thus, such diseases cannot be overlooked.

V. Recombinant feline interferon (rFeIFN):

rFeIFN is a polypeptide composed of 170 amino acids. The characterisation of the primary structure was provided.

Specifications of interferon desalted bulk
Routine controls and specifications are partly based on Ph.Eur monographs (not applicable as such to rFeIFN omega):
monograph 0784: products of recombinant DNA technology.
monograph 1110: interferon alpha-2 concentrated solution.
monograph 1440: interferon gamma-1b concentrated solution.
Details were provided in the dossier, and routine controls include physico-chemical characteristics, identification, purity and potency. Testing for bacterial endotoxins was conducted in accordance with the Ph. Eur.

Consistency of production:
To provide additional evidence of consistency during production of FeIFN omega, the following analytical methods were carried out on batches of desalted bulk (which will not, however, be conducted routinely): isoelectric focusing; determination of the molecular weight by electrophoresis under non-reducing conditions; characterisation of the primary structure of a reference FeIFN sample via peptide mapping; the absence of DNA is ensured to detect residual DNA by hybridisation.

Results on residual DNA and safety test of 5 batches manufactured post authorisation were provided and deemed to be satisfactory.

Compliance with the European note for guidance for minimising the risk of transmitting animal spongiform encephalopathy agents via veterinary medicinal products.

Use of gelatin of porcine origin as an excipient present in the final product: In accordance with the CVMP Guideline requirements, gelatin of porcine origin is used. Pigs are not recognised as suffering from transmissable spongiform encephalopathies (TSEs) and material of porcine origin is therefore not considered as presenting a risk.

Use of starting material of animal origin during production process, not present in the final product:

Foetal bovine serum: FBS originates from countries that have been identified as being free of Bovine Spongiform Encephalopathy (BSE). Potential viral contamination of FBS is eliminated by treatment with Gamma ray. The risk of transmitting TSE agents was, therefore, considered to be minimal.

BM-N cells: The risk that original seed material is contaminated with TSE infectivity was considered to be very low.

rBNV100: The high purity of the material used (mRNA), the geographical origin of the source cat (USA) and the period of isolation of these cells (early 80s) were considered to guarantee the safety of use of the cell line. The use of a sole genomic of silkworm nuclear polyhedrosis virus was also considered to be very safe. The risk of transmission of hypothetical TSE infectivity during the production steps of recombinant virus was considered to be very low.

Silkworms: Silkworms are used for mass production of recombinant feline interferon after inoculation with recombinant virus suspension. They are reared according to rigorous conditions and fed with heat-treated artificial food containing proteins of vegetable origin only. They were, therefore, considered unlikely to present any risk of transmission of TSE agents.

RousselotTM gelatin ASF/A: Gelatin ASF/A is used in the desalting chromatography column. It is produced from pig skin and was, therefore, considered to be safe as regards TSE infectivity.

In addition, the quantitative importance of all the ingredients in the active substance, that would theoretically present a risk of TSE infectivity (FBS, seed materials), is almost negligible, reducing the risk of infectivity by potential TSE agents in the final product considerably. Furthermore, the other

raw materials are from non-ruminant origin, and the product is intended for dogs and cats, which are not TSE sensitive, and so the TSE risk was considered to be satisfactorily addressed.

CONTROL TESTS DURING PRODUCTION

Preparation of the Working Seed Virus:
Ten culture flasks of BM-N cells are inoculated with Master Seed Virus and cultured at 27°C for 5 days. The pooled culture fluid is centrifuged and the supernatant collected in tubes and stored at -80°C. This constitutes the Working Seed Virus (WSV). For the inoculation step, the frozen WSV solution is thawed, diluted in TC-10 medium containing 10% heat-inactivated foetal bovine serum and kanamycin.

Silkworm inoculation and rearing:
Silkworms are introduced on day D0 into cabinets previously sterilised. Each silkworm is inoculated, with an automatic microdispenser, on day D2 with the WSV solution. The silkworms are then reared until day D7.

Extraction of body fluid:
The silkworms are incised mechanically in the abdominal region and the body fragments of the silkworms dropped into a 0.1M HCl solution. After all the incisions have been completed, the solution is stirred and the silkworm bodies are removed. The HCl solution is filtered and the remaining body fragments are further washed and extracted with HCl.

Purification, desalting and sterilisation:
The pH of the solution is neutralised to 7.0 ± 0.1 with NaOH and HCl, while stirring. Centrifugation and filtration take place. Purification is achieved with the 2-step affinity chromatography method. The rFeIFN bulk is then desalted through gel filtration chromatography and sterilised by filtration. The bulk is sampled for quality control, sealed, stored at 4°C and transported.

In process control tests:

During the rearing and inoculation of silkworms, the following parameters are tested:

parameter	norm
number of silkworms/production run	7200-8400
silkworm's status	healthy
mean weight of 1 silkworm	≥ 0.8 g
volume of inoculum of the VWS solution	2 µl
temperature of rearing	25 ± 2 °C
humidity	55 ± 15 %
quantity of food/8200 silkworms	162 kg

The following parameters are controlled during the extraction of body fluid:

parameter	norm
rFeIFN molecular weight	25 kDa
rFeIFN purity (western blot)	1 single strip
protein content	1-4 mg/ml
pH of the HCl solution	2.0 ± 0.5
pH after NaOH neutralisation	7.0 ± 0.1
temperature of neutralisation	7 ± 3 °C
autoclaving temperature of filters	121 ± 1 °C
duration of autoclaving	30 ± 1 min

The results for 7 batches were provided and were in accordance with the norms.

Various parameters were tested during blue sepharose chromatography and the results for 7 eluates were provided and were in accordance with the norms.

Further tests were performed on the interferon desalted bulk, including physico-chemical characters, purity potency sterility and specific activity.

Parameters monitored during desalting include: autoclaving temperature, duration of autoclaving, proteinic concentration, neutralisation antibodies, potency and specific activity. The results for 7 batches were provided and were in accordance with the norms.

Controls performed by Virbac include precooling to 4 °C, freezing, sublimation and dessication.

CONTROL TESTS ON THE FINISHED PRODUCT

Freeze-dried pellet:
Tests for appearance, pH, identity by viral inhibition on cells, potency, endotoxin content, sterility and purity are conducted. A test for residual humidity including a minimal limit for residual moisture is included in the release specifications for the freeze-dried pellet.

Diluent:
Tests for appearance, volume, osmolarity, endotoxin content, and sterility are conducted.

Excipients
In view of the nature to the components (gelatin, sorbitol, sodium chloride and sodium hydroxide) of the finished product, identification and assay was considered unnecessary.

The interferon obtained through the production process, using recombinant baculovirus infecting silkworms was considered as a biological product and not a vaccine. The inclusion of a safety test in animals was not, therefore, considered justified.

Results of 4 batches of the 5 MU-presentation, of 4 batches of the 10 MU-presentation and of 4 batches of the diluent were presented and were satisfactory.

STABILITY

Active substance

Intermediate results from tests up to 9 months were provided for the following parameters: appearance, pH, HPLC, potency.

Stability of finished product
Stability data on vials containing 10MU of interferon manufactured by Toray was provided under various conditions and over time periods of up to 27 months. The following parameters were checked: external appearance before and after dissolution, solubility, osmotic pressure ratio, potency, sterility, moisture content, pH, hydrogen ion concentration and insoluble extraneous substances. No change was found in any of the parameters tested over time.

The following parameters were checked for both freeze-dried pellet presentations manufactured by Virbac: appearance, pH, residual moisture, HPLC, potency and sterility up to the 27 month timepoint.

All the results provided were within the limits set, including the estimated potency, which must be of [4 MU/ml; 6.25 MU/ml] for the 5 MU presentation, and of [8 MU/ml ; 12.5 MU/ml] for the 10 MU presentation. Based on the stability data presented a shelf-life of 2 years was granted for the final product.

Stability data presented for the diluent under VICH conditions over 27 months allowed a shelf-life claim of 2 years at ambient temperature.

Stability of the reconstituted product

For the reconstituted product a recommendation is included in the SPC: "The product should be used immediately after reconstitution due to the absence of preservative". No in-use shelf-life is approved for this product.

OVERALL CONCLUSION ON QUALITY

The manufacture of the product was well described, validated and controlled. Methods and specifications for the ingredients were acceptable. The building of the recombinant virus and the use of silkworms in the production process were well described and in detail. The control tests and specifications for the finished product were suitable to demonstrate that a product of consistent quality could be produced. The proposed shelf life of 2 years for the finished product was considered acceptable.

III. SAFETY ASSESSMENT (PHARMACO-TOXICOLOGICAL)

INTRODUCTION

The side effects of interferon-α treatment found in published literature (which are useful to assess the IFN-ω properties), are listed below:

- mild to moderate flu-like symptoms in humans, with some cases of vomiting in cats after administration of rFeIFN.
- inhibition of cell growth, including hematopoïetic progenitor cells in bone marrow, which might sometimes lead to leukopenia when used long-term. In cats, a transient decrease in platelets, white blood cells and reticulocytes was also observed.
- central neurotoxicity, ranging from almost inapparent (somnolence) to very serious (coma) in humans.
- hepatotoxicity and temporary deterioration of patient's renal function.
- potential toxic effect on the optic nerve or the retrochiasmatic visual pathways.
- adverse effects on spermatogenesis, despite normal function of testes and no immunological reactions.
- a few patients have been reported to develop diabetes mellitus during IFN-α therapy.
- other exceptional adverse effects include interstitial pneumonitis, thyroid disease and autoimmunity manifestations (IFN-α therapy is able to amplify thyroid autoimmune phenomena in predisposed individuals) after long-term therapeutic use of type-I IFNs.

The side-effects of interferon-α treatment found in literature are a useful basis to assess IFN-omega properties.

LABORATORY TESTS

Pharmacodynamic laboratory trials

- **Toxicology in cats**

  Cats, aged between 9 months and 2 years of age received an intravenous dose of 2.10^7 U/kg FeIFN omega. Slight drowsiness was seen in all cats, 3 to 5 hours after administration but all recovered after 6 hours. A transient and slight increase of body temperature was observed, but remained within physiological limits. A slight increase of sinus tachycardia and respiration rate was observed, concurrent with a body temperature increase, but all the other parameters remained within normal limits. Normal limits for the water and electrolyte metabolism were observed.

- **Acute oral and intravenous toxicity in rats**

  Five weeks old SPF -SD rats both male and female were included in the study. They were given either 20MU/kg or placebo by both the oral and intravenous route. There was no mortality (LD_{50} after oral or intravenous administration ≥ 20 MU/kg). There was no significant difference in bodyweight between the paired groups. At necropsy no marked change was observed, except for a cystic kidney in 1 male.

- **Toxicity in dogs**

  Beagles (female and male), aged between 1.5 and 7 years of age received intravenously an intravenous dose of 25 MU/kg of FeIFN omega. In one dog, slight drowsiness was seen two hours after administration and a slight decrease in activity was seen 5 to 6 hours after administration; but all other parameters remained within normal limits. A slight increase in sinus tachycardia and respiratory rate was observed. Normal limits for water and electrolyte metabolism were observed.

These studies confirm the nature of the acute side-effects described in the literature: drowsiness, increase in body temperature, slight increase in sinus tachycardia and respiratory rate. These symptoms are rarely dose limiting and spontaneously dissipate after no more than a few days. They are mentioned in the SPC.

Pharmacokinetic laboratory trials

- **Pharmacokinetic trial in cats**

  Cats were administered a dose of 5 MU/kg FeIFN omega either by intravenous, subcutaneous or intramuscular injection. Autoradiography and a whole body metabolism study were carried out and pharmacokinetic parameters were determined.

- **Pharmacokinetic trial in dogs**

  Male beagles, 6 months of age, were divided in 3 groups each receiving 5 MU/kg FeIFN omega by intravenous, subcutaneous or intramuscular injection. Pharmacokinetic parameters were monitored. rFeIFN is very rapidly eliminated from the body. The results seen in dogs are very similar to those in cats.

Various pharmacokinetic findings from the literature were reported. It was concluded that the pharmacology of rFeIFN in cats was comparable to that of HuIFN in humans. FeIFN shows low tissue distribution and rapid metabolism through kidneys. Pharmacokinetic results suggest that the behaviour of rFeIFN in dogs is very similar to that in cats, although this was not demonstrated. The pharmacological effects of IFN continue for some time after its disappearance from plasma and therefore the number and type of cells interacting with IFN are far more important than merely IFN levels.

The rationale for intravenous administration in dogs was justified as follows: lower risk of appearance of anti-interferon antibodies and a tendency to an earlier recovery. In humans seroconversion rate was significantly higher in patients treated with IFN subcutaneously than intravenously. It was also presumed that intravenous administration would facilitate IFN to reach the disease foci more swiftly and exert its action earlier. The efficacy of an early therapy with feline interferon was investigated in dogs which were diagnosed as having canine parvovirus (CPV) infection by means of Parvotest and were in the early stage of CPV infection with a WBC count of ≥ 7000/µl. Feline interferon was administered at a dose of 1.0 MU/kg for 3 consecutive days and efficacy was compared with groups receiving an intravenous (Group 1) and subcutaneous injections (Group II). Earlier recovery of clinical symptoms was observed in the intravenous injection group although mortality was observed in both groups at almost the same rate: 11.8% in Group I and 12.5% in Group II.

Safety of the Administration of One Dose

DOGS

- **Safety study with Beagle dogs**

  Beagle dogs (males and females), 17-19 days old, were included in the study and given either placebo, 1.0MU/kg/day, 2.5 MU/kg/day or 5.0 MU/kg/day for 5 consecutive days intravenously.

  The dogs were clinically monitored daily including rectal temperature. Haematology and urinalysis were performed before the start of administration and at 1, 3, 7 and 14 days after the end of the fifth administration. One-way analysis of variance for each measurement point was performed and if the difference was significant, Dunnet's test for comparison of treated/control results was applied.

  No abnormal clinical signs were seen and there were no effects on rectal temperature. There was no difference in body weight between groups. Values for red blood cells, haemoglobin, haematocrit, and reticulocytes tended to decrease in all groups just before second administration. All these parameters (except reticulocytes) nearly returned to the predosing levels on the first day after the fifth administration. All other parameters remained comparable between groups. There were significant differences between the control and treated groups for the parameter « inorganic phosphate » in group 4 on day 1 after the fifth administration and « chloride » in group 3 on day 14 after the fifth administration. These changes did, however remain within the normal ranges. No differences were found for the urinalysis (pH varied greatly amongst the animals).

- **Local intravenous tolerance study in rabbits**

  It was concluded that interferon was very well tolerated after a single intravenous or perivenous administration of 10 MU.

CATS

No specific study was undertaken in cats. Reference is made to the overdose study.

Safety of an Administration of an Overdose

Dogs

- **Overdose safety study on Beagle dogs**

Beagles (males and females), about 6 months old, were included in the study and given either placebo, 1.0 MU/kg/day, 5 MU/kg/day or 25 MU/kg/day for 5 consecutive days intravenously.

The dogs were clinically monitored daily, rectal temperature was checked and haematology and urinalysis were performed. Appropriate and detailed statistical analysis was carried out.

No mortality was reported, vomiting and soft faeces occurred in several animals after the last administration. Some increase in temperature was reported (≤ 0.8°C), but temperature remained within normal limits during the monitoring period. In particular, no sex predisposition nor dose-related effects could be observed. A decrease could be observed in white blood cells, platelets, reticulocytes and red blood cells. At 25 MU/kg/day, haematologic parameters appeared to be changing. The differences registered in the urine analysis (variations in pH and protein) had no clinical consequences.

Cats

Safety Study After IV Administration of One Overdose to Cats

Cats from 9 to 24 months of age with different bodyweights were given a single overdose of 20 MU/kg by the intravenous route on a single occasion.

Clinical monitoring, effect on central nervous system, body temperature, effect on respiratory and vascular systems, effect on water and electrolyte metabolism, were all monitored in this study. No statistical analysis was conducted on this study.

Transient and mild drowsiness was seen in all cats between 3 and 5 hours after administration. All cats recovered at 6 hours. No effect on the central nervous system and no effect on water and electrolyte metabolism were seen. A slight rise in body temperature was registered 2 to 6 hours after administration. A slight increase of sinus tachycardia and respiration rate was registered, concurrently with an increase in body temperature. No other abnormal effect was seen.

An overdose of FeIFN administered intravenously can induce mild and transient hyperthermia and drowsiness. This study is conducted with a limited number of animals and not according to GLP. No control group was present. The study can only be regarded as supportive.

Safety Study of 5 Consecutive Intravenous Injections of Interferon Overdose in Cats

Cats (males and females), 5 months old, were included in the study, divided into 4 groups of 6 animals given 5,10, 20 MU/kg/day or placebo, intravenously.

Clinical monitoring, urinalysis, bodyweight, blood biochemistry and hematology were monitored. Male and females were analysed separately by one-way analysis of variance for each measurement point; if the difference was significant, Dunnet's test was used for comparison of treated/placebo results for bodyweight, and multiple comparison of treated/placebo results for clinical findings.

Episodes of vomiting were observed, with no abnormal changes in general signs or activities. Urine could not be collected on all animals but the samples available showed no abnormality.

Bodyweight: growth of animals tended to be delayed slightly during the administration period, but no dose-effect relationship could be found.

Hematology: the treatment induced a transient decrease of red blood cells, haemoglobin and hematocrit, of reticulocytes (males seems to be more sensitive), platelets (males seems to be more sensitive, with an apparent dose-dependent decrease) and white blood cells (affecting essentially lymphocytes). These changes were seen mainly after the last administration (D6 to D8) but returned to normal values within 7 to 14 days. No clear changes could be highlighted on the other hematological parameters.

Blood biochemistry: the treatment induced a transient increase of GOT, GPT (with individual fluctuations) and total cholesterol. However, these increases tended to become normal again at D19. No clear changes could be highlighted on the other biochemical parameters.

Intravenous administration of overdoses of FeIFN can induce vomiting, decreases in leukocytes (especially lymphocytes), platelets, reticulocytes and red blood cells. These changes were transient and reversible within 7 to 14 days. Increases in GOT, GPT and total cholesterol were also seen. These changes tended to recover gradually.

These trials can only be considered as indicative, because the composition of the product administered differs slightly from the one manufactured by Virbac and because intravenous administration is not the claimed administration route for cats. Thus, even if some potentially new adverse effects were seen (such as anorexia, decrease in drinking and collapse), their inclusion in the SPC is not relevant. The

adverse reactions are transient and are regarded as mild. In the case of intravenous administration in cats, increased adverse reactions may be seen, e.g. hyperthermia, soft faeces, anorexia, decreased drinking or collapse.

No hepatic or renal toxicity could be observed, even after intravenous administration: the increases of GOT, GPT and total cholesterol are not biologically significant because the other biochemical parameters remained within physiological limits. It is concluded that certain haematology parameters were transiently modified but the response was not dose-dependent.

Males appeared to be more sensitive with regard to leucocytes and platelet decreases, dose-dependent for the latter. No conclusions are possible due to the limited number of males and females used in this study and because of the different route of administration (IV instead of SC).

The study provides additional information on the evaluation of the overdose administration. Safety in cats was assessed through two studies, which were conducted by Virbac in compliance with GLP and the Ph.Eur. Studies on the safety of an overdose and on the administration of one dose and repeated administration of one dose showed that there was no difference between treated and placebo groups with regard to bodyweight.

Safety of one administration of an overdose in cats subcutaneously

SPF kittens (males and females), 9 weeks old, were included in the study, divided into 2 groups given 10MU/kg/day or placebo subcutaneously for 5 consecutive days.

Clinical monitoring, rectal temperature, bodyweight, blood biochemistry and haematology were monitored. Cats were sacrificed to collect bone marrow and thymus samples, for thymus histology and myelogram. One-way analysis of variance for each measurement point was conducted; if the difference was significant, Dunnet's test for comparison of treated/placebo results was used.

Episodes of vomiting were observed, soft faeces was seen in all the other FeIFN-treated cats at D11; all animals recovered spontaneously, without any treatment. No other abnormal reaction was observed on FeIFN-treated animals during the study. The placebo group remained healthy, except for one kitten at D4, showing anaemia of ocular conjunctiva, nasal discharge, sneezing and slight hypersalivation. It recovered spontaneously without any treatment. No local reactions after injections were registered.
Slight hyperthermia was observed in two FeIFN-treated cats at D1+ 4 hours (39.7°C), and in four treated cats at D2 + 4 hours (≤ 39.8°C); temperature of all FeIFN-treated kittens remained normal from D3 to D14.
No difference was observed in bodyweight between the groups; results in the FeIFN-treated group are however slightly lower than those of the control group.

Hematology: it seems that treatment with an overdose of FeIFN can sometimes induce a transient decrease of leucocytes (at D4 and D7), which can be considered as leucopenia in some cases. Decreases were also registered for erythrocytes from D4 to D14, for mean haematocrit (both groups were below the lower limit of 35%), for haemoglobin concentrations (sometimes slightly below the lower limit of 8 g/dl), for mean platelets count. However, these decreases were always slight, ranging between normal and slightly below minimal normal values.

An increase of reticulocytes was seen in the placebo group at D4, and in the FeIFN-treated group at D11 (the latter being possibly a consequence of the decrease of red blood cells in some kittens following the overdose treatment). Evolution of neutrophils and lymphocytes was irregular, with some inversions of formula in both groups, but apparently more important in the FeIFN-treated group. All of the other hematological parameters remained comparable between groups.

Blood biochemistry: treatment with an overdose of FeIFN can induce, in some cases, a transient increase of alanine aminotransferase (ALAT) and of total cholesterol. However, these increases remain moderate. A decrease was seen for potassium and for chloride, without any apparent biological

significance. The evolution of calcium and phosphorus curves are difficult to interpret, but the curves obtained for both FeIFN-treated and placebo groups are similar. All the other biochemical parameters remained comparable between both groups.

The percentages of neutrophils and band cells in 3 FeIFN-treated cats were below the minimal norms. The percentage of myeloblasts in one FeIFN-treated cat was below the minimal norms. All the other cell lines remained within norms. The percentages of acidophil and polychromatophil erythroblasts were above the maximal norms in 4 FeIFN-treated cats, certainly a consequence of the decrease of red blood cells. Histology of thymus: no significant lesions were observed.

Subcutaneous administration of FeIFN to 9 week old kittens can be considered as safe. An overdose of FeIFN can induce hyperthermia, vomiting, and the occurrence of minor intestinal troubles, ranging from liquid faeces to slight diarrhoea; it also induces transient and moderate leucopenia and thrombocytopenia, with values becoming normal again within 14 days. An overdose of FeIFN can induce a decrease of the red blood cells, with a stimulation of red line precursor cells. A transient and not significant increase of ALAT concentration was seen.

The SPC already stated that pyrexia, vomiting and decrease in platelets, in white and red blood cells might occur in dogs. This is extended to cats. The minor intestinal troubles (ranging from liquid faeces to slight diarrhoea) is also reported. The decrease of red blood cells probably also induced a decrease in haematocrit and haemoglobin concentration, as well as an increase in acidophil and polychromatophil erythroblasts; the high percentage of acidophil and polychromatophil erythroblasts, together with a normal to low rate of neutrophils and band cells, leads to an inversion of formula (lymphocytes > neutrophils); the decreases of serum potassium and chloride levels were possibly due to the losses after vomiting. As these results are merely indirect consequences of those already mentioned in the conclusion, they are not mentioned in the SPC.

Regarding the rise in the concentration of ALAT it is mentioned that "the statistical analysis on the time course showed no significant difference". The possible transient rise in ALAT is reflected in the SPC section 5.4.

Safety of the Repeated Administration of One Dose

Dogs

Neutralising antibody formation in young dogs after treatment

Male and female Beagles, 13-26 months old, were included in the study and given either placebo, 1.0 MU/kg/day, 5 MU/kg/day or 25 MU/kg/day for 5 consecutive days intravenously or 1MU/kg/day fifteen times every two days and once at D55 intravenously. Blood sampling before treatment and after starting the repeated administrations was undertaken. Titration of neutralising antibodies was conducted by adding a constant amount of rFeIFN to the dog serum, and measurement of the anti-virus activity.

In a second trial, male and female Beagles, 57-59 days old, were included in the study and given either placebo, 1.0 MU/kg/day, 2.5 MU/kg/day or 5 MU/kg/day for 5 consecutive days intravenously. Blood sampling was done 2 weeks after the fifth administration. Neutralising antibodies were titrated and the anti-virus activity was measured. No antibodies were found in any group.

The clinical significance of neutralising antibodies during interferon therapy remains controversial. The ability of natural antibodies to inhibit clinical efficacy of therapeutic proteins is a well-recognised phenomenon in a variety of clinical settings. However, some patients with neutralising anti-interferon antibodies continued to respond to interferon. Thus, it is clear that the presence of neutralising anti-interferon antibodies is not always associated with a loss of response. Some authors have suggested that the emergence of binding antibodies to interferon, which can modify the pharmacokinetics of interferon, may be responsible for some cases of therapeutic failure. However, the significance of

binding antibodies, which are more frequent than neutralizing antibodies, remains unclear. It was shown also that the route and frequency of administration may alter immunogenicity.

The possible emergence of antibodies after Virbagen Omega treatment in extreme conditions, although probably an uncommon event and of unknown clinical significance, is mentioned in the SPC.

It was concluded that the lack of data concerning the toxicological side-effects after repeated administration was questionable but, as it is very unlikely that a dog will be infected more than once in its lifetime with parvovirus, the submitted information was considered to be sufficient.

Cats

Safety Study After 5 Consecutive IV Administrations of Increasing Doses to Cats

SPF cats, 3 to 10 months of age, were allocated in two groups and given either FeIFN or placebo. The FeIFN treated group were given increasing doses from 1MU/kg on D1, 3 MU/kg on D7, 10 MU/kg on D14, and 30 MU/kg on both D21 and D35.

Daily clinical monitoring, body temperature, bodyweight, hematology and biochemistry were all conducted. No statistical analysis was conducted on this study.

After FeIFN administration, hyperthermia (up to + 0.8 °C), soft faeces, anorexia, decrease in drinking and collapse were seen in one to three FeIFN-treated cat, after the first injection; afterwards, only mild hyperthermia was regularly seen in cats after each administration; soft faeces were seen at day 8.

In the placebo group, collapse, anorexia and decrease in drinking were seen in all three bovine serum albumin-treated cats, during the first 6 days; soft faeces was seen and sneezing in one cat during the first 6 days; afterwards, soft faeces was seen during 3-4 days after 2nd administration and vomiting at day 22. Heart rates seem to increase after each administration but without any adverse effect. No other abnormality could be seen for all the other parameters.

Doses and overdoses of FeIFN administered at intervals intravenously induce a slight temperature increase. In some cases, soft faeces, anorexia, decrease of drinking and collapse were seen, but all cats recovered spontaneously.

Safety of The Repeated Administration of One Dose in Cats Subcutaneously

SPF kittens (males and females), about 11 weeks old when entering the study, were divided into 2 groups of animals given 1.0 MU/kg/day or placebo 3 times on 5 consecutive days, subcutaneously. The administrations were performed in 5 separate injection points.

The parameters checked were: weight, clinical monitoring, rectal temperature, haemotology and biochemistry, and sampling of thymus and bone marrow. Analysis of variance for bodyweight, temperature and general score evolution was conducted.

No difference in bodyweight between the groups could be observed. Slight hyperthermia was observed in one FeIFN-treated cat at D1 (39.7°C), and in two FeIFN-treated cats at D2 (≤ 39.8°C); temperature remained normal afterwards. The placebo group showed normal temperature values during the whole period. Statistical analysis showed that the rectal temperature evolution during the entire study was not different between both groups, but there was a significant time effect between both groups.

Clinical monitoring in FeIFN-treated cats: anaemia of ocular mucous, congestion of ocular mucous, conjunctivitis, and a small ulceration on the left nostril were observed. All placebo animals remained normal.

Hematology: repeated administration of FeIFN can induce, in some cases, a transient decrease of leucocytes; of mean hematocrit and haemoglobin concentrations. During this study, red blood cell count remained within normal ranges. Mean platelet counts were sometimes higher in the FeIFN-treated group compared to the placebo group. However, one FeIFN-treated cat showed clear thrombocytopenia from D4 to D25 and from D42 to the end of the study.

Evolution of neutrophils and lymphocytes was irregular, with some inversions of formula in both groups; rates of eosinophils, basophils and monocytes remained relatively stable in both groups. All the other hematological parameters remained comparable between both groups.

Blood biochemistry: an increase of creatinine phosphokinase was seen in both FeIFN-treated and placebo groups. A decrease was seen for ASAT, without any apparent biological significance. All the other biochemical parameters remained comparable between FeIFN-treated and placebo groups; in particular, SAP values in FeIFN-treated cats was always under those of the placebo group (including on day 0), but the shape of both curves was very similar. Low SAP values are clinically not significant.

Myelograms: deviation on eosinophil-, neutrophil - and erythroid cell lines were observed in both placebo and FeIFN-treated groups ; plasmocytes were absent in both groups and lymphocyte rate of both groups were above the maximal normal value. Thus, these findings are unlikely to be attributable to FeIFN treatment.

No significant lesions of the thymus were observed.

Repeated administration of FeIFN can induce transient clinical signs and a significant decrease of the mean leucocyte count within the third treatment period. However all individual leucocyte counts remained within normal ranges. The findings in this trial confirm the results of previous studies : FeIFN has an impact on leucocytes, ranging from a transient and moderate decrease of leucocyte count to transient and moderate leucopenia in some cases. It must however be emphasised that this occurs after repeated administrations of overdoses.

The repeated administration of interferon is performed on day 0, day 21 and day 42 to be in accordance with the requirements of Annex I of Directive 2001/82/EC. The administration according to the posology on the SPC is to be on day 0, day 14, day 60. Due to the longer time span between the first two administrations in this study, the side effects may be less pronounced initially in this study, than what could be expected following the recommended dosing interval. The side effects seen in this study (hyperthermia, decrease in WBC count) are mild and transient, and are to be expected after treatment with interferon.

Examination Of Reproductive Performance

- **Mutagenicity after treatment**

  Two mutagenicity tests of rFeIFN were carried out; the first being a reverse mutation test, the second a chromosomal aberration test. For the reverse mutation test cell suspension of the tested strain were cultured on nutrient broth medium. The results showed that the recombinant feline interferon (rFeIFN) was negative in both tests and had no mutagenic activity.

- **Teratogenicity in rats after treatment**

  Wistar strain SPF rats, virgin females, 9 weeks old, and male breeders, 12 weeks old, were included in the study. Females were mated and, after judged as pregnant, randomly divided into 4 groups. Each group received one injection of rFeIFN intravenously, daily, from day 7 to day 17 of gestation.

Necropsy of the females was performed at D21 of gestation. Appropriate and detailed statistical analysis was carried out. There were no statistical differences between treated and control groups for the following parameters: number of corpora lutea, number of implants, implantation rate, number of dead embryos, number of dead foetuses, foetal mortality, number of live foetuses, sex ratio of live foetuses and individual body weight of live foetuses.

A small number of abnormalities were seen, both external (polydactyly), skeletal, and internal. As there were no statistically significance differences between treated and control groups, and no dose-effect response, rFeIFN was considered to cause no effect in pregnant animals and their embryos or foetuses. There was no indication of delayed ossification in the observation of squama occipitalis, number of ossification centres of sternebrae, number of ossified metacarpals and metatarsals. With respect to polydactyly, a genetic factor was found in rats. Thus, those seen in the present study were judged unrelated to the treatment.

From both tests of mutagenicity submitted, no conclusions could be drawn on the mutagenic potential of the recombinant feline interferon (rFeIFN): the tests were not carried out in compliance with the OECD recommendations, and so they were not validated. A teratogenic effect cannot be excluded, although radioactivity in the foetus after intramuscular administration of ^{125}I-HuIFN-α was extremely low, compared with radioactivity in maternal plasma, suggesting that it does not pass the placenta. The impact of rFeIFN treatment on the reproductive performance of dogs is not expected.

No specific trial in cats was provided. A risk/benefit analysis shows clearly that reproductive performances are of little importance with regard to a life-threatening infection like feline leukaemia virus and/or feline immunodeficiency virus or parvovirosis. An appropriate warning is included in section 5.6 of the SPC.

Examination of Immunological Functions

Dogs

SPF Beagles (males and females), about 8-9 weeks old, were included in the study. Half of the animals selected at random, received rFeIFN omega at a dose of 10 MU/kg/day intravenously, during 3 consecutive days. The other half served as placebo/controls. All dogs were vaccinated with Virbac's vaccines for dogs on day D16 and on day D37 (when the dogs were 10/11 weeks old). All dogs were clinically monitored up to day 58. Two dogs per group were euthanased on day 16 and samples of thymus (for histology) and bone marrow (for myelogram) taken. The data were analysed with an analysis of variance on repeated measures.

There were no clinical side-effects recorded in dogs in the treated group. An increase in lymphocytes, with regard to neutrophils, was recorded at different times in both groups. Values always remained, however, within physiological limits. The rates of red blood cells, eosinophils, basophils and monocytes remained stable in both groups during follow-up. There were no histological lesions seen in the thymus. An increase of the red line was observed in 3/4 dogs (and in 1 control dog), probably due to the young age of the animals, in the myelogram. A diminution of the number of megakaryocytes was particularly observed in the two dogs in the treated group. All the animals seroconverted after vaccination with regard to all the antigens. There were no statistical differences between the groups.

This trial confirmed the impact of rFeIFN on blood cell lines. No undesirable side-effects on immunological functions, kidneys or liver, could be observed.

The possibility of the antibodies against omega interferon leading to malfunctioning of the cytokine network in treated dogs in the short and the long term was specifically addressed. As autoimmune side effects, including immune mediated thrombocytopoenia and insulin-dependent diabetes, have been associated with interferon therapy in humans, the long term effects of interferon treatment may not be considered irrelevant.

In the treatment of parvovirus infection in dogs with interferon omega, the duration of the treatment and number of injections is far lower than in human (3 days compared to several months). Therefore, the risk of appearance of antibodies is much reduced when compared to the human situation. Furthermore, this probability has to be balanced by the very low probability that a dog could be infected twice by a severe parvovirus infection, so that the probability of being administered a complete treatment twice in its lifetime is also extremely low.

Autoimmune diseases have been observed after long-term treatment of severe infections in man, particularly after type I (Alpha or Beta human interferon) in the treatment of chronic infectious disease (e.g. viral hepatitis). The conditions of administration in man and in dogs are very different. Usually, the administration of interferon in man lasts for a long period of time (weeks or months) whereas in dogs, the current indication requires a very short period (3 consecutive days). As autoimmunological side effects are usually related to the number of exposures, it can be hypothesised that the incidence of such problems is lower in dogs than in man. Generally and independent to any treatment, immunologically induced disorders are under-diagnosed in companion animals.

Cats

With regard to immunological functions in cats, the studies provided show that FeIFN displayed a slight and transient leukocyte decrease, which is also a side effect associated with high IFN doses in humans. The long-term effects of FeIFN treatment in cats was not assessed, as FeIFN treatment is intended for FIV or FeLV infections, these diseases being immunosuppressive by themselves. An appropriate warning is included in section 5.3 of the SPC.

In humans it appears that in the treatment of viral infections (mostly viral hepatitis treated with interferon alpha), the rate of seroconversion can vary between 7 to 61%. Generally, the time of appearance of antibodies to IFN is quite variable, ranging from 1 to 30 months, and 6.6% develop antibodies in the first month of the therapy. The amount of neutralising antibodies has also to be taken into account: the higher the titre of anti-IFN, the higher the risk of the loss of therapeutic effect. Thus, the appearance of antibodies has to be analysed together with the duration and frequency of treatment.

No information is available for a longer period following the last injection of interferon omega. Generally, the incidence of auto-immune disorders in companion animals is not well reported or documented and for example, the effect of interferon on immune related thrombocytopenia or insulin dependant diabetes occurring years after interferon treatment is not known. As no information is available regarding dogs and cats, section 5.10 of the SPC contains a special warning.

Interactions

Due to the indications for use, concomitant use of immunological products is not recommended. During efficacy trials, antibiotics from distinct families, rehydration products, non-steroidal anti-inflammatory products and additional treatments (hepatoprotective agents, diuretics, antifungal agents, anabolic agents, vitamins) were used without observed negative interactions.

The use of supplementary supportive treatments improves prognosis. Even if no interaction has been observed so far, supplementary supportive treatments should be used cautiously and after a thorough risk/benefit analysis. Specific information is included in section 5.7 of the SPC.

FIELD STUDIES

Dogs

A controlled, blind, randomised, monocentric, comparative (vs placebo) study involved conventional Beagles; (receiving either rFeIFN or placebo) at the age of 4-5 weeks.

Group 1 received a dose of 10 MU/kg FeIFN omega intravenously, once a day for 3 consecutive days. Group 2 received a dose of placebo intravenously, once a day for 3 consecutive days. Antibiotherapy, rehydration and gastric adsorbents were allowed.

There was no statistically significant difference for each of the haematology parameters between groups, taking into account the initial starting values at day 0. There was no statistically significant difference for each blood biochemistry parameter between groups, taking into account the initial starting values at day 0. All the myelograms were considered as normal. Due to the young age of the animals, bone marrow presented more developed red cell l and lymphoid lines than in adult bone marrow. All the thymuses were considered normal.

Details of the breeds represented in the clinical trials were submitted. No specific side effects were observed in either efficacy trial.

Cats

Study of the clinical efficacy and safety of Interferon in the treatment of infections by FeLV and/or FIV viruses in cats

Local tolerance: no adverse local reaction was seen at the site of injection. General tolerance: 7.1 % in the FeIFN-treated group and 7.0 % of the placebo group presented various adverse reactions. The difference between groups was statistically not significant. They included; general fatigue or depression; diarrhorea or enteritis; vomiting and renal failure. Although one cat showed biochemical and clinical signs of renal failure at D0 (also consistent with FeLV infection), it was still alive 2 years later.

No conclusions can be drawn with regard to the relationship between the administration of the product and the adverse effects seen during the trial.

Impact on blood cells: no negative impact on WBC, RBC and PCV could be shown.

WBC: in cats with leukopenia already at D0, there seems to be a slight and transient decrease of WBC count with regard to the placebo group at D30. The statistical comparison remains however not significant between both groups.

RBC: it appears that the physiological status of the cat at the beginning of the FeIFN treatment has an impact on the prognosis.

Administration of FeIFN is safe. The trial was well conducted and generally few side effects are seen. There is no difference in side effects seen in interferon treated cats and cats receiving placebo. All side effects whether proven to be related to treatment or not are reflected in the SPC. The SPC indicates that a slight decrease in WBC, RBC and platelets is possible (section 5.4 of the SPC).

ECOTOXICITY

An assessment of the potential environmental risk derived from the use of the product was provided. The absence of live baculovirus in the final product is ensured by the validation of the inactivation of the body fluid which guarantees that no live baculovirus is present after inactivation. The risk of finding live recombinant baculovirus in the desalted bulk and therefore in the final product, although theoretically possible, is carefully controlled and associated with an extremely low probability.

No environmental risk was identifiable through the excipients, through the active substance, through metabolites, through the target species, the route of administration and waste material (disposed of by the veterinary surgeon). As the FeIFN is administered subcutaneously in cats (no environmental pollution), there is no additional environmental risk due to the extension application.

OVERALL CONCLUSIONS ON SAFETY

Safety is well documented. The pharmacological studies of rFeIFN, although not very important for this type of product, suggest low tissue distribution and rapid metabolism through the kidneys. The pharmacological effects of IFN continue for some time after its disappearance from plasma and therefore the number and type of cells interacting with IFN are far more important than merely IFN levels.

The toxicological studies in dogs confirm the general acute side-effects described in the literature: drowsiness, increase in body temperature, slight increase of sinus tachycardia and respiration rate. These symptoms are rarely dose limiting and spontaneously dissipate after no more than a few days.

The safety studies in cats and dogs show that rFeIFN alters the haematopoietic cell lines slightly and temporarily. Increase in body temperature, some vomiting and soft faeces may occur, especially with high doses. Even if such side-effects appear, interruption of treatment is not necessary. No neurotoxicity, hepatic or renal toxicity could be observed and indicators of these values remained globally within physiological limits. The transient rise in the concentration of ALAT shown in some studies in cats is mentioned in the SPC.

No conclusion can be drawn on the mutagenic potential and teratogenicity effect of rFeIFN. They cannot be excluded, although placental passage of rFeIFN is probably low. A warning is included in the SPC.

After intravenous administration in cats, anorexia, decrease of drinking and collapse appear as potential new symptoms. Increases in GOT, GPT and total cholesterol were also seen. However, as the corresponding trials can only be considered as indicative (IV administration is not within the SPC claim for cats, and the composition of the product used differs slightly from the one manufactured by Virbac), they are not referred to on the SPC.

The impact on the cat's eye remains questionable: ocular complications for humans are described in literature, but the number of animals involved is insufficient to make a definite conclusion.

Immunological functions are not impaired after use of rFeIFN. Antibodies to anti-rFeIFN might appear in rare cases, but their clinical significance is unknown. As auto-immune secondary effects are recognised in humans following interferon administration and, in the absence of further long term toxicity data in dogs, the following warning was included in section 5.9 of the SPC: '*No information on the induction of long-term side effects is available in dog and cat, specially for auto-immune disorders. Such side-effects have been described after multiple and long-term administration of type I interferon in man. The possibility of occurrence of auto immune disorders in dogs cannot therefore be ruled out*'.

In conclusion, cats and dogs which are candidates for rFeIFN injections are seriously ill. A risk/benefit analysis clearly shows that all the side-effects, which are not systematic and remain moderate and transient, are of little importance with regard to a life-threatening infection.

IV. OVERVIEW OF EFFICACY TRIALS

INTRODUCTION

Although this subject needs further research, the following immunomodulatory properties of type-I IFNs are known:

- Type-I IFNs can affect the growth, differentiation and function of various types of cells in the immune system.
- Type-I IFNs enhance antibody-dependent cell-mediated cytotoxicity (ADCC).
- Type-I IFNs enhance the lytic ability of CTL and NK cells. In particular, IFNs can stimulate expression of class-I MHC antigens, which are crucial for recognition of foreign antigen by cytotoxic T lymphocytes.
- Type-I IFNs indirectly broaden their immunomodulatory effects by enhancing/lessening the production and/or release of various cytokines.

The mechanism of action in viral infections is still not fully understood. Type-I IFNs can impair various steps of viral replication, including penetration, uncoating and assembly of progeny virions as well as transcription and translation. In the course of evolution, some viruses have developed counter mechanisms by which they disrupt the antiviral mechanisms induced by IFNs. Whether the emergence of a resistant clone during treatment could be responsible for some therapeutic failure in humans is still not known.

Antiviral activity of IFN-ω is indirect: it does not act directly on viruses, but makes host cells resistant to them. By induction of synthesis of the double-chain RNA-dependent protein kinase and the 2',5'-oligoadenylate synthetase, viral protein synthesis is inhibited. This is a rather general antiviral mechanism, potentially able to affect a wide range of viruses.

In canine parvovirus infection, diarrhoea and vomiting are almost systematic findings, which occur within the first 3 days. These signs are followed by complete loss of appetite, loss of vigour, fever and dehydration in about 50% of the cases; around one third of the animals also show a decrease in blood cells (decrease of white blood cells is considered to be a characteristic haematological feature of canine parvovirus infection, but it is also reported to be highly variable).

It is thought that the effects of IFN-ω on canine parvovirus are largely dependent on its immunomodulatory activity rather than antiviral activity. Also, parvovirus multiplies and causes disease in tissues where active cell division is occurring such as intestinal crypt cells of intestinal mucosa. IFN-ω administration suppresses cell division, which in turn inhibits multiplication of parvovirus.

Cats

Toray Industries put on the Japanese market FeIFN intended for the protection of the cats against calicivirus infection. For this disease, the administration/dosage of FeIFN is 2.5 to 5 MU/kg administered intravenously for three times every other day, once a day.

In order to propose a suitable treatment for FeLV infections, Toray Industries has conducted several investigations. They found that: the administration/dosage for feline calicivirus infection was proven to be unsuitable for feline leukaemia; instead of every other day injection, administration every day is preferable; daily administration for at least three consecutive days is more effective; when the dose is decreased to 0.5 MU/kg the therapeutic effect is reduced. However, a high dose of more than 2.0 MU/kg increases the treatment cost without greatly increasing the efficacy.

This treatment is the subject of a United States patent (N° US 6350 443 B1). Six examples (different treatments dosage/administration) are described in this patent and discussed in the dossier. These preliminary Toray's investigations allowed Virbac to choose a dosage/administration of 1 MU/kg once

daily for 5 consecutive days. A second series of injections is recommended in order to ensure a better control of the clinical signs. This design (2 series of 5 injections) was chosen as the basic scheme for the efficacy field trial.

The selection of IFN type, dosages, routes and schedule of administration is clearly dictated by the type of disease, the pharmacokinetic pattern and the requested biological activity. In humans, the plasma disappearance of all interferons is assessed, in practice, using a two-compartment model i.e. initial distribution phase (fast) and a successive elimination phase (slow). Referring to published data, the serum half-lives of IFNs during the slow phase can vary from several minutes to several hours after IV administration of IFNs. If such results can be explained by using distinct IFN types and dosages, two characteristics can be noted i.e. high dosages of IFN induce longer half-lives and repeated daily administration tends to have similar effects. In comparison with the IV administration, IM or SC administration are mainly characterised by :

- a recorded distribution phase which can last several hours (Tmax);
- the IFN peak level (Cmax) is much lower;
- IFN plasma levels are sustained for longer ($t_{1/2\beta}$).

The IFN administration by both the IM and SC route display similar plasma curves although the IFN peak level can be slightly delayed after the SC injection. According to those pharmacokinetic properties, the SC administration of IFN-α is currently used for the prolonged treatment of chronic human viral disease (particularly hepatitis).

Three trials are presented in the dossier for the efficacy assessment in cats; a Toray trial in cats which provides information on the pharmacokinetics of rFelFN-omega and is not GLP compliant ; a trial by Virbac under GLP conditions using the proposed product for marketing to show the *in vitro* activity of Interferon 140.04 on FeLV-A virus; a trial by Virbac under GLP conditions using the proposed product conducted in FeLV and /or FIV positive cats using the subcutaneous route.

LABORATORY TRIALS

Dogs

IFN inhibits replication of various viruses through inhibition of protein synthesis. The mechanism is as follows: 2',5'-oligoadenylate synthetase (2-5As) polymerises adenilyc acid by 2',5'-phosphodiester linkage by using ATP as the substrate, which in turn activates latent RNase L in cells, which in turn degrades polysomes, leading to inhibition of viral protein synthesis. Thus, dosage of 2-5As can be considered as a good marker for rFeIFN antiviral activity.

The present study tries to establish:

- the *in vitro* activity and sensitivity of various canine cells, with regard to the rFeIFN dose and its time of addition within the medium.
- the *in vivo* induction of 2-5As activity.

In vitro* test:* rFeIFN was added at different concentrations to cultures of canine thymus-derived cells. The 2-5As activity was followed after 2, 5, 6, 24 and 49 hours of incubation.

In vivo* test:* rFeIFN was administered intravenously to 3 dogs (17 months of age) at the dose of 1 MU/kg. One other dog was used as a control. Blood samples were collected 4 and 2 days before administration, immediately before administration and at 0.25, 0.5, 1, 2, 3, 5, 7, 10 and 16 days after administration.

Intracellular activity was analysed by Radio-Immunologic Assay (RIA method) and expressed as activity per unit protein.

In vivo induction of 2-5As activity is seen in at least 2/3 dogs. The *in vitro* results also demonstrated that 2-5As activity is induced in canine cells *in vitro* by adding rFeIFN.

Comparing these results with the ones obtained in cats, it can be concluded that 2-5As activity is less in dogs than in cats. This is in line with the fact that IFNs are known to be species-specific. This study shows that 2-5As activity occurs in dogs, especially *in vitro*. Furthermore, it can be concluded that the activity is cell-specific, dose-specific and time-specific.

Determination of the Dose in Dogs

Beagles, both females and males, 102-135 days old, were included in the study. After oral infection with 10 ml of canine parvovirus type 2 (CPV), strain 238 (10^6 $TCID_{50}$/ml), they were divided into 3 groups, and given either 1 MU/kg, IV once daily, on Days 4, 5 and 6, 5 MU/kg, IV once daily, on Days 4, 5 and 6, or physiological saline, IV once daily, on Days 4, 5 and 6.

The protocol of the study established the evaluation criteria and the statistical analysis in detail. The animals were randomised amongst groups. All the animals showed clinical signs of parvovirosis before the start of the rFeIFN treatment.

There was no difference between the groups with regard to mortality rate (one dog died in the control group). Statistics show that there is no group-effect but that there is a time-effect for the evolution of body temperature. For body weight also, statistics show that there is no group-effect but that there is a time-effect and a 'product x time' effect. There is no statistically significant difference between groups with regard to diarrhoea symptoms, dehydration and vomiting, possibly because of the low number of animals in each group. However, if the markedly improved group and the improved group are put together, a significant difference is observed. There is no statistically significant difference between groups with regard to appetite, possibly because of the low number of animals. WBC decreased in all groups from day D5 on, reaching the lowest level on days D7-D9. Recovery was gradual and normal level was reached around day D12. A significant difference was observed when the markedly improved group and the improved group were put together. Evolution of lymphocytes over time showed a decrease in all groups from day D0 on, reaching the lowest level on day D4. Recovery was gradual and the normal level was reached around day D8-9. GOT values show a rapid increase on day D4, but normal levels were reached right from day D5-6 onwards.

The product undoubtedly produced a beneficial effect when administered at 1 MU/kg for 3 consecutive days. This positive effect was less obvious at 5 MU/kg.

In a second study, SPF Beagle puppies (females and males), 8-9 weeks old, were infected with 1 ml (0.25 ml in each nostril and 0.5 ml injected in the throat) of canine parvovirus type 2, strain 39 2P+3P ($10^{6.09}$ $TCID_{50}$/ml), divided into 2 groups,and given either 2.5 MU/kg, IV once daily, on D4 to D6 or D5 to D7 or placebo, IV once daily, on D4 to D6 or D5 to D7.

The protocol of the study established the evaluation criteria and the statistical analysis in detail. All the dogs in the placebo group and 2 dogs in the treated group, which presented with dehydration of 8%, received subcutaneous injections of Ringer Lactate once or twice a day until improvement was seen.

All the animals except one survived in group 1 and all the animals in group 2 died (between D6 and D10). Hypothermia was noticed in 3 dogs in group 1; during the study, they had some difficulty reaching the normal temperature range (all the animals presented normal temperature only at D14). Marked falls in temperature were seen in group 2 until death. WBC remained within the physiological limits throughout the study in group 1, except for the dog which died. In group 2, WBC decreased dramatically from D6 until death. The evolution of RBC and Haematocrit (Ht) over time was comparable in both groups. In both groups, the serological HI antibody levels clearly indicated seroconversion.

The beneficial effect of rFeIFN administration was confirmed. When administered intravenously at 2.5 MU/kg for 3 consecutive days, recovery was markedly improved.

Cats

In-vitro activity of Interferon 140.04 on FeLV-A virus

Brief description of the trial design
Four plates of 6 wells are seeded with cells sensitive to FeLV virus (named QN10S) at 5.25 x 10^4 cells per well on day D-2 and used as follows:

At D-1, six groups of 3 wells are treated each with following dilutions of FeIFN: 50 U/ml, 12.5 U/ml, 3.125 U/ml, 0.781 U/ml, 0.195 U/ml and 0.049 U/ml. Two additional group of 3 wells were used, one maintained as "cell control" and the other as "positive control", both without FeIFN.

At D0, each well is inoculated with the same amount of virus FeLV A (45 pfu), except the three referenced as "cell control". After 1h30 of incubation, the medium is removed and replaced by a new one, the quantity of FeIFN corresponding to the dose used for each well during pre-treatment being maintained.

At D3, the medium is again refreshed, the quantity of FeIFN corresponding to the dose used for each well during pre-treatment being maintained.

At D7, the percentage of inhibition of the lysis plaques induced by the virus was calculated.

Conclusion
There is a strong relationship between the dose of FeIFN and the percentage of inhibition. IFN are known to have antiviral properties. Type-I IFNs can impair various steps of viral replication, including penetration, uncoating and assembly of progeny virions as well as transcription and translation. In the course of evolution, some viruses have developed counter mechanisms by which they disrupt the antiviral mechanisms induced by IFNs. This trial demonstrates that antiviral properties of FeIFN against FeLV-A do exist *in vitro* (dose-dependent effect), and thus that no counter mechanism apparently exists for FeLV.

This study shows clear evidence of the effect of interferon 140.04 against FeLV virus type A. Subgroup A is the least pathogenic of the subtypes. Subgroups B and C arise de novo in cats infected with subgroup A and are believed to be responsible for most of the clinical syndromes associated with FeLV. Subgroup B induces immune suppression and malignant transformation. Subgroup C causes severe nonregenerative anaemia.

Justification of Administration Route in cats

Cats received a dose of 5 MU/kg FeIFN omega, and the following tests were conducted: pharmacokinetics in blood (via IV, SC or IM administration), whole-body autoradiography (IV route, ^{125}I-rFeIFN), excretion tests (IV route) faecal/urinary excretion.

For the autoradiography and metabolism study (in whole body), desalted rFeIFN was labelled with ^{125}I (purity of 98.0 %). The antiviral activity of rFeIFN was used as starting material to produce the labelled rFeIFN. There was no statistical difference in the antiviral activity before and after labelling.

Stability of rFeIFN in serum, urine and faeces was checked. Blood was sampled at different time intervals for each route of administration, to establish pharmacokinetic parameters. Euthanasia of the animals was performed. Autoradiograms were conducted after intravenous administration. Urine samples were taken before administration and at different timepoints after intravenous administration. Faeces of a single defecation were collected.

Stability: rFeIFN is not inactivated after 7 hours. Urine and faecal excretion: no rFeIFN activity was detected at any time of sampling. It was shown that rFeIFN is not inactivated in urine, but that it is inactivated in faeces. Whole-body autoradiography: initially labelled IFN is mainly seen in liver and thyroid, and to a much lesser extend in spleen, adrenal gland and urinary bladder; after 3 hours, the labelled IFN is mainly seen in urinary bladder, thyroid, liver, stomach and contents in digestive tract, and to a much lesser extend in laryngeal mucosa.

This study shows that the FeIFN displayed similar pharmacokinetic properties in cats to those observed with human interferon in humans. Consequently, as for the treatment of chronic viral disease in humans, SC administration seems to be the appropriate route for the treatment of chronic viral infections in cats such as FeLV and/or FIV diseases. This view is indeed supported by efficacy trials. It should also be noted that IV or IM routes are not very convenient in cats, especially if multiple administrations are requested.

Antibody induction to FeIFN in cats

A study was conducted to demonstrate that Virbagen Omega does not induce antibodies to FeIFN that may compromise longer-term treatment, as the induction of antibodies is a recognised risk with this type of product. A study to detect anti-rFeIFN-ω neutralising antibodies after repeated administrations of 140.04 was conducted.

Kittens (males and females), 13 weeks old, received 3 series of 5 subcutaneous treatments (1 MU/kg per injection), on days D0-D4, D14-D18 and D63-D67.

The results showed that three 5-day treatments were well tolerated. No neutralising antibodies were detected. This is also supported by indirect evidence, because the recombinant feline interferon of Virbagen Omega (rFeIFN-ω) is quite homologous to the feline interferon (FeIFN). The amino acid sequence of rFeIFN-ω consists of 170 amino acids and is homologous to the amino acid sequence of FeIFN except for one missing amino acid.

Validation of the seroneutralising test was provided, and the sensitivity confirmed that Virbagen Omega does not induce antibodies to FelFN in cats.

The proposed dosage regime (i.e. 1 MU/kg once daily, injected subcutaneously, once daily for five consecutive days; 3 treatments to be given starting on days 0, 14 and 60) is supported by weak pharmacokinetic data. The United States Patent no. 6.350.443 summarised the preliminary Toray's investigations justifying the choice of the dosage/administration for the development of Virbagen Omega in cat. According to example nos. 1 and 5 of this Patent, a second series of injections was recommended in order to ensure a better control of the clinical signs. This design (2 series of 5 injections) was considered as the basic scheme for the efficacy field trial performed by Virbac.

FIELD TRIALS

Dogs

Different experimental sites were involved in the study with both female and male dogs, varying in age from 1 month to 11 years. The inclusion criteria were 1+2 or 1+3, as follows:

1. More than one major clinical symptom observed which included diarrhoea, vomiting, dehydration and other findings, and decrease of white blood cells in hematological examination, and the attending veterinarian diagnosed the case as parvovirosis,
2. CPV was detected in faeces,
3. Virus was not detectable or not examined, but the dog lived with a household in which parvovirosis was diagnosed, and presented with similar symptoms as those of the household.

The dogs were randomly divided into 3 groups,and given 2.5 MU/kg, IV once daily, for 3 days, 1.0 MU/kg, IV once daily, for 3 days or no rFeIFN.

Clinical, haematological and biochemical parameters to be evaluated and the statistical analysis were well defined in the protocol.

The severity of parvovirosis was established and the evolution of the symptoms was monitored by 2 different teams (practitioners and experts), according to a five-grade classification scheme: markedly effective, effective, slightly effective, not effective, aggravated. Virus isolation was carried out in 73 cases, with 72 being positive. All the other included cases satisfied inclusion criteria 1+3.

There is a statistically significant difference between groups. The probability of survival was higher in the treated groups (1+2) than in control group 3. The efficacy rate differs significantly between groups. The efficacy rate (number of animals in which treatment was considered effective or better/number of animals in the group) differs significantly between groups and according to severity of the infection.

The beneficial effect of rFeIFN administration was confirmed. When administered intravenously at 1.0 or 2.5 MU/kg for 3 consecutive days, recovery was markedly improved.

1.0 MU/kg dose of FeIFN

Dogs (females and males), of at least 5 weeks, with clinically diagnosed parvovirus infection, confirmed by a positive finding of CPV-antigens in the faeces were randomly divided into 2 groups for this study. They were given 1.0 MU/kg, IV once daily, for 3 days or no rFeIFN.

All the dogs were rehydrated and all dogs received antibiotics. The differences between groups for each clinical symptom taken separately and for WBC were statistically not significant. There were no statistical difference found with regard to mortality rate.

In this trial, where 1 MU/kg rFeIFN was administered intravenously once daily for 3 consecutive days to dogs with parvovirosis, there was no evidence of a beneficial effect.

2.5 MU/kg dose of FeIFN

Dogs (females and males) of at least 5 weeks, with clinically diagnosed parvovirus infection, confirmed by a positive finding of CPV-antigens in the faeces were randomly divided into 2 groups for this study. They were given 2.5 MU/kg, IV once daily, for 3 days or no rFeIFN.

This difference in mortality rate is statistically significant (calculated relative risk of death in placebo group compared to treated group was equal to 4.4). In vaccinated animals, the difference is not statistically significant. In unvaccinated animals however, the difference is statistically significant (calculated relative risk of death in placebo group compared to treated group was equal to 6.4).

At 2.5 MU/kg administered intravenously once daily for 3 consecutive days, rFeIFN gives a specific advantage in the treatment of parvovirosis, when compared to symptomatic treatment of the disease.

In view of the minimum age of the animals used in the clinical trials it was considered that treatment of animals from 1 month of age could be allowed.

Cats

Study of the clinical efficacy and safety of Virbagen Omega in the treatment of infections by FeLV and/or FIV viruses in cats

This was a controlled, double blinded and randomised study in 7 different breeds of cat, 3 months to 20 years of age. Cats to be included in the trial had to present clinical signs of an FeLV and/or FIV infection and an ELISA test positive to FeLV or FIV or both viruses. Exclusion and non-inclusion criteria were defined: amongst them, animals positive to ELISA test with a tumorous form of the infection by FeLV, or animals in critical clinical stages were not included in the trial; use of any immunomodulatory treatment (ie corticosteroids) was forbidden.

Cats received either 2 sets of 5 injections (on days D0-D4 and D14-D18) or 3 sets of 5 injections (on days D0-D4, D14-D18 and D60-D64), with either FeIFN (1 MU/kg.day) or a placebo. Both products were administered by the subcutaneous route. Additional supportive treatments, such as fluid therapy, vitamins, antibiotherapy if necessary, were used concomitantly.

Of the population receiving 2 series of 5 injections: the percentage of mortality during the 6 months period was 31.3 % in FeIFN-treated group, versus 25.0 % in placebo group. As treatment did not improve the survival rate, no further analysis was undertaken.

Of the population receiving 3 sets of 5 injections: the results at D0 showed that there was no statistically significant difference between FeIFN-treated and placebo groups at D0 on the following parameters: age, bodyweight, living conditions (house with garden, apartment, cattery), ELISA results, rectal temperature (increased or decreased), general behaviour, appetite, thirst and dehydration, aspect of mucosa, percentages of stomatitis, total clinical score, WBC and RBC (decreased, normal, increased), PCV.

During experimentation, there was no statistically significant difference between FeIFN-treated and placebo groups on the following parameters: concomitant treatments (rehydration, antibiotics, vitamins, others) and ELISA results (7.9% in the FeIFN-treated group and 8.5% in placebo group became negative at D120).

FeIFN-treated and placebo group are comparable at the beginning of the experiment. Concomitant treatments did not create any bias.

Mortality rate was always lower in the FeIFN-treated group: these results were not statistically significant at time point “4-months”, but became statistically significant at all the other time points (“6-months”, “9-months” and “1-year”). Anaemia effect was significant at each time point. This means that cats without anaemia on day 0 had a longer survival than cats with anaemia. This is reflected in the SPC. The survival rate was also studied on two sub-populations of cats, the cats positive to FeLV at least and the cats positive to FIV only. Mortality rate in the “FeLV at least” group was always higher than the mortality rate in the “FIV only” group.

Administration of FeIFN increases the 6-month, 9-month and 1-year survival rate and improves the clinical score of the treated cat on a 4-month period of observation.

Both groups can be considered as comparable at the beginning of the trial, because no statistically significant difference could be seen on major parameters. The course of the trial was not biased because of the supportive treatments received (no statistically significant difference either between groups) and the number of animals involved in each group. Thus, the differences in the outcome can only be attributed to the FeIFN-treatment.

The initial design of two series of 1 injections daily for 5 consecutive days is not supported by this trial: it appears that three series of 5 injections of 1MU/kg once daily are necessary to increase the survival probability up to 12 months, and to decrease the clinical severity of the FeLV and/or FIV infections. Many cats were eliminated from the study, leaving only about 40 cats left for study of clinical effect after day 120. The SPC reflects that a prolongation of life up to 12 months can be expected, but the quality of life in that period is just as important from a cost/benefit point of view.

The change of the serological status of some cats (tested positive on day 0 but tested negative with the ELISA test on day 120), and whether these cats are expected to have cleared virus due to interferon treatment was further investigated. In the final study report, it was specified that approximately 8% of tested cats on D0 became negative on D120 with an ELISA test.
7.9% in the group A (IFN) and 8.5% in the group B (placebo)
There was no statistically significant difference between groups (p=1.0000).

A positive FeLV cat being re-tested negative 4 months later may be explained by different hypothese:

1. The cat could have been a false positive result on D0, which is possible with such an in-practice test kit (sensibility = 90.9%, specificity = 97.8%).
2. The treatment with interferon helped the cat to clear its virus.
3. The cats could be transiently viraemic, with a progressive elimination of the FeLV in the blood and developed a latent carrier stage. In this case, the cat can be tested negative a few months later.

The first hypothesis is not the most relevant one since the animals were symptomatic (symptoms possibly related to a retrovirus). If the second hypothesis is possible for the cats from the treated group, it is not appropriate for the placebo group cats. The third hypothesis is, the most relevant because in the FeLV case, the antigenaemia can be transient.

POSITIVE FIV CASES ON D0 THAT CHANGED TO NEGATIVE ON D120

Cats positive to FIV on D0, became negative to FIV on D120; in both the interferon group and in the placebo group. The in-practice test kit that was used in this trial on D0 and D120 detected anti-virus antibodies directed against the viral core protein p24 and the transmembrane envelope protein gp40. It is very unlikely that a cat clears its virus; and if so, antibodies should have persisted during several months. In this case, there are two hypotheses:

1. The case was a false positive on D0 and a true negative on D120
2. The case was a true positive on D0 and a false negative on D120.

This test is based on enzyme-linked immunosorbent assays (ELISA). Specificity of the test is very high and positive results are generally reliable, especially when the cat is symptomatic. Nevertheless, as with all test kits, there is a possibility for false positive and false negative results.

It is likely that the cats are false negative on D120 rather than false positive on D0 because the clinical signs observed are in accordance with a retroviral infection. To eliminate any doubt about the possible inclusion of FIV negative cats, the significance of the statistical analysis excluding the doubtful cases was re-checked. The results obtained after the exclusion of these cases that can be considered as doubtful do not question the conclusions of the trial. Moreover, the results are more convincing, which can be explained by the exclusion from the analysis of the healthy cases.

- For FeLV infection, the test allows to check for antigenaemia. It is not possible to conclude that a clearance of the virus induced by the treatment with Virbagen Omega due to a common transient antigenaemia for this disease occurs.
- For FIV, the test allows to check for antibody, therefore clearance of the virus could not be detected. False positives on D0 or false negatives on D120 have to be considered. However, in the worst case (false inclusion), the conclusion of the study remains unchanged.

The apparent change of the serological status is probably the result of a transient antigenemia for FeLV disease and false negative results on D120 for FIV.

Data of clinical scores for the cats that were followed up on days 180, 270 and 360 were presented and analysed - although their numbers are relatively low. A statistical analysis of the time course of the relative clinical score (% reduction) was made on days 180, 270, 360 between groups A (140.04 treated) and B (placebo treated). This statistical analysis was not significant whatever the time point. This study period corresponds for the majority of the cats to an asymptomatic phase of the FeLV infection (chronic phase). The time course of the relative clinical score is significantly different

between groups during the symptomatic phase of the FeLV infection (from D0 to D120). Section 5.2. of the SPC was amended to reflect this.

Although more cats in the field trial tested positive to FIV, the mortality rates of FIV positive cats are lower than in the FeLV at least positive group of cats (FeLV at least positive includes FeLV positive and cats concomitantly infected with FeLV and FIV). The FIV positive group shows less difference in mortality between the placebo group and the interferon treated group. Nevertheless, since survival of FIV infected cats might be very long (up to several years), the duration of the observation period was not long enough (one year) for demonstrating beneficial effect of the product.

Survival length is not convincingly longer in the FIV positive interferon treated group compared to the FIV positive placebo group. The pharmacodynamic study conducted only demonstrates an effect against FeLV virus. The survival claim in the SPC is only made for FeLV infected or FIV coinfected cats.

Analysis of the Field Study

The field study in cats was analysed, using an All Randomised Study Animals (ARSA) analysis approach. There are several differences in the p-values obtained but there are only three changes of statistical significance:

- concomitant treatment: the difference in the antibiotic taken is no more significant in the ARSA analysis.
- four-months survival: the difference becomes significant in the ARSA analysis ($p = 0.0385$).
- time course of WBC count: the difference becomes significant in the ARSA analysis for cats with leukocytosis at D0 ($p = 0.0330$).

In conclusion, using the ARSA analysis, the results are even better since four-months survival became significant and the p-values of the other survival (six-months, nine-months, one year) are slightly reduced. On the whole, both methods gave similar statistical conclusions from a clinical and biological point of view.

Justification of anaemia as covariate

FeLV and/or FIV infections are known to induce anaemia in cats which is an important prognostic factor due to the difference in disease outcome. The latter is demonstrated by the criteria “mortality rate” at 4, 6, 9 and 12 months according to the anaemia status of cats on the first treatment day. A difference of mortality between cats with and without anaemia appeared clearly in both IFN and placebo groups. The anaemia parameter must be then considered as a source of variability which causes a loss of power in statistical tests. Statistical analysis of the survival and the time course of the total clinical score was then made with adjustment on covariate anaemia on day 0.

Analysis with and without the covariate anaemia

Survival

A comparison of survival between cats treated with IFN and placebo was presented at 4, 6, 9 months and one year. With adjustment, the differences between groups at 6, 9 and 12 months are significant. In the analysis without adjustment, they become not significant. The increase of the p-values is logical: an important prognostic factor is not taken into account and so the power of the statistical test is reduced. But the p-value keep weak values, next to the significance level of 5%.

Total clinical score

Results of the time course of the total clinical score between D0 and D120 are presented. Without adjustment, the model of repeated measures showed a significant difference between treatment groups ($p=0.0437$). With adjustment, the model of repeated measures already showed a significant difference between treatment groups with a very low p-value ($p=0.0064$). Anaemia does not seem

to be an important prognostic factor for the total clinical score, the difference between treatment groups is always significant.

OVERALL CONCLUSION ON EFFICACY

The activity study shows that activity of 2',5'-oligoadenylate synthetase, which is a parameter of antiviral activity, is induced in canine cells *in vitro* by adding rFeIFN, and to some extent *in vivo* by administrating rFeIFN. The antiviral activity of FeIFN is confirmed, both by an *in vitro* test (activity) and an *in vivo* trial (efficacy), even if its mechanism of action remains still somewhat obscure. No counter mechanism, by which some viruses are able to disrupt the antiviral mechanisms induced by IFNs, could be identified for FeLV.

Efficacy studies presented were conducted in the target species both cats and dogs. rFeIFN undoubtedly shows a beneficial effect.

For the dogs the posology and route of administration proposed in the SPC was tested: the dose of 2.5 MU/kg intravenously for 3 consecutive days gives the best results. For the cats the initial hypothesis (two series of 1 injection daily for 5 consecutive days) was not supported. On the contrary, three series of 5 injections of 1 MU/kg once daily subcutaneously are necessary to increase the survival probability up to 12 months, and to decrease the clinical severity of the FeLV and/or FIV infections in cats.

The SC route of administration in cats is supported by literature (currently used in humans for prolonged treatment of chronic diseases) and by the pharmacokinetic results, which showed that FeIFN properties are comparable to those obtained in humans treated with IFN-α, however, it is noted that information on IFN-ω remains very scarce. IFN-α properties appear to be very useful to assess IFN-ω properties because of the close relationship of IFN-ω mainly with IFN-α and because of the now general use of IFN-α/β in human therapeutics. The SC route is also very convenient for use in cats.

The efficacy was also investigated in presence of anti-CPV antibodies. Results are better in animals without antibodies. This is in line with what can be expected, as rFeIFN is thought to have an immunomodulatory activity rather than antiviral activity.

The SPC (section 5.2) reflects the fact that treated anaemic cats infected with FeLV and/or FIV showed a slightly greater mortality than treated non-anaemic cats.

Literature mentions the fact that cats can become refractory to continued treatment with high-dose IFN-α (apparently due to the development of neutralising antibodies). Such an effect was not seen during the field trial presented, when the appropriate posology and method of administration are respected.

V. RISK-BENEFIT ASSESSMENT

The data submitted confirms the acceptability of the proposed formulation and presentations and the suitability of the specification for the active substance. The construction of the recombinant virus and the use of silkworms in the production process have been well described. The stability tests provided for the finished product show that the product is stable for 24 months.

The product is considered compliant with the European note for guidance for minimising the risk of transmitting animal spongiform encephalopathy agents via veterinary medicinal products.

The toxicological studies in dogs confirm the general acute side-effects described in the literature: drowsiness, increase in body temperature, slight increase in sinus tachycardia and respiratory rate. These symptoms are rarely dose limiting and spontaneously dissipate after no more than a few days.

The safety studies in dogs show that rFeIFN alters the hematopoietic cell lines slightly and temporarily. Increases in body temperature, some vomiting and soft faeces may occur, especially with high doses, however interruption of treatment is not necessary. No neurotoxicity, hepatic or renal toxicity could be observed.

Safety studies in cats show that after intravenous administration, some additional adverse reactions were seen however, this route is not proposed for cats with this product. The studies also show that after repeated administration of high doses, mild to moderate side effects, with low frequency appear, as documented in the SPC, however interruption of treatment is not necessary. The other side effects of IFN-α treatment found in literature are not confirmed in these trials: no neurotoxicity, no hepatic or renal toxicity could be observed. The transient rise in the concentration of ALAT shown in two studies is mentioned in the SPC, section 5.4. The impact on the cat's eye remains open to interpretation as the number of animals involved is insufficient to make a definite conclusion.

No conclusion can be drawn on the mutagenic potential and teratogenicity effect of rFeIFN. They cannot be excluded, although placental passage of rFeIFn is probably low. The following warning has been included in Section 5.6 of the SPC: *'The safety of the veterinary medicinal product has not been established during pregnancy and lactation'*.

The immunological functions are not impaired after the use of rFeIFN. Antibodies anti-rFeIFN might appear in rare cases, but their clinical significance is unknown. As auto-immune secondary effects are known in humans following interferon administration and, in the absence of further long term toxicity data, the following warning was included in the SPC: *'No information on the induction of long-term side effects is available in dog and cat, specially for auto-immune disorders. Such side-effects have been described after multiple and long-term administration of type I interferon in man. The possibility of occurrence of auto immune disorders in treated animals cannot therefore be ruled out.*

All efficacy studies were conducted in the target species. rFeIFN undoubtedly shows a beneficial effect. The posology and route of administration proposed in the SPC was tested and a dose of 2.5 MU/kg in dogs for 3 consecutive days intravenously and 1MU/kg in cats subcutaneously for 5 consecutive days on 3 occasions gave the best results.

In conclusion, animals which are candidates for rFeIFN injections are seriously ill animals (parvoviral infection in dogs and cats with FeLV and/or FIV in symptomatic phase). A risk/benefit analysis clearly shows that all the side-effects, which are not systematic and remain moderate and transient, are of little importance with regard to a life-threatening infection. The product was efficacious as there was a reduction in mortality and the clinical signs of parvovirosis (enteric form) in dogs from one month of age. The antiviral activity of FeIFN in cats is confirmed, both by an *in vitro* test (activity) and an *in vivo* trial (efficacy). The SPC reflects the fact that treated anaemic cats infected with FeLV and/or FIV showed a slightly greater mortality than treated non-anaemic cats.

Based on the original and the complementary data presented, the Committee for Medicinal Products for Veterinary Use concluded that the quality, safety and efficacy of the product were considered to be in accordance with the requirements of Directive 2001/82/EC.