The opinions of this expert report are the considerations of Professor Sebesteny on CV 247 following his long-term experience with the product. The summaries presented should not be construed as demonstrating the safety or efficacy of CV 247 since the product as not received regulatory approval to date.

EXPERT REPORT ON CLINICAL DOCUMENTATION

CV247 is under investigation and its safety and efficacy have not yet been demonstrated

Prepared by:

Prof A Sebesteny BVSc, Dip Bact, MRCVS

Consultant at
Cancer Research UK and
Ludwig Institute of Cancer Research

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Problem Statement

A wide range of cancer therapies have been previously studied. These include physical agents such as gamma-radiation and chemical agents such as synthetically produced drugs as well as natural substances produced by the body e.g. interferon, tumour necrosis factor, hormones, growth factors and cytokines. However all treatments, to date, share a common problem, that their action is not limited to neoplastic tissue, but also to healthy tissue, especially those reproducing at a high rate, such as bone marrow, gastric or intestinal epithelium and skin. This results in a wide range of side effects which may be temporary and unpleasant (e.g. loss of hair, nausea) or result in permanent damage to vital tissues or organs (such as liver or kidney). For animals these side-effects severely limit the scope of treatment as the owner (being unable to communicate with them) may overestimate or may fear underestimating the discomfort or suffering involved and may opt for euthanasia at any stage.

Current Approaches to Animal Cancer

When cancer is diagnosed in pets and other animals, there are currently three options:

- Euthanasia
- Conventional treatment (surgery, chemo- or radiotherapy)
- Withholding treatment (other than tonics and painkillers) until death or until euthanasia seems inevitable even to the most determined owner.

In veterinary practice, many owners choose the first option, not wishing to subject their pet to the inevitable side effects and risks of conventional treatment, whilst a hard core of owners will take the third option for the same reasons.

Other Approaches to Treatment

In order to overcome the problem of side effects, various methods of targeting the treatment solely to neoplastic cells have been studied. The most obvious concept is the aiming of physical agents such as destructive gamma radiation to the tumour itself. This raises the problems of precision in aiming and of correct dosing of the rays together with finding and recognising all neoplastic tissues, some of which could have already spread to a wider diffuse area, or by transport via lymph or blood to more remote parts of the body. This is overcome in practice by use of various diagnostic and scanning methods, mostly requiring expensive, complex equipment and expertise in interpreting the results.

More sophisticated methods utilised include the coupling of the drugs, toxins and other agents (such as gene particles or repair enzymes) with antibodies, specifically recognising tumour cells and attaching themselves to them. Alternatively use can be made of inactivated harmless viruses that prefer rapidly dividing cells, or have a predilection of the very type of tissue from which the tumour arises, thereby delivering their agent only to the site where needed. These methods are being constantly refined and perfected, but are still fraught with difficulties (early

detachment of their active agent, false or failed recognition, detoxification of the agent en route, development of resistant tumour cells etc).

Alternative methods are constantly emerging, especially from those workers who believe that neoplasia is a condition arising from and involving the patient as a whole; therefore its treatment or prevention should involve the same ("holistic") philosophy. This approach is also substantiated by population studies highlighting the role of lifestyle, environment, toxic substances and feeding habits.

It is a widely accepted fact that from cells reproducing in the body some are frequently emerging with faulty, damaged or otherwise altered gene fragments and some of these could be potentially neoplastic. Other cells already present may be damaged in-situ by external insults (radiation, chemical carcinogens) or by internal insults from some byproducts of the body's metabolism (endogenous damage). These are normally recognised and eliminated by the defence mechanisms of the body such as immune responses, "killer" lymphocytes or phagocytic cells, or their faults are reversed by special gene (DNA) repair enzymes within cells, etc. (Such gene repair mechanisms are present even in primitive cells such as E. coli bacteria). The effectiveness of these functions is influenced by the general health status of the host, the integrity of other biochemical processes as well as the presence or absence of other specific factors. A single failure in recognising and dealing with a neoplastic cell could result in a tumour.

Rationale for the development of CV247

CV 247 is a treatment regime proposed for neoplasia involving mainly naturally occurring substances. It was developed on the theory that cancer involves the deficiency of certain essential naturally occurring substances in the body. It has been postulated that by replacing these substances CV 247 can bring about an improvement in the quality of life and in many instances regression of the tumour. The product is initially intended for use in dogs for which no other treatment option is possible, or authorised by the owner.
CV247 is a combination of 4 well characterised active components.

Vitamin C (Ascorbic Acid) Ph Eur
Manganese Gluconate USP
Copper Gluconate USP
Sodium Salicylate Ph Eur

CV247 is prepared as a solution for oral administration. The dose used both for veterinary and clinical use was based upon doses which might be administered when the individual components were taken either as dietary supplements or, in the case of sodium salicylate, as recommended as a therapeutic medicament. The dose ranges between 0.2ml/kg and 0.39ml/kg depending upon the subjects weight and where they fit into the dosing table.

<15lb	15-30lb	30-45lb	45-60lb	60-75lb	>75lb
1.5ml	3ml	4.5ml	6ml	7.5ml	9ml

The maximum dose per kg would thus contain:

97.5 microgram Mn (0.78 mg Mn gluconate)
109.2 microgram Cu (0.78 mg Cu gluconate)
15.6 mg vitamin C
13.65 mg Na salicylate

The scientific rationale for the inclusion of each component has been summarised in the Pharmaco-Toxicological Expert report and is repeated here as follows:

Manganese (Mn)

In both animals and man, Mn is essential trace element for normal brain functioning and for many ubiquitous enzymatic reactions, including hexokinase, superoxide dismutase and xanthine oxidase. Superoxide dismutases (SODs), are part of the defence mechanism against reactive oxygen species, and altered amounts of copper/zinc SOD and MnSOD have been implicated in multistage carcinogenesis in both rodents and man (Davis, 1999).
The catalytic reaction of SOD in detoxifying superoxide involves a redox reaction that utilises either copper (cytosolic and extracellular Cu/Zn SODs) or Mn (mitochondrial MnSOD). In the active site of the SOD enzymes, copper or Mn is alternatively reduced or oxidised by superoxide to produce hydrogen peroxide (which is then further metabolised by either catalase or glutathione peroxidase). MnSOD within animal tumours has been reported to be low (Markland,1982), and since both low Cu/ZnSOD and MnSOD have been reported to be associated with cancer susceptibility (Finley and Davis, 1999), the need to maintain adequate levels of Mn is important.
Studies have shown that even marginal trace element deprivation, including Cu and Mn, can significantly alter immunologic function and have been shown to affect the initiation and progression of a large variety of neoplasia.

Copper (Cu)

Copper, like manganese and zinc, is chemically classified as a transitional element in the periodic table. It's essential role in animals and man was first realised in 1926 after it was found to be required for haemaglobin synthesis in rats (Mann, 2000).
Copper can form complexes in which the metal serves as a central atom and as a result its function is closely associated with its binding to biological ligands, particularly in enzyme systems. Like manganese, Cu can adopt distinct redox states allowing the metal to play a pivotal role in cell physiology as a catalytic co-factor in the redox chemistry of enzymes, mitochondrial respiration, iron absorption, free radical scavenging and elastin cross-linking.
The importance of Cu can be attributed to its role as a co-factor in a number of enzymes that are involved in the defence against oxidative stress, and a deficiency compromises the anti-oxidant defence system of cells thus increasing their susceptibility to oxidative DNA damage (Pan, 2000). Copper containing superoxide dismutase (Cu/ZnSOD) has been found to be lower in malignant cell lines as compared to normal tissues (Marklund,1982), and reduced amounts of copper/zinc SOD and MnSOD have been implicated in multistage carcinogenesis in both rodents and man (Davis, 1999). Copper loading of rats has been shown to increase both liver

cytosol and mitochondrial SOD activity (Russanov, 1986). Copper gluconate has been claimed to have tumour inhibiting properties and has been used in high doses for the treatment of cancer tumours (Nieper, 1979).
Copper deficiency is known to impair immune function as a consequence of reduced production of both neutrophils and T lymphocytes. Copper deficiency is also known to inhibit the proliferation of T cells in response to mitogens (Stipanouk, 2000).

Vitamin C (Ascorbic Acid, AA)

Vitamin C is regarded as the most important anti-oxidant in mammalian cells. With the exception of primates, it is synthesised in animals from D-glucose or D-galactose via the glucuronic acid pathway.
As a strong reducing agent, vitamin C forms a part of the body's antioxidant defences against reactive oxygen species (ROS) and free radicals.
Oxidative stress, whether from an increased production of oxidants or from a failure of physiological anti-oxidant systems, can cause cancer (Bjelakovic, 2004), and consequently the use of anti-oxidant supplements is widespread. Ascorbic acid is the single nutrient supplement most commonly used by cancer patients (Block, 2003).
Cells involved in the immune response normally contain very high concentrations of vitamin C. Aging and chronic disorders, such as cancer, are associated with depression of immunity and are accompanied by depleted levels of both plasma and leucocyte vitamin C (Basu, 1996). Proposed mechanisms of action for ascorbic acid in the prevention and treatment of cancer include enhancement of the immune system (Head, 1998); notably it may promote chemotaxis, reduce allergic reactions and raise interferon production (Anderson, 1981).
Numerous, albeit uncontrolled, epidemiological studies in man, have pointed to the importance of both dietary and supplementary ascorbic acid in the prevention of various types of cancer including bladder, breast, cervical, colorectal, oesophageal, lung, pancreatic, prostate, salivary gland, stomach. leukaemia and non-Hodgkins lymphoma (Head, 1998).

Sodium salicylate (SS)

Sodium salicylate is a well documented and widely monographed, non-steroidal anti-inflammatory drug (NSAID).There is much current interest in the NSAIDs as possible chemotherapeutic agents.
Though SS and other NSAIDs maybe able to act independently of cyclo-oxygenase (COX) activity and prostaglandin synthesis activity (Tegeder, 2001), the salicylates, in general, are known to be able to exert an anti-inflammatory effect, by direct blocking of prostanoid synthesis from arachidonic acid, and by virtue of their inhibition of the COX enzymes. COX 2, in particular, is induced in response to cell activation by pro-inflammatory cytokines, growth factors and tumour promoters, and thus may have a pathophysiological role connected to inflammation and carcinogenesis.
It has been demonstrated that elevated levels of PGE2 (prostaglandin E2) are synthesised in tumour cells over-expressing COX-2, which results in secretion of growth factors such as VEGF (vascular endothelial growth factor) to induce angiogenesis. PGE2 also has several inhibitory effects on lymphocytes, including suppressing lymphokine-activated killer cells and cell-cell mediated tumour cell killing. It has also been postulated that cells over expressing COX are associated with

decreased expression of transforming factor-B2 responsible for inhibiting the growth of epithelial cells and also associated with decreased expression of E-caderin which is responsible for cell adhesion leading to invasion and metastasis. Tumours with higher COX-2 expression have a greater resistance to chemotherapy and radiotherapy and this over expression is increased by agents which activate tyrosine kinase signalling.
Thus COX 2 or PGE2 inhibition would be anti-angiogenic, and could stimulate anti-tumour immuno-surveillance (Simmons, 2001). Though salicylates have been reported to be only weak inhibitors of both isolated COX 1 and COX 2, they are potent inhibitors of prostaglandin (PG) synthesis in intact cells, and salicylate, or its metabolites, may selectively inhibit PGE2 synthesis (Graham, 2003).
The COX 2 tumour hypothesis has been supported by various experimental animal models but does not preclude the role of other factors, such as the role that COX 1 plays in carcinogenesis, and the fact that SS prevents nuclear factor kappa B activation and hence can cause tumour cell apoptosis (Wu, 2001).

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Justification for the selected doses

Three of the ingredients of CV247 are commonly taken as supplements, and recommended daily allowances (RDA) or similar, have been published. However RDAs is the average daily dietary intake level that is considered sufficient to meet the nutrient requirements of the majority of healthy individuals. In cases where there is either a deficiency of the supplement or there is a need to enhance the level, such as in certain chronic diseases then there is sometimes a need to increase these allowances to well above that recommended. The following is based upon doses recommended in man and animals

Vitamin C

Ascorbic acid has been used extensively as a supplement in both animals and man.
Several studies have stated that doses of Vitamin C well above the RDA are required to be protective against cancer (Fleischauer, 2001). It has been suggested that Vitamin C must be used continuously and indefinitely in the treatment of cancer (Cameron, 1991), and that protection is better derived if consumed as a complex mixture with other nutrients (Byers, 1995).
The current RDA for man is 60mg/d (0.86mg/kg based upon an average 70kg man), but this is based upon the mean level of 46mg/d to prevent scurvy. A level of 90-100mg/d (1.43mg/kg) is considered to more appropriate in healthy subjects to reduce the risk of chronic disease (Carr, 1999), and up to 200mg/d (2.86mg/kg) to enhance the immune response. Studies in man have shown that 10g/d (142mg/kg) may be taken for years without ill effect (Bendich, 1995). The dose of Vitamin C in CV247 equates to between 8 and 16mg/kg, which would not be considered harmful to man, and is aimed to enhance levels in immunologically compromised cancer patients. Though most animals endogenously synthesise ascorbic acid to levels which are homeostatically controlled, in those suffering from cancer it is likely that the same deficiencies exist as for man and that supplementation with doses of vitamin C up to 16mg/kg will have no harmful effect.

Copper gluconate

There are a plethora of copper containing veterinary compounds available at doses considerably in excess of those proposed on a daily basis for CV 247.
The Feeding Stuffs Regulations (Statutory Instrument 2000 No 2481) allows a maximum content of copper between 15 and 175 mg/kg of feed depending on age and type of animal.

There is no RDA for Cu in man, but the National Research Council has stated that the estimated safe and adequate daily dietary intake (ESADDI) is up to 3mg (43 microgram/kg based upon an average 70kg man). Most diets do not furnish this amount even for healthy subjects, and that therefore supplementation is to be desired. Cu gluconate is regarded as the safest Cu salt and therapy with up to 9mg/d (130 microgram/kg) have been used for several months without ill effect. The dose of Cu gluconate in CV247 equates to up to 110 microgram Cu/kg, which is not considered toxic to animals, but would enhance the Cu loading to increase Cu/Zn SOD activity in Cu deficient animals with cancer.

Manganese gluconate

Normal daily intake of manganese for healthy dogs is considered to be around 2.3 mg for every pound of dog food eaten (on a dry matter basis). The Feeding Stuffs Regulations lists maximum content for manganese in mg/kg in complete feeding stuffs. For manganese carbonate, chloride, oxide and sulphate 250 mg/kg are allowed. This is considered higher than in the context of the amount of manganese proposed per daily dose of CV 247.
The Salt Institute website provides information on manganese requirements for a range of animals. In particular references suggest a levels of 50 ppm of total diet have been routinely supplied to dogs**.**

The ESADDI for Mn in man is 2 to 5mg/d (up to 71mg/kg in average 70kg man), though individuals have been reported to consume more than 10mg/d without apparent ill effect. Toxicity with Mn salts is mainly as a consequence of parenteral administration, and animals show a high resistance to oral Mn toxicity. It is not considered that a dose of up to 100 microgram/kg in CV247 is a toxicological risk.

Although studies on the interaction of trace metals and cancer have not been carried out to quantify the trace element requirements in response to a neoplastic challenge, the doses of Cu and Mn selected for use in CV247 are in keeping with their known properties, their toxicological profiles and the likely requirements for supplementation in cancer patients, whether animal or man.

Sodium salicylate

Sodium salicylate is monographed in both clinical and veterinary pharmacopoeias as an anti-inflammatory drug. In man the recommended dose is up to 650mg every 4 hours, and in some case up to 5.4g/day. This equates to a maximum daily dose of 77mg/kg (based upon an average 70kg man), approximately 5-10 times the dose being used in CV247. It is considered that the dose is high enough to have the desired therapeutic effect without being toxic to either animals or man.

Data presented in support of the application

Data has been presented from 2 sources, firstly, pharmacology and clinical studies undertaken on the combination CV247, and secondly information derived from the literature on the individual components. The literature derived pharmacology data from animals other than man has been included in Part III of the application, whilst that data from studies in man has been included in Part IV.

CLINICAL PHARMACOLOGY

Two studies were undertaken to ascertain the antitumour properties of CV247.

1. A preliminary study to test the effect of CV247 on the rate of growth of implanted RMA thymoma cells in C57BL/6 mice.

A total of 50 male C57BL/6 mice were each injected subcutaneously with a dose of RMA thymoma cells known to give 100% tumour "take". 24 experimental mice were treated with 0.1 ml of CV 247, administered once daily by gavage, whilst 26 control mice received water also by gavage. Experimental mice also received CV247 in their drinking water at 0900, 1700 and 2000 hr each day. All mice were maintained on a standardised diet. Tumour diameters were measured twice daily and all mice were sacrificed on day 17, after which tumours were excised and weighed.

There was no significant difference between experimental and control animals during the early time points, but there was a statistically significant difference in the size of the tumours measured on day 17 (median 0.53cm for the experimental animals compared with 1.31cm for the control group, p=0.012) and also for the weight of the excised tumours (median 0.4g for the experimental group compared with 1.0g for the control group, p= 0.0025).
In addition, at day 17, 4 tumours were too small to measure in the animals treated with CV247, compared with only 1 in the control group. In a number of mice more than 1 tumour grew along the injection needle tract, but this was considerably more frequent in the control group (10 mice) compared with the experimental group (1 mouse). In 3 control mice, tumours could not be excised because they were infiltrating deeper tissues.
There were no side effects attributable to CV247.

Group	Size(cm)	Size(cm)	Size(cm)	Weight(g)
	day 13	day 15	day 17	day 17
CV247	0.22	0.39	0.63	0.46
Control	0.23	0.69	1.14	0.92

At the doses used in this controlled study, CV247 demonstrated a measurable effect on the growth rate of RMA thymoma in mice. CV 247 did not prevent, but reduced the growth of tumours in the treated group in comparison to the controls.

2. A Study to investigate the anti-cancer potential of CV 247 and its constituents dosed to C57BL mice bearing a syngeneic tumor

This study assessed the anti-tumour activity of CV247 and combinations of the formulation's constituents against LL2/LLc1 tumours in C57BL mice. Lewis Lung Carcinoma (LLc) is a highly metastatic and drug resistant murine non-small cell lung (NSCL). The effect of the agents was compared to untreated control animals bearing tumours. Gemcitabine (Gem), a highly active anti-tumour agent, was used as a positive control. All treatments were assessed over a period of 2 weeks.

Macroscopic examination of tumours on excision revealed differences in tumour structure between treatment groups. Tumours treated with CV247 and SS + AA + Mn appeared to be fluid filled and spongy in comparison to the untreated controls and other component groups. Gemcitabine treated tumours were slightly smaller and did not appear to contain much fluid. SS+AA+Mn appeared to have some effect in reducing tumour volume (growth), but significant differences in tumour weights between CV247, Gemcitabine and the untreated controls were observed. This reduction was most marked for Gemcitabine, and though not significant, was more apparent for CV247 than its components, notably sodium salicylate alone. Microscopic examination was less conclusive. All treatments, when compared to the untreated mice, suggested a similar positive, though insignificant, benefit with regard to intra-tumoural necrosis, but not intra-tumoural inflammation.

Mean group scores for the intra-tumoural necrosis

Untreated	1.7
Gemcitabine	1.11
SS + AA + Mn	1.25
SS + AA	1.37
CV247	1.2

This study demonstrated potential for CV247 to be an anti-tumour agent. The decrease in final tumour weight indicates a mechanism for tumour reduction that is not necessarily related to tumour volume. Speculatively it may be suggested that an immunological process is initiated that results in breakdown of the tumour core, therefore reducing the weight (but not size) of LL2/LLc1 carcinomas. The LL2/LLc1 cell line is particularly aggressive, which may explain why Gemcitabine did not demonstrate significant efficacy in this model.

Pharmacokinetics

A Pharmacokinetic assessment of CV247 as part of a target animal safety study following oral administration to Beagle dogs for 26 weeks

A pharmacokinetic assessment of sodium salicylate has been carried out as part of the species tolerance study conducted at Huntingdon Life Sciences. In addition manganese and copper levels were analysed at intervals to assess any changes as a consequence of repeat dosing of CV247.

Each test animal received oral doses of CV 247 once daily for 26 weeks. Test animals received the formulation either as 0.44 ml/kg/day or 0.88 ml/kg/day.
Samples of venous blood (5 ml) were withdrawn from the jugular/cephalic vein of two male and two female animals in each group on day 1 and during weeks 6, 13 and 26, at pre-dose and 1,2,4,6,8,10,12 and 24 hours after dosing.
Pharmacokinetic processing of the data indicated that the rate and extent of systemic exposure of the dogs to sodium salicylate increased with increasing dose, although the increases were less than the proportionate dose increment for Cmax. For AUC the increases appeared to be proportional to the dose increment on day 1 and during week 6 but slightly greater than the dose increment during weeks 13 and 26.
The rate (Cmax) and extent (AUC_{24}) of systemic exposure of female dogs to sodium salicylate were generally similar to those indices of exposure in male dogs.
After repeated oral doses (weeks 6, 13 and 26) the extent (AUC) of systemic exposure to dogs to sodium salicylate were generally less than or similar to, those values after a single dose except for females administered the 30.8 mg/kg/day dose during weeks 13 and 26, when exposure was higher than on day 1.
In conclusion, the extent of systemic exposure of dogs to sodium salicylate appeared to be characterized by linear (dose independent) kinetics over the dose range 15.4-30.8 mg/day.
The plasma levels of copper and manganese were analysed at intervals throughout the study. An increase in copper in males of both the high and the low dose groups from day 1 during week 6 and 26 was observed. At the low dose level, an increase in manganese in both sexes from day 1 to week 26 was observed. In the high dose level, an increase in manganese in both sexes from day 1 during weeks 6, 13 and 26 was observed. However individual results were variable and increases were not considered significant.

CLINICAL STUDIES

Two studies of CV247 have been undertaken in dogs

A Clinical Study of CV247 for the Treatment of Cancer in Dogs

The study objectives were to assess disease response, quality of life, and tolerability to CV247 in dogs with diagnosed cancer.
The study was designed as an open label evaluation of the effectiveness and tolerability of CV247 in 50 dogs with malignant disease over a 6 month period.
Dogs were to have uncontrolled progressive disease at trial entry, ideally histologically proven. CV247 was administered orally using a syringe, at a dose level range, which was weight dependant, and continued daily until the point where either the owner wished to withdraw, or the investigator recommended withdrawal due to disease progression, or death. Though the investigative period was for 6 months, dogs could remain on the medication indefinitely, if benefit continued. Owners were required to ensure that their pets adhered to a diet which provided high quantities of nutrients, vitamins and minerals but which avoided so far as was possible, artificial colourants, flavourings and preservatives.
The primary efficacy assessment was made by the investigator using a 10-point quality of life check list. This was based upon both the investigators observations and

the owner's quality of life questionnaire. The latter included questions relating to general appearance and activity levels.

1. Poor. Dull, listless and moribund
2. Poor. Marginal improvement on 1.
3. Below average. Eating and drinking
4. Below average. Marginal improvement on 3
5. Satisfactory. Good appetite
6. Above average. 5+Animal is alert
7. Above average. Marginal improvement on 6
8. Good. Active with good exercise tolerance
9. Very good. Health restored to previous level
10. Excellent. Health exceeding that before illness

Tumour regression or progression was either observed or measured. When not measurable, tumour status was judged by changes in symptoms. Life expectancy was a subjective judgement made from the time of diagnosis, taking into account age and disease severity.
A global judgement of tumour status for each animal, was made by an independent veterinary expert on completion of the trial and recorded as either disease progression, stabilisation or regression. For the purpose of analysis, the degree of change observed in quality of life was separated into 3 categories, no change, significant change and highly significant change. A significant change equated to a difference of 2 from the start of the study to termination/withdrawal, whilst a difference of 4 constituted a highly significant change.
Adverse events were recorded following both spontaneous declarations by owners at the clinic visits and by direct questioning by the investigators.

A total of 53 dogs with a wide variety of cancers were recruited (25 female and 26 male, 2 were not recorded) with a mean age of 9.8 years (range 3-17). The total duration of dosing with CV247 varied between 1 and 30 months with a mean duration of 7.4 months per animal.
A quality of life score was recorded for 48 animals at the start of the study and 50 upon completion at 6 months, or earlier if the dog was withdrawn. No dog had a lower score for quality of life upon completion or termination. No improvement was recorded for 15 (28%) animals; whilst 38 (72%) dogs showed a marked improvement in quality of life, with 29 (55%) having an increased score of 4 or more. There was no clear trend for response according to cancer type, though more dogs with carcinomas had a highly significant change (4 or more) in quality of life score, compared to animals with other cancers.
The combined data from the primary and secondary end-points was assessed by the investigators and subsequently independently examined and verified by an expert veterinary surgeon, who assigned an overall disease response to CV247 summarised as follows:

disease progressed (worsened)	15 (28%)
disease stabilised	25 (47%)
disease regressed (improved)	13 (25%)

This open label study was designed to evaluate the properties of CV247 in treating dogs with malignant disease, with particular regard to effects on quality of life and disease stabilisation over a 6 month treatment period.

A total of 53 dogs with progressive malignant disease were recruited into the study, all of whom were required to have a diagnosis verified by histological evidence. Though this was sometimes obtained, it was found that in the majority of cases the investigating veterinary surgeons preferred to use This was in most cases obtained, but when it was not available or possible, investigating veterinary surgeons had to use their professional judgement based on clinical signs and symptoms, if possible supported by other clinical tests (haematology, biochemistry, X-ray, CT, ultrasonics etc) so as to avoid traumatising already very sick animals. Dogs with both carcinomas and other cancer types were recruited. It was not always possible for owners to visit the clinic at the required 2 weekly intervals, and thus data was not available for some dogs with that level of frequency.

The primary objective of the study was to evaluate any quality of life changes in the recruited animals, all of whom had a guarded or poor prognosis on entry, and for whom, euthanasia would often have been the recommended course of action, so as to avoid unnecessary suffering. It was considered that for the majority of owners, the most important requirement for treatment was to reduce suffering and improve well-being. In dogs such improvement is easily recognised, and the quality of life questionnaire included in the protocol for completion at regular intervals by the owners, was designed to identify any such changes. The questionnaire was then used as a basis, together with any signs of clinical improvement, for the investigators to assign a quality of life score using the 10 point scoring system referred to in the study design. A comparison of the scores between entry and study completion suggests that CV247 promoted an improvement in quality of life in over 70% of the dogs recruited into the study, sometimes significantly so, even if only for a brief period before the animal succumbed to their disease. For many dogs, however, this improvement in well being was apparent for many months, and though estimates of life extension was not undertaken on entry into the study, it appeared that lives were extended in a number of animals.

Of more significance with regard to the study objectives, was an evaluation of disease stabilisation. This was assessed by each investigator, taking into account any changes in quality of life and tumour regression. The latter was determined at intervals, either by physical measurement, tumour palpation, and/or clinical symptoms. The assessment of disease stabilisation was subsequently independently evaluated by a veterinary expert, the outcome of which, showed that disease progressed in 15 (28%) of the dogs recruited, was stabilised in 25 (47%), and was found to regress in 13 (25%) of the animals treated with CV247. There did not appear to be a trend with regard to dog type, gender, age, disease type or prognosis. The recommended diet was followed closely by the owners of all the dogs in the study but was not observed in the context of this study to affect the outcome of treatment.

CV247 has been evaluated in man and has been found to have a minimal side effect profile, usually confined to mild gastro-intestinal events. In this study there were no reports of any adverse events.

This open study was undertaken to assess the effect of CV247 in treating neoplasms in dogs (and cats). A total of 51 dogs were recruited into the study. Again all cases were independently monitored and were firmly diagnosed by Veterinary Hospitals, independent veterinary surgeries or laboratories.

A number of different parameters were monitored during the course of the study and the effectiveness of treatment was analysed against:

1. Tumour regression or remission after surgery
2. Extension of life beyond forecast.
3. Quality of remaining life.
4. An Overall success factor

Tumour regression was either be observed or measured or both
The difference between life expectancy, as agreed by an independent panel for each dog, and actual life span was used as the measure of life extension beyond forecast.
Quality of life was determined using the same hierarchal scale referred to in the first study discussed.
A subjective assessment of the overall effectiveness of the treatment was made by reference to all measures including tumour regression, life expectancy and quality of life.

The duration of treatment ranged between 0.5 and 25 months.

Duration of treatment in months	total number of dogs
0.5-3	20
4-6	12
7-9	9
10-12	2
>12	7

No adverse effects were reported.

Of the 51 dogs that form this study 38 (74.5%) exhibited an increase in their life expectancy beyond that which would have been a "reasonable expectation" at the time the prognosis was made. The mean total life beyond total expectancy was 44.2 weeks for all dogs for all cancer types. The average life extension for dogs with carcinomas was 27.2 weeks, which was worse than those with sarcomas (55.4 weeks). For those dogs with sarcomas, then mean life extension was longer for soft tissue sarcomas (70.3 weeks) than for hard tissue cases (10.7 weeks).
A total of 17 tumours were observed to have regressed as tabulated below.

tumour type	total presenting	total regressed
sarcoma	21	5 (24%)
carcinoma	24	9 (38%)
lymphoma	3	2 (66%)
melanoma	2	1(50%)

There is also evidence to show that some tumours did not regress nor reduce but were held in abeyance.
Of the 51 dogs treated, 2 had inadequate records, but only 4 dogs showed no improvement in quality of life scores, leaving 45 (88%) that showed an improvement.
A rating of 8 and above (good or better) is considered the normal life style for the majority of animals whilst a rating of 10 is considered excellent and provides a life style not previously seen in the animal.
The table below shows the number of cases that scored 8 or above during treatment with CV 247.

	Quality of life good or better (8,9 &10)	Quality of life Excellent (10)	Sample size
Dogs	34 (66.6%)	6 (11.8%)	51

The Veterinary panel met as a body and considered each case in the study to assess the overall effectiveness of treatment. Their findings are shown in the following table.

	Success	Qualified Success	Failure (inconclusive)
Dogs	19 (37.3%)	18 (35.3%)	13 (1) (27.5%)

From the data presented it was concluded that CV 247 has a beneficial effect on the quality of life of most canine cancer patients. Its antitumour effects were observed in a number of dogs though it seemed to be more effective against those arising from, or invading, soft tissue than hard (such as bone).
In the majority of cases it was considered that animals experience an extended life span from that predicted at the time of diagnosis. In addition there was a quantitative increase in the quality of life assessment.
CV247 appears to be a very safe treatment without any adverse events recorded.

Global Analysis of Safety and Efficacy in dogs

A total of 104 dogs have been treated with CV247 for between 0.5 and 30 months (mean 6.9) in the 2 clinical studies. There were no reports of any adverse events.
The dogs were presented with a wide variety of tumour types, often in a poor state of health with a reduced quality of life. Most were given a guarded or poor prognosis. Though the earlier assessment examined a variety of endpoints, the primary endpoint in both studies was quality of life. In this regard, a comparison of the scores at the beginning and end of treatment clearly showed an improvement in a significant number of animals which suggested that considerable benefit could be derived from treatment with CV247, even if only for a limited period. For some dogs the improvement resulted in a return to a level of life quality equal to that experienced prior to their illness taking hold. As a minimum this would include all dogs for whom

a change in the quality of life score was 7 or more, i.e. 29% of all dogs recruited as tabulated below:

Change in quality of life scores	Total number of dogs (study 1)	Total number of dogs (study 2)	TOTAL
9	0	3	3
8	2	11	13
7	11	3	14
6	6	4	10
5	5	5	10
4	6	4	10
3	6	6	12
2	3	6	9
1	1	3	4
0	8	4	12
not recorded	5	2	7
TOTAL	53	51	104

Other Clinical Data

CV247 has been used to treat cancer in man and has been the subject a pilot study and a retrospective assessment following use in "named patients". It is also the subject of 2 on-going clinical trials. A summary of the available data is included below.

Does CV247 with dietary advice influence quality of life and malignant progression ? (Study CV247-2)

This pilot study was designed to evaluate the tumour static properties of CV247.
A total of 37 patients with progressive malignant disease were recruited into the study, 11 with colo-rectal cancer, 2 with ovarian cancer, 14 with prostate cancer and 10 with miscellaneous cancers. Though no patient showed any marked improvement using the Rotterdam Symptom checklist, based upon the period of treatment as a marker of clinical benefit, then a total of 15 patients were treated for periods of between 6 and more than 22 months as summarised in the following table:

Cancer	**Pat Nos**	**Av Age**	**Range**	**Duration**	**Range**
Colo-rectal	11	62yr	43-73	4m	2-7
Prostate	14	73yr	58-79	7.8m	2-22+
Misc	10	54yr	50-82	4.6m	1-10
Ovarian	2	57yr	57-58	14m	9-19
Total	37	67yr	43-82	6m	1-22+

Stabilisation of biomarkers notably in 7 of the prostate patients and 1 of the ovarian cancer patients supported the clinical benefit. It was particularly noteworthy that 4 of the patients with progressive prostate cancer were continuing to receive treatment with CV247 after the study was terminated, and that of these 4, 3 had received no previous treatment for their disease. CV247 was very well tolerated with only a

minimal side effect profile attributable to the drug. There were no serious side effects. Twelve patients died during the study, 7 with colo-rectal cancer. All deaths were as a consequence of the disease and were not related to the test medication.

A randomised double blind Phase II study of CV247 versus sodium salicylate in patients with early progressive prostate cancer (Study CV247-3)

The objective of this ongoing NCRN sponsored trial is to test the anti-neoplastic properties of CV247 in a large cohort of men with early stage, progressive prostate cancer, and to compare the properties of CV 247 with sodium salicylate alone so as to evaluate the specific benefits of adding copper, manganese and ascorbic acid to sodium salicylate.
The trial is a randomised, double blind, phase II study. Patients eligible for trial entry are required to have progressive disease measured by two consecutive increases in PSA greater than 20%, over a 3-month period. Patients are reviewed at 1 month for toxicity assessment and at 4, 7, 10, 12 and thereafter every 3 months for safety and efficacy assessment. Patients still exhibiting progressive disease at 4 or more months will be offered CV247 (open phase) regardless of the original medication allocated, or conventional managements according to West Anglia Cancer Network Guidelines depending on their preferences and disease severity.
101 patients have been recruited to date. 31% have been stabilised with either CV247 or sodium salicylate for 12 months or more. The majority of patients who have been withdrawn, have ceased treatment at 4 months. More than 80% of these patients had received previous therapy, mainly radiotherapy or hormones, or both.
There have been no reports of serious adverse events attributable to the test medications.

Does CV247 influence the quality of life and malignant progression in patients with cancer who have completed conventional treatment? An open prospective Phase II study. (Study CV 247-4).

This is an ongoing open label prospective Phase II study during which up to 40 patients diagnosed with progressive malignant cancer, will be assessed at monthly intervals**.** Patients recruited have either completed conventional therapy or declined such treatment.
The primary objective is the evaluation of the Quality of Life using The European Organisation for the Research and Treatment of Cancer QLQ - C30 (EORTC QLQ - C 30) version 3 questionnaire**,** and tolerability of CV247 in patients with advanced malignant disease, initially observed over a six month period.
Data derived from the study will be assessed to see whether the patients' symptoms and / or quality of life had improved, stabilised or worsened. Comparison will be made with anticipated outcomes in similar patients based both upon experience. The Hospice and published observations. 34 patients with a variety of cancers have been recruited to date, of whom, 9 are continuing treatment beyond the 6 month period.
An interim analysis of the duration of treatment has shown that:

16 patients (47%) have been withdrawn (8 within 1m)
18 patients (53%) ongoing (3 for 1m, 7 > 6m)
21 patients were/are in the study for 2m or more

Patients who have been withdrawn include 4 for adverse events (indigestion, bad taste and "bloatedness"), 2 patients have died, and 10 for poor protocol compliance (failure to attend more than one clinic visits), worsening symptoms, or hospitalisation.
An interim assessment of changes to quality of life has been made by evaluation of the global assessment scores for overall health and quality of life from the EORTC questionnaire. A score at the last clinic visit at least 2 greater than that on study entry for the patients in the study for 2 or more months has been classified as an improvement. A score of +/-1 between the 2 visits has been classified as no change (stabilisation), whilst a score at least 2 less is deemed as being worse. The results to date are:

Improved (score at last visit >2 compared with score at entry) 9 (41%)
Same (score at last visit +/-1 compared with score at entry) 12 (55%)
Worse (score at last visit <2 compared with score at entry) 1 (5%)

A retrospective analysis of the safety and efficacy of CV247 used on a "named patient" basis

Over a 5 year period, 109 patients in the age range 14-85 have been treated with CV247 on a compassionate basis, under the medical supervision of a specialist in palliative care who generally saw each patient on a monthly or two months basis.

31 of the patients did not progress with treatment beyond 3 months as they were too far advanced in their disease and could not cope with the journey to the clinic.
Of the remaining 78 patients, 48 patients (61%) were on CV247 between 3 months and 1 year, 13 patients (17 %) were on CV247 for 1 – 2 years, and 17 patients (22 %) have been taking the CV247 for 2 years or more.

Cancer	**Pat Nos**	**3m or <**	**6m or >**	**12m or >**
lung	20*	7	11 (55%)	7 (35%)*
mesothelioma	4	1	2 (50%)	2 (50%)
ovarian	12	6	4 (33%)	0 (0%)
prostate	16	2	10 (63%)	6 (38%)
bowel	11*	3	7 (64%)	1 (9%)*
breast	15	6	7 (47%)	4 (27%)
renal	7*	1	3 (43%)	2 (29%)*
pancreatic	5	1	3 (60%)	2 (40%)
oesophageal	4	3	0 (0%)	0 (0%)
others	17	4	10 (59%)	5 (29%)
TOTALS	**109***	**34 (31%)**	**57 (52%)**	**27 (25%)***

* 1 patient with bowel and 1 with renal cancer had lung metasteses, and though counted in both the lung, bowel and renal rows are not included twice in the totals.
Others include, thyroid, bladder, liver, endometrial, cervical, sarcoma, stomach, astrocytoma, gall bladder and glioblastoma.

A 10 point scale was devised to assess the response to CV247. Of the 48 patients on CV247 for 3 months to 1 year, 31 patients (65%) scored 6 or above i.e. significant symptom improvement or disease stabilisation, beyond what was expected and this

could often not be accounted for by other modes of treatment (eg., steroids or radiotherapy). Of the 30 patients on CV247 for 1 year or more, all scored 6 or above.
In the whole group of 78 patients who took CV247 for more than 3 months, there were 21 patients (27%), where there was some evidence that the disease had stabilised, regressed and even disappeared in some cases. Overall, in 60 patients (77%) there was evidence of symptom burden reduction, suggestive of disease stabilisation.The most startling responses were several cases of lung malignancy where patients lived 2 years or longer with excellent quality of life.
Other symptoms over the course of the study that showed most improvement were pain and general energy levels.
Adverse events were minimal. The only people who found the medication hard to tolerate were those who were already in the very terminal phase with difficulty eating and drinking generally. 2 patients experienced mild nausea or indigestion and stopped treatment, though these were not serious.

In the absence of sequential objective testing, in terms of scans and blood tests, it is difficult to definitely conclude that symptom reduction was indicative of disease stabilisation, but in a significant proportion of patients on CV247 for longer than 3 months, there was an improvement in symptom burden, general quality of life and in several patients, significant life extension beyond normal prognostic projections. It is of note that in the group of 21 patients who did exceptionally well, there were several who had never had any conventional treatment with chemotherapy or radiotherapy. The remainder of this group had ceased conventional treatment by the time CV247 was commenced**.**

Global Analysis of safety and efficacy in man

Completed and ongoing studies in man have recruited a total of 281 patients with a variety of cancers, and treated with CV247 for between 1month and 60 months

Cancer	named pats	CV247-4	CV247-3	CV247-2	TOTAL
lung & meso	24	7	-	-	31
ovarian	12	3	-	2	17
prostate	16	7	101	14	138
colo-rectal	11	2	-	11	24
breast	15	7	-	2	24
other	31	8	-	8	47
TOTAL	109	34	101	37	**281**

Of the 4 studies reported above, 2 are ongoing and therefore conclusions cannot be drawn as the level of efficacy, though an initial observation derived from the duration of treatment would suggest that some patients are deriving benefit, including quality of life, for extensive periods. From the observations made in the pilot study and from the named patient experience, it would seem that certain cancers might respond better than others, notably lung and prostate, though the numbers are too small to be conclusive. It does seem, however, that previous treatment notably chemo and radiotherapy results in patients being less responsive to CV247.
There have been no reports of serious adverse reactions to CV247. The only adverse events that could be attributed to CV247 have been the occasional complaint of

abdominal discomfort, including gastric irritation, bloatedness and nausea, the majority of which have been mild in nature.

PHARMACOLOGY AND CLINICAL DATA FOR THE COMPONENTS OF CV247 DERIVED FROM THE LITERATURE

Manganese

In both animals and man, Mn is essential trace element for normal brain functioning and for many ubiquitous enzymatic reactions, including hexokinase, superoxide dismutase and xanthine oxidase. Superoxide dismutases (SODs), are part of the defence mechanism against reactive oxygen species, and altered amounts of copper/zinc SOD and MnSOD have been implicated in multistage carcinogenesis in both rodents and man (Davis, 1999).
Animals deficient in Mn can be characterised by impaired insulin production, alterations in lipoprotein metabolism, an impaired oxidant defence system, peturbations in growth factor metabolism, and, in early development, pronounced skeletal abnormalities (Keen, 1999). In man it has been calculated that the theoretical mean dietary level of Mn required to maintain positive balance is 3.5mg/d or 50 microgram/kg (Freeland-Graves, 1988).
Normal daily intake of manganese for healthy dogs is considered to be around 2.3 mg for every pound of dog food eaten (on a dry matter basis). The Feeding Stuffs Regulations lists maximum content for manganese in mg/kg in complete feeding stuffs. For manganese carbonate, chloride, oxide and sulphate 250 mg/kg are allowed. This is considered higher than in the context of the amount of manganese proposed per daily dose of CV 247.

Absorption

Homeostatic mechanisms, which primarily involves the liver, limit the availability of the Mn absorbed from the gastrointestinal tract.
Uptake of dietary Mn in rats is controlled by several dose dependant processes, including biliary excretion, intestinal absorption, and intestinal elimination (Anderson, 1999). Dietary fibre supplementation in rats decreased apparent absorption and significantly increased excretion of both Mn and copper in faeces, the extent dependant upon the type of high fibre supplement (Gralak, 1996).
Mn absorbed in the divalent form from the gut via the portal vein is complexed with plasma proteins such as gamma-2 macroglobulin, which are efficiently removed by the liver. Mn may also be oxidised by ceruloplasmin to Mn3+. Trivalent Mn binds to the iron carrying protein transferrin, which is not as readily removed by the liver (Andersen, 1999).
Absorption in man follows a biphasic pattern of uptake with an initial transient phase followed by steady state conditions. Uptake versus Mn concentrations showed saturation type kinetics. The transport characteristics in steady state conditions exhibited 2 components, saturable and nonsaturable resulting in absorption efficiency falling as the dietary level of the mineral increases. It is evident that efficient mechanisms operate to maintain Mn homeostasis regardless of dose or diet and suggest that doses as high as 20mg/day do not result in any signs of toxicity in healthy adults (Finley, 2003).

High dietary iron intake has been reported to inhibit Mn absorption (Davis, 1992), whereas Mn uptake is upregulated by iron deficiency and mediated by divalent metal transporter 1, an iron regulated factor known to play a role in dietary iron absorption (Heilig, 2005). Iron status, however, has not been shown to affect Mn absorption; rather it is serum ferritin concentrations found that it is strongly associated with the amount of Mn absorbed (Finley, 1999).
In man the fractional Mn absorption from human milk is considerably higher than from cows milk or soy formula (Davidsson, 1989), only the addition of calcium resulted in a significant decrease in Mn absorption (Davidsson, 1991).There is no effect of ascorbic acid on Mn absorption (Davidsson, 1995).

Distribution

Following absorption, Mn is thought to bind to gamma-2 macroglobulin for delivery to the liver. Because Mn can be oxidised to the Mn3+ state it can bind to transferrin for subsequent delivery to other tissues
Transferrin has been proposed as the mobilisation protein for both iron and Mn. in the hypotransferrinemic (Hp) mouse mutant model, the absence of transferrin was found to be associated with an accumulation of abnormally high levels of Mn in the liver, though distribution to the heart, brain and sternum was not affected, suggesting that another transport mechanism exists (Dickinson, 1996). It has been reported that Mn can cross the blood brain barrier possibly by both a saturable divalent Mn transport and transferrin conjugated Mn transport system (Takeda,2000). Pharmacokinetic studies have shown that the Mn salt will also determine the rate of transport into the brain with Mn chloride being greater than Mn sulphate, which in turn is greater than the phosphate salt (Erikson, 2002).
It has been found in rats that the liver concentration of Mn was not affected by the the form or route of administration, but Mn concentrations were significantly increased in the cortex and cerebellum following gavage, than after the intraperitoneal or intratracheal routes (Roels, 1997).
Within the CNS, Mn accumulates primarily within astrocytes (Aschner, 1999).
Distribution in rat brains has been found to gradually decrease from 15 days to 60 days. The biological half-lives of Mn was determined to be 51-74 days with the longest being those in the hypothalmic nuclei and thalamus (Takeda, 1995).

Metabolism and excretion

The liver is the primary organ involved in Mn homeostasis. When Mn is transported into the liver it either rapidly enters the mitochondria, to be incorporated into mitochondrial SOD, or it is sequestered into lysosomes. Lysosomal Mn is then actively transported into the bile and is concentrated in the gallbladder to a concentration 150 fold greater than that seen in plasma (Stipanuk, 2000). Rates of excretion in man on an interindividual basis have been observed to vary considerably (Davidsson 1989a). Under normal circumstances very little Mn is lost through urine or through cutaneous losses.
Data from immature rats, mice and cats have suggested that elimination of Mn undergoes a period of maturation with adult patterns of excretion developing at the time of weaning (Fechter, 1999). Compared to Mn2+, Mn3+ has a slower elimination rate and may have a greater tendency to accumulate in tissues (Aschner, 1999).

Clinical Experience with manganese

Although commonly used as a nutritional supplement, there are no reports of formal studies on the clinical use of manganese salts, except for one study with copper gluconate referred to below.

Copper

Copper (Cu) like manganese and zinc is chemically classified as a transitional element in the periodic table, and third most abundant trace element in the body, in which it is, widely distributed (Mann, 2002). Copper can form complexes in which the metal serves as a central atom and as a result its function is closely associated with its binding to biological ligands, particularly in vertebrate enzyme systems. Like manganese, Cu can adopt distinct redox states allowing the metal to play a pivotal role in cell physiology as a catalytic co-factor in the redox chemistry of enzymes, mitochondrial respiration, iron absorption, free radical scavenging and elastin cross-linking. Copper is also important in neurosecretion (Hartter, 1988).

Copper homeostasis

If present in excess, free Cu ions can cause damage to cellular components, due to the production of highly reactive hydroxyl radicals. In response mammals have evolved means of minimising levels of free Cu ions and destructive Cu complexes which involves a delicate balance between uptake and efflux determines the amount of cellular Cu. The liver has a pivotal role in the homeostasis of several essential trace metals including Cu which is co-ordinated by several proteins including glutathione, metallothionein, membrane bound Cu transporting P-type ATPases, Menkes and Wilson proteins and by cytoplasmic transport proteins called Cu chaperones that ensure delivery to specific sub-cellular compartments. These homeostatic mechanisms prevent accumulation of Cu to toxic levels (Schuemann, 2002).
As a consequence, except under certain genetic conditions, Cu is likely to be benign to most mammals and not responsible for genomic instability, including fragmentation of and/or alterations to DNA, induction of mutation, or apoptosis, or other toxic events (Linder, 2001).

Copper deficiency

The importance of Cu can be attributed to its role as a co-factor in a number of enzymes including cytochrome oxidase and particularly SOD, that are involved in the defence against oxidative stress, and a deficiency compromises the anti-oxidant defence system of cells thus increasing their susceptibility to oxidative DNA damage (Pan, 2000). Reduced amounts of copper/zinc SOD and MnSOD have been implicated in multistage carcinogenesis in both rodents and man (Davis, 1999).
Copper deficiency is also known to impair immune function.

There are many copper containing veterinary compounds available at doses considerably in excess of those proposed on a daily basis for CV 247.
The Feeding Stuffs Regulations (Statutory Instrument 2000 No 2481) allows a maximum content of copper between 15 and 175 mg/kg of feed depending on age and type of animal. Studies from animal models and human volunteers suggest that

acceptable intakes of Cu that would avoid Cu deficiency and/or toxicity may vary between 10 and 50mg/kg body weight (Aggett, 1999).

Absorption

The bioavailability of Cu from the diet is about 65-70% in man resulting in net absorption of about 1mg/day. Copper can be absorbed in the stomach and throughout the length if the small intestine, but predominantly in the jejunum. Intestinal absorption of Cu has been reported to be inhibited by zinc (Sandstead, 1995), and Vitamin B6 depletion in young women has been found to inhibit Cu absorption (Turnlund, 1991). Calcium supplements improve body Cu retention, whilst ascorbic acid is known to inhibit Cu absorption (Kies, 1989), though this is dose dependant (Jacob, 1987). Copper uptake is energy dependant and is affected by the valency state of Cu, preferring Cu 2+ over Cu 1+ (Ferruzza, 2000).
Plasma concentrations in rats have been reported to be lower for orally administered Cu oxide than for Cu sulphate and Cu lysate (Rojas, 1996).
Copper from plant foods is absorbed equally as well as from animal foods (Johnson, 1988), though absorption from the gastrointestinal tract in monogastric animals differs from that in ruminants (Nederbragt, 1984).

The biological t1/2 of 67Cu from the diet is about 13-33 days in man (Johnson, 1992). Gastrointestinal symptoms can occur at whole blood concentrations around 3mg Cu/L (Barceloux, 1999).

Distribution

Once absorbed into the blood plasma, Cu is primarily bound to albumin and transported to the liver where it is incorporated into ceruloplasmin. The complex is then released into the circulation for delivery to the peripheral tissues. The mechanism of Cu uptake into cells is through the binding of the ceruloplasmin-Cu complex to a cell surface receptor, following which Cu is taken up as the free metal (Percival,1990). Intracellular Cu is initially bound mainly to low molecular weight components such as glutathione and subsequently shifted to high molecular weight components such as metallothionein and SOD (Ferruzza, 2000).

Metabolism and Excretion

Copper metabolism can vary between species; rats and cows, for example, exhibit grossly different Cu metabolism patterns (Blincoe, 1992). Under normal circumstances less than 3% of dietary Cu is lost through urine or through cutaneous losses. Although some Cu can be lost by direct sloughing off of intestinal cells, especially when intake is high, almost all Cu excretion is via the bile, complexed in such a way that re-aborption cannot occur. Biliary Cu excretion in man ranges from 0.5 to 1.3 mg/day.

There is evidence, from studies in rats, for the existence of 2 distinct biliary excretion pathways for Cu, a slow, capacity limited one which is independent of glutathione, and a rapid pathway which is glutathione dependant (Houwen, 1990).

Clinical experience with copper gluconate

Cu gluconate has been used to treat the haematologic disorders associated with copper deficiency at a dose of 9mg/day for 6 months (Bartner, 2005), and to help modulate, as part of a micronutrient supplement, the blood anti-oxidant status in patients with major trauma (Berger, 2001). Other clinical uses of Cu gluconate as a part of a cocktail of substances, include the treatment of melanoma (De Oliveria, 1998).
Copper gluconate at a dose of 10mg/day for 12 weeks, has also been investigated as a treatment for low back pain (Pratt, 1985). Copper gluconate has been claimed to have tumour-inhibiting properties and has been used in high doses for the direct treatment of cancer tumours (Nieper, 1979), though details are not available
Cu gluconate has also been used in combination with manganese gluconate. A randomised double blind, placebo controlled study was undertaken in 97 patients for the local treatment of breast fissures related to breast feeding, in which it was found that the combination after 5 days was significantly better in the treatment of fissures less than 14 days old, when compared with placebo (Dreno, 1997). The combination has also been evaluated for the treatment of superficial wounds (Mallet, 1994).

Vitamin C

Vitamin C is a natural product contained in many fruits and vegetables. It is comprised essentially of 2 compounds, L-ascorbic acid (MW 176) and its oxidised derivative, L-dehydroascorbic acid. Although most vitamin C in the body is in its reduced form, both ascorbic acid and dehydroascorbic acid have biological activity and are interconvertible by an oxidation and reduction (redox) reaction, requiring the presence of the enzymes ascorbate oxidase and glutathione dehydrogenase. Ascorbic acid is a 6-carbon lactone which is synthesised in many animals from D-glucose or D-galactose via the glucuronic acid pathway. However several species including man and primates are unable to synthesise ascorbate because they lack hepatic gulonolactone oxidase, the terminal enzyme in the biosynthetic pathway. As a consequence such animals must ingest ascorbic acid to survive. Ascorbic acid is a strong reducing agent and as such has many biochemical functions. Ascorbic acid is oxidised to dehydroascorbate through a short-lived intermediate , ascorbate free radical or monodehydroascorbate, which forms a part of the body's antioxidant defences against reactive oxygen species (ROS) and free radicals. Consequently,Vitamin C is regarded as the most important water- soluble anti-oxidant in human plasma and mammalian cells (Duarte, 2005).
Ascorbic acid plays an important role in many reactions involving mono-oxygenases or di-oxygenases, reactions, which also require molecular oxygen and Fe2+ or Cu2+ as a cofactor. Essentially, ascorbic acid plays either of 2 roles, as a direct source of electrons for the reduction of oxygen, or as a protective agent for maintaining Fe and Cu in their reducing states.
A high concentration of vitamin C is found in the adrenal medulla of mammals. It is a cofactor, acting as a hydrogen donor, for dopamine beta-mono-oxygenase, which catalyses the synthesis of noradrenaline, in neurons, and the adrenal medulla. This mono-oxygenase system contains copper, which is an integral part of the functioning of vitamin C, and which accepts electrons from ascorbate as it is reduced to the cuprous ion.
In total, vitamin C acts as an electron donor for 8 mammalian enzymes. Three participate in collagen hydroxylation, 2 in carnitine biosynthesis, and one each is

required for noradrenaline biosynthesis, peptide hormone amidation, and tyrosine metabolism.
Cells involved in the immune response contain very high concentrations of vitamin C. Aging and chronic disorders, such as cancer, are associated with depression of immunity and are accompanied by low levels of both plasma and leucocyte vitamin C (Basu, 1996).

Ascorbic acid is used extensively as a supplement in both animals and man, and is available in a large number of preparations. Tablets typically contain from 50 to 1500mg of the vitamin.

Absorption

Absorption in man occurs in the buccal cavity, stomach and small intestine. Gastrointestinal absorption is via an active carrier mediated transport system, which can become saturated, and may account for the fact that dietary vitamin C absorption decreases with increasing intake (Hornig, 1980). In man following oral administration Cmax seemed to plateau with increasing dose and suggesteds that vitamin C plasma concentrations are tightly controlled when taken orally (Padayatty, 2004).

Distribution

Following absorption, ascorbate is found in the plasma of man and animals. Ascorbate is transported by a carrier protein that is Na+ and energy dependant, whereas dehydroascorbic acid, transport of which is 10-20 fold faster than ascorbate, is Na+ and energy independent (Welch, 1995) Because it is water soluble and not protein bound, ascorbate rapidly equilibrates in intra and extracellular compartments. Vitamin C exists mainly in the body in the reduced form as ascorbate, the oxidised form accounting for less than 10%. Ascorbate and dehydroascorbic acid transport depend upon substrate availability. When oxidants are generated, dehydroascorbic acid is formed, transported into cells and reduced to ascorbate. The cycle of extracellular ascorbate oxidation and subsequent intracellular reduction is known as ascorbate recycling and is most likely to occur during the time that diffusible oxidants are present and increased intracellular ascorbate could be utilised for oxidant quenching.

Metabolism and Excretion

Metabolism of vitamin C in man occurs by the irreversible hydrolysis of dehydroascorbic acid to diketogulonic acid, followed by oxidation to oxalic acid and threonic acid. These metabolites with some ascorbate-2-sulphate are excreted in the urine along with unmetabolised ascorbic acid.
As well as these metabolites, excretion of vitamin C in man can also occur in the forms of lyxonic acid, xylonic acid and xylose, which are the breakdown products of diketogulonic acid following decarboxylation. In addition a small portion of vitamin C (< 2%) is metabolised to CO2 and exhaled during respiration. In guinea pigs, on the other hand, 60%-70% of vitamin C is excreted as CO2. The overall metabolism of vitamin C is affected by the level of its intake. At a physiological level, less than 10% is excreted as ascorbic acid and 90% as metabolites, whereas a reverse occurs when large doses are ingested.

Oxidative stress, can cause cancer (Bjelakovic, 2004), and consequently the use of anti-oxidant supplements is widespread. Ascorbic acid, a deficiency of which is often seen in cancer patients (Head, 1998), is the single nutrient supplement most commonly used by cancer patients (Block, 2003).

Some Scottish and Japanese studies have pointed to the potential benefit of high dose vitamin C for the treatment of terminal cancer (Head, 1998), and to increase survival (Block, 2003), though these findings are controversial, having been, on the whole, derived from uncontrolled trials or anecdotal reports. Nonetheless, numerous epidemiological studies have pointed to the importance of both dietary and supplementary ascorbic acid in the prevention of various types of cancer (Head, 1998).

Analysis of the survival time curves in one open study indicated that death occurred for the vitamin C treated patients at about one third the rate for the controls (Cameron, 1976), whilst another showed that ascorbate supplemented cancer patients had a median overall survival almost double that of the non-supplemented group (Cameron, 1991). However a double blind study at the Mayo Clinic on patients with advanced cancer comparing high dose vitamin C (10g/day) and flavoured lactose placebo did not, however, support these findings. (Creagan, 1979), and though several studies suggest some benefit, many were unable to conclude that vitamin C is useful in the treatment of cancer.

However it has been suggested that the primary form of vitamin induced tumour cell death is autoschizis (Taper, 2001). It has been shown that vitamins C and K3 can induce autoschizis through activation of acid and alkaline DNAse's respectively (which are known to be deficient in all non-necrotic cancer cells in animals and man), at an early stage of cancer cell death (Verrax, 2003). Several studies have found that vitamin C is effective to protect against the development of cancer, and as a chemoprotective agent when used as adjunct to conventional chemotherapy.

High dietary intake of vitamin C has been observed to modify the relation between Helicobacter pylori and gastric cancer. Recent follow up studies on high- risk populations suggest that ascorbic acid protects against gastric cancer for which H pylori is a significant risk factor (Feiz, 2002).

In vitro and animal studies suggest a benefit of vitamin C in breast cancer (Kurbacher, 1996). In addition the effect of ascorbic acid on the growth of human mammary tumour xenografts was investigated , and was found to inhibit the growth of tumour fragments implanted beneath the renal capsule of immuno-competent mice. Administration in the mouse diet did not affect growth. However it was inhibited when the diet containing vitamin C was supplemented with copper sulphate suggesting an important additive or synergistic affect of copper with vitamin C (Tsao, 1988).

Treatment with anti-oxidant vitamins has been found to lower the recurrence rate of adenomas of the large bowel (Roncucci, 1993). However, high dose (10g/day) vitamin C has been investigated in the treatment of advanced colorectal cancer, and was found to be ineffective. (Moertel, 1985).

The majority of cancer patients combine some form of complementary medicine with conventional therapy (Drisko, 2003). Concern has been raised that anti-oxidants might reduce oxidising free radicals created by radiotherapy and some forms of chemotherapy and thereby decrease the effectiveness of the therapy. However a review of the evidence has demonstrated that exogenous anti-oxidants alone produce

beneficial effects in various cancers and both animal and human studies have not found any reduction in efficacy of either radio- or chemotherapy when given with anti-oxidants. In fact the evidence suggests that the effectiveness of many cancer therapeutic agents is increased, as well as a decrease in while adverse effects are decreased when given with anti-oxidants (Lamson, 1999).
It has been proposed that the combined vitamins C and K3 be used as adjuvant therapy, based upon the observation in studies in mice that the combination selectively potentiated both tumour chemo and radiotherapies (Buc, 2002).
Vitamin C also appears to increase the drug sensitivity of cervical carcinoma cells by stabilising P53 (Reddy, 2001).
An investigation into the treatment of acute myeloid leukaemia, found that ascorbic acid enhanced the apoptotic effect of arsenic trioxide, a drug which induces remission in a high proportion of patients (Bachleitner, 2001).
A study carried out in patients with advanced breast cancer evaluated the benefit of ascorbic acid as a chemosensitiser of polychemotherapy. It was found that in the group in which ascorbic acid was given as a supplement there was a significantly higher reduction in average breast lump size and tumour cell DNA content (Goel, 1999).

Sodium salicylate (SS)

The properties of the salicylates are well documented and salts of salicylic acid have been used as anti-inflammatory drugs for over 100 years. These salts are effectively pro-drugs of salicylic acid (SA). Sodium salicylate is monographed in several pharmacopoeias including Europe, Japan and the USA, as well as BP Vet, as an anti-inflammatory drug.
SA is a substrate of the liver cytochrome P450 enzyme CYP2E1 (Wu, 2001) and may interact with other NSAIDs and with other drugs which are extensively bound to plasma proteins. The salicylates should only be co-administered with anti-coagulant drugs with caution.

Pharmacokinetics

The concentration of salicylate in serum and perilymph following intraperitoneal injection of SS in chinchillas peaked 2-4 hours after a single injection. Clearance was approximately complete after 16 hours (Boettcher, 1990).
The biological half- life and the apparent volume of distribution of salicylate were reported to be increased as a consequence of pregnancy in rats after i/v injection, but there was no observed effect in absolute total clearance (Dean, 1989).
In another rat study it was concluded that the physiological state and nutrition status can affect salicylate pharmacokinetics (Varma, 1984).
A pharmacokinetic study of SS undertaken as a part of the target animal safety study in dogs has been referred to in the section on CV247. Pharmacokinetic analysis of the data indicated that overall, the extent of systemic exposure in dogs to SS appeared to be characterised by linear (dose independent) kinetics over the dose range tested.

Pharmacokinetic studies in man have found that the absolute bioavailability of SS is 100% after oral administration of a solution of a dose of 9mg/kg to normal male and female subjects. There were sex differences with regard to tmax, but not the Cmax.of SA; there were no differences with regard to apparent volume of distribution, plasma

clearance, or AUC (Miaskiewicz, 1982). Following orally administered doses of SS the mean peak plasma concentrations of SA appears to be dose related (Cerletti, 1984), but the tmax occurs sooner after lower doses (Seymour, 1984).
Urinary recovery of SA and its metabolites after a single oral dose of SS essentially accounts for all the administered dose and was not influenced by age (Abdallah, 1991). Another study in which subjects received single oral doses of SS observed that AUCinf did not correlate with age and nor was there any gender difference. Clearance was only affected by serum albumin concentrations a reduction in which, as may be seen with increasing age, decreased serum protein binding resulting in slower elimination of salicylate (Netter, 1985).

Absorption

Absorption of SA begins in the stomach by passive diffusion. Studies in rats, as a model of absorption, found that the glandular and non-glandular parts of the stomach showed different concentration levels following oral administration (Brune, 1977). The uptake of salicylate into the small intestine has been investigated in isolated rat small intestinal epithelial cells, when uptake was found to reach an equilibrium within 15 minutes by a mechanism possibly other than simple diffusion (Suzuka, 1988).

Distribution

After absorption SA shows a concentration dependent, plasma protein binding. (Dromgoole, 1981). Since only unbound drug is available for distribution, an increase in free drug leads to an increase in the volume of distribution. It has been shown that high concentrations of absorbed SS are found in vivo in the glandular region of the stomach, in kidney tubules and in inflamed tissues, probably because SA is a weak acid and is likely to accumulate within cells which are surrounded by bordering acidic fluids (Brune, 1977).The ionised form of salicylate is significantly taken up into human red blood cells (Nishihata, 1984).

Metabolism

SA is metabolised by linear and saturable processes. It may be conjugated with glucuronic acid to form acyl or phenolic glucuronides, or with glycine to form salicyluric acid (salicylglycine, o-hydroxyhippurate). In addition hydroxylation leads to the formation of 2,3 dihydroxybenzoic acid and gentisic acid (2,5 dihydroxybenzoic acid) which in turn is either eliminated or conjugated with glycine to form gentisuric acid. Experiments with anti-oxidant inhibitors indicate that superoxide dismutase, haem protein inhibitors and glutathione block gentisic acid formation (Davis, 1989). The formation of salicyluric acid is the preferred metabolic pathway, but the capacity for the formation of both this and salicyl phenolic glucuronide is limited leading to an increase in the elimination of the other metabolites if the concentration of SA in the body exceeds 600mg. The overall elimination of salicylate proceeds by first order kinetics at low doses and by both zero and first order kinetics at higher doses. Consequently the elimination plasma half life increases with dose, and plasma concentrations increase disproportionately which is most pronounced with respect to unbound plasma SA due to decreased protein binding (Levy, 1980). Such capacity limited metabolism has been shown to increase the half life of SA from about 3.5hr to over 30hr. This non linearity in SA metabolism

is of importance with long term, high dose therapy with salicylates, though it has been observed that SA is likely to induce its own metabolism associated with a decrease in plasma levels and an increase in the the urinary elimination of of salicyluric acid by approximately 50% after 3 days of treatment (Dromgoole, 1981).

Excretion

SA and its metabolites are mainly eliminated by urinary excretion, though renal elimination is strongly pH dependant. Raising the pH leads to an increase in the excretion of SA per se as only unionised molecules can be re-absorbed. The fact that alkaline urine increases the renal clearance of SA is common to many animal species (Roch-Ramel, 1979).
The elimination of SA in goats and cattle following administration of SS both orally and intravenously found that in these species only the parent compound and the glycine conjugated metabolite, salicyluric acid, were found in urine (Short 1990).

Clinical experience with sodium salicylate

The role of the salicylates in the treatment of various disease states has been widely researched. In particular the salicylates, have been demonstrated in almost every model to exert an anti-inflammatory effect, and specifically the blocking of prostanoid synthesis from arachidonic acid by inhibition of the cyclo-oxygenase (COX) enzymes, or, in the case of SA, predominantly affect the metabolism of arachidonate via the lipoxygenase pathway. Both SS and aspirin have similar anti-inflammatory potency and both drugs cause a dose dependant reduction in the concentration of PGE2 in experimental inflammation.
Recent interest in the NSAIDs, in general, has been a possible role as chemotherapeutic agents, possibly via induction of apoptosis, and inhibition of angiogenesis resulting in prevention of tumour growth by anoxia. Both COX selective and nonselective NSAIDs inhibit angiogenesis through direct effects on epithelial cells involving inhibition of mitogen activated protein (MAP) kinase (ERK2) activity (Jones, 1999). The proposed mechanisms of the anti-neoplastic effects of NSAIDs via the inhibition of COX-2 has been supported by various experimental animal models but does not preclude the role of other factors, such as the role that COX 1 plays in carcinogenesis, and the fact that SS prevents nuclear factor kappa B activation and can cause apoptosis (Wu, 2001).

CONCLUSION

CV247 has been developed as a treatment for cancer, and specifically the improvement of well-being in dogs diagnosed with progressive cancer for whom the prognosis is often poor, quality of life significantly impaired, and for whom euthenasia is often the recommended course of action. The latter is anathema to many owners who predominantly seek a safe therapy which will improve the well being of their pets, and, possibly provide a chance of producing disease regression and increasing life expectancy. The application seeks approval for the improvement of well being following treatment with CV247, a combination therapy based on sound scientific principles for the contribution of each component in cancer treatment. Both copper and manganese are essential trace elements found in superoxide dismutase,

altered amounts of which have been implicated in carcinogenesis. In addition deprivation of both elements can alter immunological function. Vitamin C is the most important anti-oxidant to be found in mammalian cells and is an important factor in immune surveillance against cancer, whilst sodium salicylate is an approved anti-inflammatory drug which, because of its COX enzyme inhibitory properties, may have benefit as a chemotherapeutic agent. The doses selected are sufficient to boost depleted levels in cancer patients without being toxic, and with regard to vitamin C at a dose level which would not inhibit copper absorption.

Early proof of concept studies to investigate anti-cancer potential in mouse lymphoma models provided evidence of efficacy, which was supported, albeit to a lesser extent in a syngeneic Lewis lung model, though the latter was probably negatively influenced by the aggressive nature of the cancer line used. Importantly the latter study provided some evidence for the need of the combination rather than for any one component. CV247 was found to slow the development of cancer but was not a cure. Both studies demonstrated that CV247 was safe.

CV247 has been investigated in dogs and man. Two open label studies in a total of 104 dogs provided convincing evidence, albeit in an uncontrolled setting, that quality of life was improved in over 70% of the animals treated. Treatment periods were sometimes short, and both studies relied heavily on owner compliance with the protocol, but in several cases, all independently reviewed, quality of life was improved, sometimes to a level better than that observed before the animals became (noticeably) ill. In addition there was some evidence of tumour regression and prolonged life expectancy. It was not possible to identify with any degree of certainty, as to whether any particular cancer was most responsive to CV247, but sarcomas affecting hard tissues seemed to be the least responsive. Any future study, which would need to have a control arm, might benefit by recruiting a less diverse population of tumour types. The studies did provide irrefutable evidence of safety. Data from studies in man have been included for the sake of completion, though 2 studies are ongoing and little evidence of relevance with regard to efficacy can thus be gleaned. Nonetheless it is evident that some patients appear to have derived benefit from CV247 (or in the case of the ongoing study in prostate cancer), one of its components, sodium salicylate, for considerable periods of time, and in excess of that, which might have been expected from such a patient population. The studies, which to date have included 281 patients, again add considerable weight to the safety of CV247, there having been few adverse reactions, mainly gastro-intestinal in origin.

The clinical experience and pharmacology of the individual components have been extensively researched in the literature. Of particular note are the homeostatic mechanisms that exist, predominantly in the liver, which limit systemic exposure to both copper and manganese, and thus minimise any toxicological risk from these 2 components. If there was any note of caution it might be with regard to treatment of Bedlington Terriers and Doberman Pinschers, types, which for genetic reasons, are more vulnerable to copper toxicosis. A chronic toxicity study undertaken in dogs included a pharmacokinetic analysis of sodium salicylate and found that systemic exposure is characterised by linear kinetics. Clinical experience with the individual components is extensive for both vitamin C and sodium salicylate, with both being identified as potential chemotherapeutic agents in their own right. However evidence of efficacy is limited, and in the case of vitamin C is largely derived from epidemiological and uncontrolled data, though high dose intravenous vitamin C has been used successfully to treat a variety of tumours.

The evidence provided demonstrates that, overall, there is a need for a treatment such as CV247 to improve well-being, and increase the chance of life expectancy, in dogs diagnosed with progressive cancer. CV247 is a combination of 4 active ingredients all of which have been theoretically shown to make a contribution to cancer treatment. Experience in both dogs and man have found that CV247 can be of considerable benefit without any of the side effects commonly associated with current cancer therapy, which induces many pet owners to decline therapy or opt for prompt, possibly premature, euthanasia. A definitive study, adequately controlled, maybe required to provide for irrefutable evidence of efficacy, but for this there would be more serious limitations than in humans for the control of such variables as breed, gender, neutering, age, nutrition status, cancer type, and owner compliance. The latter includes opting for early euthanasia, refusal for monitoring tests and, where necessary, postmortem examination.
There is no doubt that many dogs would benefit, with regard to improved quality of life, from the availability of CV247.

REFERENCES

Details of the publications used in this report are included in the list of references at the end of Part IV of the application.

INFORMATION ABOUT THE EXPERT

Andor Sebesteny BVSc, Dip.Bact., MRCVS, qualified as a veterinary surgeon in 1960 from Bristol University and has been in practice, both full and part time ever since. Since 1964 he has also been the veterinary supervisor of laboratory animals at the Imperial Cancer Research Fund (now CR UK), where he is currently a consultant veterinary pathologist. In addition he has been the “named vet” at the Ludwig Institute of Cancer Research since 1988. Andor Sebesteny has published over 30 papers in health control and cancer research, and has presented papers at over 60 National and International meetings. He was recently appointed Honorary Professor of Laboratory Animal Science at Budapest Veterinary University.