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620512

COMMONWEALTH OF AUSTRALIA

Patents Act 1952

APPLICATION FOR A STANDARD PATENT

I, MICHAEL ARLINGTON, of 3 Windham Place, Westleigh, New South Wales, Australia hereby apply for the grant of a standard patent for an invention entitled:

THERAPEUTIC USES OF CYANOCOBALIMIN

which is described in the accompanying provisional specification.

My address for service is:

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DATED this 2nd day of May 1989

MICHAEL ARLINGTON
by his Patent Attorneys
HALFORD & CO.

To: Commissioner of Patents
Fee: $65.00
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NOTICE OF ENTITLEMENT

I, Michael Arlington, of 3 Windham Place, Westleigh, New South Wales, 2120, Australia, the applicant in respect of Application No. 54534/90, state that I am the actual inventor.

M. A. Arlington — 31 Oct 91.

Michael Arlington (Date)

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(57) As used in this specification, the expression "average daily dosage" means the dosage administered to the patient divided by the period (in days) between administrations. Thus, administration of 10mg twice daily will be equivalent to an average daily dosage of 20mg while administration of 10mg every second day is equivalent to an average daily dosage of 5mg. In the case of continuous IV administration, the "average daily dosage" is the dosage administered per day.

CLAIM

1. A method of treatment or prevention of neoplastic disease or immune-system related disease in a human or animal in need of such treatment or prevention comprising administering an average daily dosage (as herein defined) of from 5 to 1000mg of cyanocobalamin to the human or animal.

7. A method of treatment or prevention according to claim 6 wherein the cyanocobalamin is administered in an aqueous solution containing from 0.5% to 1.1% by weight of cyanocobalamin.

.../2

27. A method of treatment of neoplastic disease or immune system related disease in a human or animal in need of such treatment comprising administering an average daily dosage (as herein defined) of from 5 to 1000mg of cyanocobalamin to the human or animal until symptoms of the disease show improvement and thereafter administering cyanocobalamin at an average daily dosage of less than 5mg.

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COMPLETE SPECIFICATION

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TO BE COMPLETED BY APPLICANT

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Complete Specification for the invention entitled: THERAPEUTIC USES OF CYANOCOBALAMIN

The following statement is a full description of this invention, including the best method of performing it known to me:

SO 14915 30/04/70

This invention relates to therapeutic uses of cyanocobalamin (vitamin B12), and particularly to the the therapeutic effects of high dosages of cyanocobalamin in the treatment of neoplastic disease and also immune-system related diseases such as auto-immune disease, Aquired Immune Deficiency Syndrome (AIDS) and many other forms of immunodeficiency disease. The invention may also be useful in the treatment and modification of most viral and many bacterial infections.

Experiments in the effects of cyanocobalamin in tumors have been carried out at least since 1950. Bodian (1,2) described the use of vitamin B12 in the treatment of neuroblastoma in children. Parenteral dosages of 1mg intramuscularly every second day, and in later trials up to 2mg i.m. on alternate days produced encouraging results, in many cases resulting in regression and sometimes the disappearance of the tumor. The mode of action of vitamin B12 on the tumors was inexplicable, and the dosage chosen empirically.

On the other hand, Langman (3) described a retrospective study of treatment of 108 children with neuroblastoma, which failed to show beneficial results from vitamin B12 therapy. Most of the treated patients had received frequent injections of 1mg. or more, and the intensity of initial treatment did not seem to be related to the length of survival. Similarly, Sawitsky and Desposito (4) reported a retrospective study which also suggested that vitamin B12, in similar dosages, had no useful effect on neuroblastoma.

Welsh (5) reported a trial of vitamin B12 therapy (1mg. i.m. daily) on 25 patients with acute

leukaemia. There was no evidence to suggest any beneficial effect.

Bodian and Rigby (6) examined the effects of cyanocobalamin in two animal tumour systems, namely C1300 neuroblastoma/sarcoma in mice and PWA2 fibrosarcoma in rats. They found a very significant inhibition of growth in the murine tumours, and a very significant enhancement of growth in the rat tumours. These paradoxical results suggest the possibility of a genetic basis for the effect of cyanocobalamin on some tumor cells.

The dosages used in the prior art were, as has been noted, empirical, and appear to have been based on the available concentrated preparations of the vitamin. The present invention is based on studies which have been made of the effects of significantly higher dosages, which strongly suggest that cyanocabalamin in the dosage range 5-1000mg preferably 10-200mg, and more preferably 20-50 mg per day, will constitute a useful and non- toxic chemotherapeutic agent in the treatment of a wide spectrum of neoplastic disease and immune system related disease. Also contributing to the invention is a hypothesis as to the biochemical mechanisms involved.

The present invention thus provides a method of treatment or prevention of neoplastic disease or immune-system related disease in a human or animal comprising administering an average daily dosage (as herein defined) of from 5 to 1000mg of cyanocobalamin to the human or animal.

As used in this specification, the expression "average daily dosage" means the dosage administered to the patient divided by the period (in days) between

administrations. Thus, administration of 10mg twice daily will be equivalent to an average daily dosage of 20mg while administration of 10mg every second day is equivalent to an average daily dosage of 5mg. In the case of continuous IV administration, the "average daily dosage" is the dosage administered per day.

It is strongly preferred that the cyanocobalamin be administered at a frequency of at least thrice per week, preferably at least once each day.

The cyanocobalamin preferably is administered parenterally, desirably as an aqueous solution containing between 0.5 and 1.1% cyanocobalamin, and optionally containing conventional excipients. Ideally, the solution should contain about 1% cyanocobalamin.

For conditions where topical application is desirable, the cyanocobalamin may be administered as a topical preparation containing from about 0.5% to 5% cyanocobalamin, in a conventional carrier such as dimethylsulphoxide.

Vitamin B12 in the coenzyme form methylcobalamin is essential for the clonal expansion of human B lymphocytes in large-scale antibody production (27). B lymphocytes are the cells in the human imune system which are responsible for the synthesis of antibodies in response to antigenic stimulus.

Methylcobalamin has a remarkable enhancing effect on protein and immunoglobulin synthesis in human lymphocytes (28), and also on helper and suppressor T lymphocytes (T4 and T8 cells respectively) in the presence of mitogenic/antigenic stimulus (29). Cyanocobalamin, the biologically inert, synthetic form

of Vitamin B12, is converted to the biologically active form methylcobalamin by human lymphocytes (28).

From these observations it is evident that cyanocobalamin has the capacity to function as a potent immunostimulant and immunomodulator in the human immune system.

In the course of its metabolism to biologically active coenzyme forms of Vitamin B12, such as methylcobalamin and adenosylcobalamin (7, 8), conversion of the cyanocobalamin produces cyanide radicals.

The normal cell detoxifies small amounts of free cyanide easily (9). It is hypothesised that the in vivo, human neoplastic cell is unable to detoxify free cyanide and is therefore destroyed. It is further hypothesised that this inability arises from the lack of the enzyme (and therefore the gene) required to effect this process, because of chromosomal aberration and gene deletion. Intracellular cyanide detoxification is a single-enzyme process, mediated by the enzyme thiosulphate: cyanide sulphurtransferase (TCST) and utilising sodium thiosulphate from the plasma.

It is further postulated that the gene from the TCST enzyme is co-located with the normal cell's "oncogenes", i.e. those genes responsible for regulation of cell division, cell-to-cell recognition, intercellular cement formation, etc, so that carcinogenic factors which ablate these regulatory "oncogenes" and thus render a cell neoplastic, automatically ablate the TCST gene responsible for intracellular cyanide detoxification. It has been known for many years that tumour cells are remarkably low in cytochrome content, and therefore presumably

rely largely on anaerobic metabolism for their continued survival (10). This observation suggests that small amounts of free cyanide derived from cyanocobalamin conversion are therefore more effective in the destruction of tumour cells, in that there are much lesser quantities of the target enzyme (cytochrome oxidase) requiring inactivation, compared to normal cells. The observation also supports the genetic considerations proposed immediately above, as the genes for cellular cytochrome endowment are probably largely co-located with regulatory "oncogenes" and the TCST gene, so that most of them suffer the same fate as regulatory "oncogenes" and the TCST gene in oncogenesis.

The differing results obtained by Bodian and Rigby (6) can therefore be explained on the basis of differing gene locus for the cyanide detoxification enzyme in mice and rats.

Recent work in the cytogenetic study of human neuroblastoma cell hybrids has shown evidence of aberrant DNA formation, with chromosomal fragility, breakage and loss of genetic material (11,12,13), and identification of a high incidence of regular chromosomal abnormalities, such as deletions, translocations, insertions and pericentric inversions. These abnormalities are consistent with those being increasingly observed as characteristic chromosomal abnormalities in other tumour systems, eg, retinoblastoma, nephroblastoma and chronic myeloid leukaemia (11,14,15).

The most recent work from the Walter and Eliza Hall Institute of Medical Research, on gene mapping in human Burkitt lymphomas and murine plasmacytomas, has also identified characteristic chromosomal

abnormalities in these tumour systems, with predictable deletion of gene sequences of variable length (16, 17, 18, 19, 20, 21).

The work in neuroblastoma cell hybrids and also Burkitt lymphma/murine plasmacytoma points strongly to the critical role played by chromosomal aberration and gene deletion in oncogenesis.

It is believed that similar considerations apply in the demonstrated effect of cyanocobalamin against auto-immune diseases. Auto-immune disease may occur when defects in the lymphocytes result in the production of antibodies which attack the body's own cells. As part of normal operation of the immune system, T lymphocytes have a role as messenger cells recognising foreign antigens such as viruses and relaying this information to the B lymphocytes, in addition to the anti-infection function of the T lymphocytes themselves. The B lymphocytes then produce antibodies based on the information provided by the T lymphocyte. Auto-immune disease may occur either if the T lymphocytes are defective, and pass on incorrect information to the B lymphocytes to produce aberrant antibodies, or if the B lymphocytes are defective and produce incorrect antibodies when given the correct information. These abnormal lymphocyte populations probably constitute tumour systems within the reticulo-endothelial system, with their expression being in aberrant antibody production directed against normal body tissues. These abnormal lymphocytes also seem to lack the TCST gene responsible for cyanide detoxification.

The present applicant has been accumulating over the past 5 1/2 years, since the initiation of the therapy described below, evidence of the efficacy of very high

dosages of cyanocobalamin in the treatment of neoplastic disease and immune system related disease.

The unfavourable reports of Sawitsky and Desposito (4) and of Langman (3) relating to Vitamin B12 therapy in neuroblastoma, and the absence of evidence of efficacy in the work of Welsh in acute leukaemia (5) are explained, it is believed, by the dosages used in the treatments which were the subject of the studies. It is now believed, as a result of the applicant's investigations, that the dosages then employed were inadequate, especially so when considered against the cyanide effect in cyanocobalamin therapy of neoplastic disease, as disclosed above.

The studies leading to the present invention strongly suggest a highly disparate response of tumours to cyanocobalamin, and suggest that this response reflects the dependence upon, or affinity for, Vitamin B12 of the tumour's histological tissue of origin. High-affinity tumours, for example, osteoclastoma, seem to regress very rapidly with relatively low doses of cyanocobalamin, probably because the tumour cells have an ongoing metabolic requirement for Vitamin B12. Low-affinity tumours (eg, ovarian adenocarcinoma) are probably only dependent upon Vitamin B12, and therefore susceptible to the action of cyanocobalamin, at the time their cells are about to divide, thus requiring much higher doses of cyanocobalamin to produce a favourable response. Other factors of importance in the determination of dosage are tumour density (eg, scirrhous or cystic), degree of malignancy and extent of disease. The exponential effect of tumour growth becomes very apparent in the therapy of low-affinity tumours.

The methods of the present invention will now be

described by way of example, with reference to particular patients treated during trials of the invention.

Patient 1

A 66 year old female suffering from extensive pelvic recurrence of an endometrial adenocarcinoma. After radiotherapy and hormone therapy, cyanocobalamin therapy followed for 3 months at a dosage of 4mg IM daily. The patient's bowel symptoms disappeared and the treatment appeared to be preventing progression of the primary disease.

Patient 2

A 49 year old male with extensive liver metastases from a resected primary, anaplastic adenocarcinoma of the rectum. His liver enzymes had been steadily elevating. Liver function tests showed improvement after a continuous intravenous infusion of cyanocobalamin, at a dosage of 100mg daily for five days from 27 September, 1984.

Both patients 1 and 2 ultimately succumbed to their disease.

Patient 3

A 29 year old male found to be HIV positive on routine serology by local doctor July 1985. Rapidly deteriorating T cell function and ratios were evident by October/November, 1985 although the patient was clinically well.

Cyanocobalamin therapy 5mg intramuscularly daily commenced 12 November, 1985, reducing to 5mg

intramuscularly second daily from 7th June, 1986. Marked improvement in IgM levels and T cell ratios evident by May, 1986.

Slight deterioration evident in T cell ratios following reduction in dosage as above.

A trial of isoprinosine was commenced in February, 1987 and ceased February, 1988 with no apparent benefit. Cyanocobalamin was maintained at 5mg second daily during this trial, until August, 1987, then increased to 5mg IM daily by the patient.

Cyanocobalamin dosage was increased to 25mg subcutaneously daily from late February, 1988. Close follow-up on this patient has not been possible due to geographic limitations, but the patient has apparently continued on 25mg cyanocobalamin daily, and recent lymphocyte marker tests show that the patients T4 counts have remained stable. Haemoglobin levels have also remained stable, with normal platelet counts. The patient has developed Karposi's sarcoma, a low-affinity tumour for B12 and a low-grade malignancy of vascular origin with a high incidence in HIV infection.

The patient has shown no evidence of toxic effects from therapy.

Patient 4

A 39 year old male diagnosed 12 months previously as suffering from chronic lymphocytic leukaemia.

Cyanocobalamin therapy was commenced 2nd May, 1988 at 50mg subcutaneously daily, reducing to 25mg subcutaneously daily from 27th June, 1988, and

increasing again to 25mg subcutaneously twice daily from 18th August, 1988.

Reduction in splenomegaly by 5cm was evident after three weeks of therapy, necessitating monitoring of spleen size by ultrasound. Resolution of lymphadenopathy was evident after two months of therapy.

Significant increase in blood platelet count was evident by 12th August, 1988, with regression of the disease process from Stage II to Stage I within three months of commencement of therapy.

It is stressed that cyanocobalamin will exert its effect only on the precursors to mature leukaemic lymphocytes, i.e., lymphoblasts, not on mature, end-of-the-line leukaemic lymphocytes, which will remain in the peripheral blood until the end of their natural life cycle (i.e. possibly up to two years).

The B12 dosage was reduced to 10mg parenterally twice weekly with effect 25th March 1989, and ceased altogether September 1989. A review on 6th March 1989 (the first in 12 months) showed slight recurrence of splenomegaly at 3-4cm below (L) costal margin, with recurrence of mild, rubbery lymphadenopathy in the neck, axillae and inguinal regions. There has obviously been recurrence of the leukaemic process, but it does not seem to have advanced beyond the stage it was at 2 years ago, just prior to the commencement of B12 therapy.

This particular tumour system gives every indication of being a low sensitivity tumour to B12, but significant response has been demonstrated to the B12 as above, with significant fall in white cell count

2-3 months after the commencement of B12 therapy. There was no evidence of elevation in serum alkaline phosphatase or serum transaminase levels at any stage during therapy. There is considerable promise for a much more effective result in this type of low-affinity tumour by combining the B12 therapy with effective conventional chemotherapy, with a strong possibility of marked alleviation of toxic side effects from the chemotherapy.

Patient 5

A 54 year old female sufferring from scleroderma, an auto-immune collagen disease. Cyanocobalamin therapy 25mg subcutaneously daily commenced 16th June, 1988, reducing to 25mg three times weekly from 16th September, 1988. There had been no previous specific therapy for this disorder.

After the first three months of therapy improvements were mainly clinical in nature, and were summarised as follows:

a) Marked improvement in Raynaud's phenomena in fingers.

b) Moderate reduction in swelling of fingers - although her fingers are still slightly swollen, she could no longer be described as having "sausage" digits.

c) Almost complete resolution of the clinical signs of interstitial disease (crepitations) in the mid and lower zones of both lungs.

d) Gradual but incomplete fading of telangiectasia on face and hands.

e) Suggestion of slight fall in chronically elevated ESR (erythrocyte sedimentation rate) in serial pathology tests.

f) Suggesti . early improvement in chest X- ray appearances.

Gradual improvement continued on the reduced dosage as above, with ESR remaining at normal levels. While in the early stages of treatment the patients ANF (anti-nuclear factor) titres consisted of a low-titre and a high-titre pattern, the low titre pattern disappeared. Immunopathology showed an apparent drop in ANF titre accompanied by continued improvement in skin condition and pulmonary signs.

The B12 dosage was decreased to 250 micrograms orally twice daily from 20th February 1989. A return of high-titre ANF to previous levels was evident by 15th June 1989, but with no evidence of return of low-titre ANF as at 25th January 1990. Significant deterioration is now evident in Raynaud's phenomenon in the patient's fingers, but the improvement is maintained in other respects. Only mild swelling is evident in the fingers, with minimal signs of interstitial disease is evident at both lung bases. The ESR has remained within normal levels and significant elevation of serum B12 levels is being maintained by the oral B12 administration.

Patient 6

A 60 year old male with gastric carcinoma and extensive hepatic metastases. No previous oncological therapy. Cyanocobalamin therapy 25mg subcutaneously twice daily commenced 30th April, 1988.

Therapy interrupted on two occasions, from 17-23 May, 1988 inclusive, and 13-21 July, 1988 inclusive. Despite this, significant regression of large liver metastases was observed in the first three months of therapy, followed by probable replacement fibrosis to a large extent. Serial abdominal ultrasound studies

showed no significant progression of liver metastases during therapy, allowing for the interruptions mentioned above.

Increasing serum transaminase/alkaline phosphatase levels were observed during therapy, superimposed on the already-elevated levels from the liver metastases, when the liver metastases were in regression. This probably reflects the kill rate of gastric carcinoma cells. The levels were observed to fall rapidly when therapy was interrupted from 13-21 July, 1988 in hospital, and rise again rapidly on reinstitution of therapy.

Gastrojejunostomy was performed 25th July, 1988 for severe pyloric stenosis.

No toxic effects were observed during therapy.

Cyanocobalamin therapy was ceased 26th September, 1988 as the patient elected to undergo a course of conventional cyclic chemotherapy (CEM - cysplatin, epirubicin and mitomycin C) which was commenced 28th September, 1988. This has produced severe toxic effects including marked leucopaenia, thrombocytopaenia and septicaemia with alopecia and considerable weight loss. One month following the first course of this therapy there was no clinical evidence of regression of the enlarged liver. The patient died on 3rd March 1989.

From serial liver function tests, it appears that disease progression in this patient had been halted by the cyanocobalamin before the start of conventional chemotherapy. It is most likely that the treatment at the hospital was mainly treating replacement fibrosis in the patient's liver.

Patient 7

A 22 year old female with a rapidly progressive squamous carcinoma involving the right cheek and sub-mandibular area. Cyanocobalamin therapy with 50mg subcutaneously twice daily commenced 25th May, 1988, two weeks after cessation of radiotherapy on 14th May, 1988. The tumour mass remained static in this intervening period between therapies.

Rapid and marked elevation of serum transaminase/alkaline phosphatase levels were observed following commencement of cyanocobalamin therapy. This coincided with increasing regression of the tumour mass over the ensuing two months, and probably reflects the kill rate of squamous carcinoma cells, especially in view of the fact that the liver remained clinically and ultrasonically normal throughout therapy.

Infection developed in the tumour and surrounding facial tissues 23rd June, 1988, and the oedema from this, combined with obstruction from the tumour mass, lymphoedema and post-radiation vasculitis, resulted in severe impairment of arterial circulation into the whole area. This in turn severely compromised the chemotherapeutic approach of cyanocobalamin delivery to the tumour, with marked reduction in kill rate of the tumour cells (as evident in significant fall of serum transaminase/alkaline phosphatase levels), and consequent progression of the tumour.

Cyanocobalamin dosage was increased to 500mg daily by subcutaneous infusion for ten days from 17th July, 1988 in an attempt to offset the ischaemia in the area, with only limited and transient success. There

was no evidence of toxicity from this very high dosage, or at any stage during therapy.

The patient ultimately succumbed to her tumour (22nd August, 1988).

Patient 8

83-year old female with 3 large lipofibromas of (R) buttock, present for some time and gradually increasing in size to the point where surgical excision was being seriously considered. Cyanocobalamin therapy was commenced on 8th March 1989 at a dosage of 10mg parenterally once daily, when the dimensions of the 2 largest tumours were 9cm x 9cm and 8cm x 6cm in their maximum diameters.

Complete resolution of tumours was evident after 3 months of B12 therapy, which was ceased 11th June 1989. There was no recurrence of tumours as at 2nd February 1990.

Patient 9

61-year old male with extensive broncho-alveolar carcinoma of both lungs diagnosed July 1988. He underwent radiotherapy for cerebral metastases October 1988 with marked regression, and chemotherapy November 1988 with some improvement in respiratory symptoms. There was evidence of early progression of cerebral metastases on CT scan of brain, at start of cyanocobalamin therapy on 10th February 1989 at a dosage of 50mg parenterally twice daily.

There was objective evidence of some improvement in respiratory status and general condition by 17th March 1989, and evidence of some regression in

cerebral metastases on CT scan of brain on 4th May 1989, with no appearance of new lesions. The B12 dosage was reduced to 25mg parenterally twice daily from 5th May 1989. Gradual development of respiratory infection with (L) pleural effusion 1-8th May 1989, resulting in hospital admission. Cytology on pleural aspirate 13th May 1989 and 15th May 1989 was negative for malignant cells.

Patient decreased 31st May 1989 as a direct result of pneumonia, evident at autopsy on 2nd June 1989. Review of the autopsy slides with the pathologist who performed the examination, showed small areas of necrosis (as distinct to post-mortem autolysis) in the tumour masses. Necrosis is not normally a feature of the histopathology of broncho-alveolar carcinoma, which tends to be a slowly progressive, infiltrative rather than invasive carcinoma.

This tumour demonstrated a low sensitivity to the B12 therapy, and in view of the above, a considerably lower dose of B12 probably would have achieved a similar result. The patient tolerated the sustained high dosage of B12 well, with no evidence of toxicity, either clinically or on routine blood tests. There was no evidence of elevated serum transaminase or alkaline phosphatase lenels at any stage during B12 therapy.

Patient 10

54-year old female with extensive and severe, chronic eczema of over 4 years duration, resulting in several hospital admissions. Baseline pathology on 11th April 1989 showed a markedly elevated serum level of Ig E, with twice normal values for 24-hour urinary histamine excretion and methylhistamine excretion,

together with a pronounced allergic state evident on RAST screening, all consistent with cutaneous mastocytosis. Cutaneous mastocytosis is an extensive infiltration of the skin by mast cells, almost certainly a benign neoplastic process of the myelo-proliferative variety.

Cyanocobalamin therapy was commenced on 20th April 1989 at a dosage of 25mg parenterally once daily, and has been continued to present. Marked improvement was evident in eczema after 6 months therapy, with only minimal involvement in elbow flexures and behind knees. Modification of allergic state is evident on progress RAST screening. 24-hour urinary histamines showed normalisation of histamine excretion and only marginal elevation of methylhistamine excretion on 17th October 1989. Ig E continues to fluctuate widely at markedly elevated levels, but the mildly elevated ESR at the start of treatment has returned to normal.

Mild exacerbation of eczema occurred with significant stress January-February 1990, and 24-hour urinary histamines showed only a slight increase in methyl-histamine excretion. There has been no evidence of adverse effect from the B12 therapy so far.

Patient 11

48-year old male with adenocarcinoma of prostate diagnosed November 1988. Bilateral orchidectomy was performed immediately following diagnosis. Extensive skeletal metastases were evident on X-rays and CT scan at the time of diagnosis. Progression of bone metastases was evident on bone scan of 17th April 1989, particularly in the areas of active

haemopoiesis.

Cyanocobalamin therapy commenced on 5th June 1989 at a dosage of 50mg twice daily parenterally, when the patient was experiencing severe and increasing bone pain. B12 dosage reduced to 25mg parenterally twice daily from 7th July 1989. Rapid and marked elevation of serum alkaline phosphatase, lactic dehydrogenase and in particular the tumour-specific enzyme prostatic acid phosphatase occurred following the start of B12 therapy, demonstrating a probable moderate-to-high sensitivity of the tumour system to B12. These enzyme levels peaked from 31st July-14th August 1989, then rapidly declined toward normal values with continued B12 therapy. This probably represents a high kill rate of prostatic carcinoma cells by the B12, with the viable tumour mass rapidly decreasing after the peak in enzyme levels, especially in view of the fact that the patient had lost most of his bone pain and required virtually no analgesia after 6 weeks of B12 therapy.

Unfortunately, the destruction of large areas of active haemopoietic marrow by the tumour resulted in a severe deficiency of blood clotting factors including platelets, and the patient developed a subdural haematoma following a fall. This required neurosurgical evacuation on 18th August 1989. Persistent bleeding problems followed the surgery, and the patient succumbed to recurrent intracranical bleeding on 3rd October 1989. B12 therapy was continued until 2nd October 1989, and there was no evidence of toxicity. The patient's liver remained clinically normal throughout B12 therapy.

Patient 12

51-year old female with metastatic adenocarcinoma of breast diagnosed following lumpectomy of breast December 1987. Extensive pulmonary metastases evident, and excellent response to chemotherapy January-July 1988. Development of multiple cutaneous metastases May-June 1989 with gradual progression, particularly in scalp.

Cyanocobalamin therapy commenced on 22nd August 1989 at a dosage of 20mg parenterally once daily. Baseline liver function tests and abdominal ultrasound were normal, and chest X-ray was reported as normal with no evidence of pulmonary metastases (poor quality film, however). A mild elevation was evident in serum enzyme levels of alkaline phosphatase and GGT by 27th September 1989, with deveopment of respiratory symptoms (cough and slight dyspnoea). X-rays and CT scan of the chest on 4th October 1989 showed a (R) pleural effusion with a small metastasis in the mid-zone of the (L) lung, and also the (L) adrenal gland. A large (L) axillary mass was also detected, but no abnormality was demonstrated in the liver.

The B12 dosage was increased to 20mg parenterally twice daily from 30th October 1989 and further mild elevation of alkaline phosphatase and GGT levels was evident on 15th and 30th November 1989, indicating a tumour system in the lower range of sensitivity to B12. The patient was admitted to hospital on 8th December 1989 for investigation of respiratory status and persistent fatigue. Chest X-ray on admission shoed resolution of the small metastasis in the mid-zone of the (L) lung.

B12 therapy was suspended 10th December 1989, and conventional chemotherapy commenced 12th December

1989. Significant fall evident in serum alkaline phosphatase and GGT levels on 28th December 1989.

B12 therapy recommenced on 2nd January 1990 at a dosage of 20mg parenterally once daily, and continued in conjunction with conventional chemotherapy. This has resulted in considerable alleviation of toxic side effects from the chemotherapy. Overall, there has been marked regression in most of the cutaneous metastases and in particular the large (L) axillary mass, which is now barely palpable. The patient has been free of respiratory symptoms for the past 2 months.

Patient 13

HIV positive since 1984, with gradual deterioration in immune function consistent with chronic HIV infection. Lymphocyte marker studies of 16th January 1989 showed a depressed T4 lymphocyte count of 384 and an abnormal T4:T8 ratio of 0.52, with signs of bone marrow suppression and mild anaemia. Just prior to commencement of B12 therapy there was marked fatigue evident on clinical examination, and signs of HIV encephalopathy with some confusion, and some impairment of memory and concentration.

Cyanocobalamin therapy was commenced on 16th February 1989 at a dosage of 25mg parenterally once daily. There was resolution of fatigue and cerebral signs within one month of starting B12 therapy. T4:T8 ratios improved to 0.76 on 26th May 1989 and 0.94 on 25th July 1989. T4 count had improved to 494 with resolution of anaemia by 17th October 1989, and T4:T8 ratio was 0.46. There has been fairly rapid fluctuation of T4 counts, T4:T8 ratios and haemoglobin levels since October 1989, but he remains

well and able to pursue normal activities.

Patient 14

HIV positive since 1987, with Kaposi's sarcoma diagnosed on skin biopsy February 1989.

Cyanocobalamin therapy commenced on 31st March 1989 at a dosage of 25mg parenterally daily. Slow regression evident in scattered, small cutaneous KS lesions initially during first 4 months of therapy, followed by slow progression. B12 dosage was increased to 25mg parenterally twice daily on 21st September 1989, with subsequent slow regression evident again in KS lesions. T4:T8 ration improved from 0.53 on 14th March 1989 to 1.17 on 4th August 1989, and T4 counts from 336 to 471 on those dates respectively. T4 count 420 and T4:T8 ration 0.68 on 1st November 1989.

Patient 15

Rheumatoid arthritis definitively diagnosed in 1984, despite negative rheumatoid factor on blood testing. The patient was treated with naproxen only over past few years. RA Latex and Rose-Waaler tests negative, but C-reactive (complement-reactive) protein 90 times the upper limit of normal on blood tests of 20th February 1989. X-rays of both hands and feet on 23rd March 1989 showed changes typical of rheumatoid arthritis.

Cyanocobalamin therapy commenced on 17th Mach 1989 at a dosage of 20mg parenterally once daily, decreasing to 20mg three times weekly on 16th June 1989, and further to 250 micrograms orally twice daily on 21st September 1989. C-reactive protein returned to normal values by 21st April 1989, with marked

improvement in symptoms and signs of rheumatoid arthritis in hands and feet after 6 weeks of therapy. The patient was able to resume playing tennis after 3 months of therapy. There has been no subsequent elevation of C-reactive protein on reduced dosages as above.

Patient 16

Seropositive rheumatoid arthritis was diagnosed July 1987, followed by intermittent therapy with d-penicillamine and prednisone. Baseline pathology on 22nd June 1989 showed positive RA Latex and Rose-Waaler (1:16) tests with moderately elevated C-reactive protein. X-rays of both hands and feet on 23rd June 1989 showed mild changes typical of rheumatoid arthritis.

Cyanocobalamin therapy commenced on 6th July 1989 at a dosage of 20mg parenterally daily, decreasing to 20mg parenterally 3 times weekly from 31st October 1989. Evidence of seroconversion occurring was evident on 8th August 1989 with negative Rose-Waaler and mildly elevated C-reactive protein, but positive RA Latex. Seroconversion was complete on 11th September 1989 with negative RA Latex. Complete resolution also of symptoms and signs of rheumatoid arthritis in hands and feet was apparent by 11th September 1989. RA Latex has remained negative despite reduction in dosage as above. Persistent mild elevation of C-reactive protein may well be related to low-grade biliary inflammation due to recently diagnosed gallstone (there has been evidence of mildly elevated liver function tests since 1983).

Patient 17

Systemic lupus erythematosus with significant renal involvement was diagnosed in 1983. Regular treatment with prednisone and intravenous cyclophosphamide resulted in chronic respiratory infection due to immunosuppression. Cyclophosphamide and prednisone therapy was suspended June-July 1989, and cyanocobalamin therapy commenced on 23rd August 1989 at a dosage of 25mg parenterally twice daily, with rapid resolution of chronic respiratory infection. Baseline pathology on 17th August 1989 showed ANF positive at 1:80, DNA binding positive at 71%, and in particular abnormally low complement fractions C3 and C4, which had been persistently low since diagnosis in 1983.

Prednisone and IV cyclophosphamide therapy was reinstated with exacerbation of SLE in November 1989. B12 dosage decreased to 25mg once daily from 17th November 1989, with no recurrence of respiratory infection to date. C3 and C4 levels were normal on 7th February 1990, for the first time since diagnosis in 1983 (also confirmed by recent results). DNA binding on 7th February 1990 was 1:10, which correlates well with results of 17% in February 1990, the lowest level recorded since diagnosis.

Patient 18

16-plus-year history of auto-immune muscle wasting disease (various diagnoses entertained) with persistent high elevation of creatine kinase levels except when under effective treatment. Baseline pathology on 29th March 1989 showed positive smooth muscle antibody (titre 1:40) with elevated muscle enzymes CK (711) and LDH (337).

Cyanocobalamin therapy was commenced on 7th April

1989 at a dosage of 25mg parenterally once daily, decreasing to 15mg parenterally once daily on 16th June 1989, and increasing again to 25mg parenterally once daily from 5th October 1989. Smooth muscle antibody was negative by 9th May 1989 and there has been no recurrence to date. Significant decreases were evident in CK and LDH levels by 6th June 1989, but with slow fluctuations of an episodic nature evident since then. Anti-skeletal muscle antibody negative on 1st August 1989.

From the results described above, it appears that the progress of cyanocobalamin therapy against neoplastic disease is reflected in the patient's levels serum alkaline phosphatase, alanine transminase and gamma GT (GGT) levels, which may readily be measured by standard liver function tests. Successful treatment seems to be characterised by a rapid rise in the serum levels of these enzymes as the enzymes are released during the destruction of the tumour cells, with the levels peaking and declining as regression of the tumour is achieved.

There appears to be wide differences in susceptibility of different tumours to the cyanocobalamin therapy, and it is believed that these differences can be explained according to the function of the tumour's histological tissue of origin. Cyanocobalamin therapy appears to be most successful where the tumour cells have a moderate or high metabolic B12 requirement, for example in enzyme-secreting cells.

For tumours with a low requirement of B12, and therefore a low sensitivity to cyanocobalamin, the cyanocobalamin therapy may be used as an adjunct to conventional chemotherapeutic agents such as cyclophosphamide, mitozantrone, vincristine or

epirubicin. Apart from enhancing the tumouricidal effect of conventional chemotherapy through its own anti-tumour effect, the cyanocobalamin treatment appears to have the additional advantage of considerable alleviation of the toxic side effects of the chemotherapy.

Immune system related diseases also display differing sensitivities to cyanocobalamin therapy. Rheumatoid arthritis, for example, is an auto-immune disease which is giving every indication of being highly sensitive to cyanocobalamin, and is probably due purely to abnormal clones of antibody-secreting B lymphocytes. Systemic lupus erythematosus, on the other hand, is an auto-immune disease which seems to have a low order of sensitivity to cyanocobalamin, and is therefore probably due almost entirely to abnormal populations of T lymphocytes. B lymphocytes or plasma cells are short-lived "factory" cells in large scale antibody production, and therefore have a relatively high and ongoing requirement for Vitamin B12 in order to synthesise thse protein structures. T lymphocytes have more or less a scouting function in immune response, and pass back immunological intelligence to the reticulo-endothelial system, to facilitate the large scale production of effective antibodies by B lymphocytes (plasma cells). T cells are long-lived and rarely divide, with a resultant very low requirement for Vitamin B12. Thus, diseases due to abnormal clones of B lymphocytes appear to have high sensitivity, while disease due to abnormal T lymphocyte populations have low sensitivity.

Patient 18 is showing definite evidence of the co-existence of both abnormal clones of B cells and abnormal populations of T cells in the production of her auto-immune muscle wasting disease, in that there

is clear evidence of both high-sensitivity and low-sensitivity components to cyanocobalamin therapy in her condition.

In addition to the discovery of the effects of high dosages of cyanocobalamin, the applicant has developed a dermatological preparation of cyanocobalamin for topical use, and it is proving effective in the treatment of the premalignant conditions of solar keratosis/hyperkeratosis, with excellent cosmetic results. The preparation consists of cyanocobalamin 1% in a penetrant vehicle, for example dimethyl sulphoxide.

Although the Examples refer to methods of treatment of disease, it is believed that cyanocobalamin therapy may also be useful as a method of prophylaxis suitable for those in high risk groups for neoplastic or immune system related disease, for example for persons having been exposed to inhalation of asbestos fibres.

Cyanocobalamin was determined to be non-toxic in extremely high doses, shortly after its isolation in 1948 (22), and is well tolerated and extremely safe when given by the intramuscular or deep subcutaneous route (23,24). Tissue uptake, storage and utilisation depend on the availability of transcobalamin II in the plasma, and doses in excess of 100mcg (ug) saturate this transport system and tissue binding sites. The excess cyanocobalamin is then rapidly cleared from the plasma by glomerular filtration and excreted in the urine, the majority within 8 hours and more than 90% within 24 hours (23, 24).

Sustained high dosage results in augmented renal excretion (18). It would seem that the plasma acts as

a temporary reservoir of cyanocobalamin following parenteral administration, with only a minority of intracellular Vitamin B12 appearing as cyanocobalamin 24 hours after injection (2). This transient plasma store of cyanocobalamin is partly bound to transcobalamins I and III, awaiting utilisation and avoiding renal excretion whilst bound (21). Radioactive Vitamin B12 studies have confirmed that the liver is the main repository of Vitamin B12 in humans, and that the relative distribution of Vitamin B12 among the organs is similar, regardless of the absolute Vitamin B12 content of the liver (23). Results of animal studies with Vitamin B12 may not be applicable to man, because of species differences in the organ distribution of Vitamin B12 (23).

Therapeutic applications may be found in connection with animals however, and veterinary application of the therapeutic method of the present invention are within the scope of the present disclosure.

The applicant has maintained patients on dosages of cyanocobalamin in the range 1mg-100mg daily by intramuscular/deep subcutaneous injection for months on end, with no evidence of untoward side effects. The main consideration seems to be the development of discomfort at injection sites in markedly cachectic patients because of volumetric impositions, and this has necessitated cessation of therapy in only one patient, who was extremely cachectic and in an advanced, terminal stage of malignant disease.

Continuous intravenous infusions have been conducted on a total of 12 occasions in 10 different patients, with dosages of cyanocobalamin, ranging from 24-100mg daily for periods ranging from 1-30 days. The only untoward event with these infusions was the

development of a phlebitis in the infused arm of one of the patients after 24 hours. This necessitated administration of the dosage (40mg daily) by frequent intramuscular injection for a further 4 days, and there was no further problem on cessation of the intravenous drip after the first day. As Betadine was used as a skin cleanser by the anaesthetist who installed the drip, it was impossible to determine whether the phlebitis was caused by the cyanocabalamin, the Betadine or the mere act of intravenous infusion of 5% dextrose.

No evidence of renal toxicity was found in a patient infused with cyanocobalamin 24mg daily for 4 days, despite the fact that he had virtually only one functional kidney at the time of the infusion, and following which his serum creatinine returned to normal, having been mildly elevated prior to infusion. No evidence of hepatoxicity was found in a patient infused with cyanocobalamin 100mg daily for 5 days, nor was there evidence of renal toxicity, in that his routine urinalysis remained normal (no proteinuria or haematuria was detected), despite the fact that he had extensive liver metastases. Another patient with extensive liver metastases was infused with cyanocobalamin 80mg daily for 5 days, and maintained steady liver function tests and normal routine urinalyses. These patients all showed visible improvement in their general condition with the infusions, and, within 6 hours of cessation of infusion, their urine had returned to normal colour, from being bright pink during the infusions. Radio-isotopic assay of 24-hour urinary excretion of Vitamin B12 has been carried out in two other patients, both with low-affinity tumours. One patient, on dosage of cyanocobalamin 1mg intramuscularly daily, showed 24-hour excretion of 95%, and the other, on dosage of

cyanocobalamin 2mg intramuscularly daily, showed 24-hour excretion of 91%.

There has been an extremely low incidence of definite adverse reaction to cyanocobalamin reported in Australia over the past 20 years, and only a very low incidence of possible adverse reaction reported in the same period (25, 26). The most likely adverse reactions, even in the very high dosages used by this investigator, would seem to be hypokalaemic or allergic in nature. Hypokalaemia is easily counteracted by oral potassium supplements, which are routinely used as a part of this therapy. Crystalluria is probably extremely unlikely in parenteral dosages of less than 5 grams daily, based on the solubility of cyanocabalamin in water of 1:80 (23, 24).

Cyanocabalamin has a molecular weight of 1355.4 (23), and calculation of the real osmolality of 1% solution yields a value of 7.38 mmol/litre, with an osmotic pressure of 0.19 atmosphere at 38oC. In comparison, human serum has a real osmolality of 302.1 mmol/litre, with an osmotic pressure of 7.66 atmospheres at 38oC (26). It is difficult to envisage such an osmotically weak solution of cyanocobalamin, an essentially physiological substance, causing problems of toxicity or irritation locally at sites of injection. This assumption is supported by observations on the dermatological preparation of cyanocobalamin referred to above, and its topical use in 8 patients with multiple lesions has produced no evidence of cutaneous or subcutaneous toxicity or irritation, or systemic toxicity. Cyanocobalamin is saturated in aqueous solution at 1.25% concentration, but its use at 1% concentration will avoid significant problems with crystallisation at low temperatures.

References:

1. Bodian M. Neuroblastoma. Pediat. Clin N. Amer. 1959, 6, 2, 449-472.
2. Bodian M. Neuroblastoma. Arch. Dis. Childh. 1963, 38, 606- 619.
3. Langman M. J. S. Arch. Dis. Childh. 1970, 45, 385.
4. Sawitsky A. and Desposito, F., J. Pediat. 1965, 67, 99.
5. Welsh I., B.M.J. Nov. 22, 1952, 1133.
6. Bodian M. and Rigby C.C., Brit. J. Cancer, 1963, 17: 90.
7. Matthews D. M. and Linnell J. C. B.M.J. 1979, 2, 533.
8. Abe T. et al. Brit. J. Haematol., 1975, 31, 493.
9. Datta S. P. and Ottaway J. H. Aids to Biochemistry. 6th Ed. (1962) 239.
10. White A. et al. Principles of Biochemistry 2nd Edition (1959), 239.
11. Balaban-Malenbaum G. and Gilbert F. Advances in Neuroblastomer Research, 1980, 59.
12. Biedler J. L. Advances in Neuroblastomer Research, 1980, 81.
13. Balaban-Malenbaum G. and Gilbert F. Advances in Neuroblastomer Research, 1980, 97.
14. Broduer G.M. Advances in Neuroblastomer Research, 1980, 81.
15. Moorhead and Evans A.E. Advances in Neuroblastomer Research, 1980, 109.
16. Cory S. et al. EMBO J. 2: 213-216 (1983)
17. Adams J.M. et al. Proc. Natl. Acad. Sci. 79: 6966-6970 (1982).
18. Adams J.M. et al. Proc. Natl. Acad. Sci. 80: 1982-1986 (1983).
19. Cory S. et al. EMBO J. 2: 697-703 (1983).
20. Cory S. et al. The Walter & Eliza Hall Institute of Medical Research Annual Review 1983-84, 122-123.

21. Gerondakis S.D. et al. Cell 36: 973-982 (1984).
22. Matthews D.M. and Linnell J.C. B.M.J., 2, 533-535.
23. Goodman and Gilman The Pharmacological Basis of Therapeutics. (6th Edition), 1980.
24. Martindale: The Extra Pharmacopoeia. (26th Edition), 1973.
25. Aust. Drug Evaluation Committee. Report of Suspected Adverse Drug Reactions No. 5, August 1964-September 1980.
26. Aust. Drug Evaluation Committee. Update to report of Suspected Adverse Drug Reactions No. 5, October 1980-September 1983.
27. The Role of Vitamin B12 and its Transport Globulins in the Production of Antibodies. Hitzig, W.H. and Kenny, A.B. J. Clin. Exp. Immunol. (1975) 20, 105-111.
28. The Uptake of Vitamin B12 by Human Lymphocytes and the Relationships to the Cell Cycle. Hall, C.A. J. Lab. Clin. Med., January 1984 Vol. 103, No. 1.
29. Effects of Methyl-B12 on the in-Vitro Immune Functions of Human T Lymphocytes. Sakane, T. et al. J. Clin. Immunol. (1982) Vol. 2, No. 2.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of treatment or prevention of neoplastic disease or immune-system related disease in a human or animal in need of such treatment or prevention comprising administering an average daily dosage (as herein defined) of from 5 to 1000mg of cyanocobalamin to the human or animal.

2. A method of treatment or prevention according to claim 1 wherein the average daily dosage (as herein defined) of cyanocobalamin is from 10 to 200mg.

3. A method of treatment or prevention according to claim 2 wherein the average daily dosage (as herein defined) of cyanocobalamin is from 20 to 50mg.

4. A method of treatment or prevention according to any of claims 1 to 3 wherein the cyanocobalamin is administered at least thrice per week.

5. A method of treatment or prevention according to claim 4 wherein the cyanocobalamin is administered at least once per day.

6. A method of treatment or prevention according to any of claims 1 and 5 wherein the cyanocobalamin is administered parenterally.

7. A method of treatment or prevention according to claim 6 wherein the cyanocobalamin is administered in an aqueous solution containing from 0.5% to 1.1% by weight of cyanocobalamin.

8. A method of treatment or prevention according to claim 6 wherein the solution contains about 1% by

weight of cyanocobalamin.

9. A method of treatment or prevention of neoplastic disease according to any of claims 1 to 8.

10. A method of treatment or prevention according to claim 9 wherein the neoplastic disease is a tumour of cells having a moderate or high sensitivity to cyanocobalamin.

11. A method of treatment or prevention according to claim 10 wherein the neoplastic disease is a tumour of cells having a moderate or high metabolic requirement of cyanocobalamin.

12. A method of treatment or prevention according to claim 11 wherein said cells have a protein structure synthesis function.

13. A method of treatment or prevention according to claim 12 wherein said cells have an enzyme- or hormone- synthesis function.

14. A method of treatment or prevention according to claim 9 wherein the tumour cells have reduced intracellular cyanide detoxification mechanisms compared to the tumour's histological tissue of origin.

15. A method of treatment or prevention according to claim 14 wherein the tumour cells substantially lack intracellular cyanide detoxification enzymes.

16. A method of treatment or prevention according to claim 15 wherein the tumour cells lack the gene for thiosulphate:cyanide sulphurtransferase.

17. A method of treatment or prevention according to claim 14 wherein the tumour cells have lower levels of cytochrome oxidase than the tumour's histological tissue of origin.

18. A method of treatment or prevention according to claim 9 wherein the cyanocobalamin is administered in addition to conventional chemotherapy.

19. A method of treatment or prevention of immune-system related disease according to any of claims 1 to 8.

20. A method of treatment or prevention according to claim 19 wherein the immune system related disease is immunodeficiency disease.

21. A method of treatment or prevention according to claim 20 wherein the immunodeficiency disease is Acquired Immune Deficiency Syndrome.

22. A method of treatment or prevention according to claim 19 wherein the immune system related disease is auto-immune disease.

23. A method of treatment or prevention according to claim 19 wherein the immune system related disease is caused at least in part by abnormal populations of lymphocytes.

24. A method of treatment or prevention according to claim 23 wherein the immune system related disease is caused at least in part by abnormal clones of B lymphocytes.

25. A method of treatment or prevention according to claim 23 or 24 wherein the lymphocytes have a reduced

intracellular cyanide detoxification mechanism.

26. A method of treatment or prevention of rheumatoid arthritis according to any of claims 1 to 8.

27. A method of treatment of neoplastic disease or immune system related disease in a human or animal in need of such treatment comprising administering an average daily dosage (as herein defined) of from 5 to 1000mg of cyanocobalamin to the human or animal until symptoms of the disease show improvement and thereafter administering cyanocobalamin at an average daily dosage of less than 5mg.

28. A method of treatment of neoplastic disease or immune system related disease in a human or animal in need of such treatment comprising administering an average daily dosage (as herein defined) of from 5 to 1000mg of cyanocobalamin to the human or animal at a frequency of at least once per day until symptoms of the disease show improvement and thereafter administering cyanocobalamin at a reduced frequency.

29. A method of treatment of neoplastic disease substantially as herein described with reference to any one of Examples 1, 2, 4, 6, 7, 8, 9, 11 and 12.

30. A method of treatment of immune system related disease substantially as herein described with reference to any one of Examples 3, 5, 10, 13, 14, 17 and 18.

31. A method of treatment of rheumatoid arthritis substantially as herein described with reference to any one of Examples 15 and 16.

DATED this 5th day of November, 1991.

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By his Patent Attorneys
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