A phase I trial of hypoxoside as an oral prodrug for cancer therapy — absence of toxicity

B. J. Smit, C. F. Albrecht, R. W. Liebenberg, P. B. Kruger, M. Freestone, L. Gouws, E. Theron, P. J. D. Bouic, S. Etsebeth, P. P. van Jaarsveld

Objective. To assess the toxicity of hypoxoside taken orally by 24 patients with lung cancer.

Design. Open study with patients taking 1 200 - 3 200 mg standardised *Hypoxis* plant extract (200 mg capsules) per day divided in 3 doses in order to maintain metabolite blood levels near 100 µg/ml.

Participants and setting. Patients with histologically proven squamous, large-cell or adenocarcinoma were hospitalised initially at the radiation oncology ward, Karl Bremer Hospital, Bellville, W. Cape. Thereafter they returned every 2 weeks for full clinical examinations.

Methods. Routine biochemical and haematological measurements were done. Patients underwent regular full clinical examinations including radiographs and computed tomography scanning according to the discretion of the principal investigator.

Results. Nineteen patients on hypoxoside therapy survived for an average of 4 months with progression of their primary tumours and metastases, while 5 survived for more than a year. One of them survived for 5 years and histological examination of the primary lesion showed absence of cancer. No toxic effects, in clinical examinations or biochemical or haematological measurements, were found that could be ascribed to the ingestion of hypoxoside. Only one occasion of possible drug intolerance, with anxiety, nausea, vomiting and diarrhoea, was noted.

Departments of Radiotherapy and Pharmacology, University of Stellenbosch, Tygerberg, W. Cape

B. J. Smit, M.MED.

C. F. Albrecht, PH.D.

P. B. Kruger, PH.D.

L. Gouws, M.MED.

E. Theron, M.SC.

P. J. D. Bouic, PH.D.

S. Etsebeth, M.SC.

P. P. van Jaarsveld, PH.D.

Essential Sterolin Products, Halfway House, Gauteng

R. W. Liebenberg, B.COMM.

M. Freestone, R.N.

Conclusion. **The absence of toxicity warrants further investigation of hypoxoside as an oral prodrug, especially in patients with slow-growing necrotising tumours that are inoperable and have high concentrations of β-glucuronidase and sulphatase as well as a high sensitivity for rooperol.**

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Fig. 1 summarises the major results of our research on hypoxoside.[1-3] It is non-toxic to cancer cells in tissue culture, but when deconjugated to rooperol significant cytotoxicity is found at relatively low concentrations. After oral ingestion no hypoxoside or rooperol are found in the circulation.[3] Only phase II metabolites (glucuronides and sulphates) of rooperol are present. Like the glucoside, the conjugated metabolites are also non-toxic to cells in tissue culture, but they can be activated by treatment with glucuronidase.[1] Since it is known that certain tumours contain relatively high levels of glucuronidase[4-7] activation of rooperol metabolites at the site of the tumour therefore seemed to be an attractive approach to achieve selectivity in cancer chemotherapy.

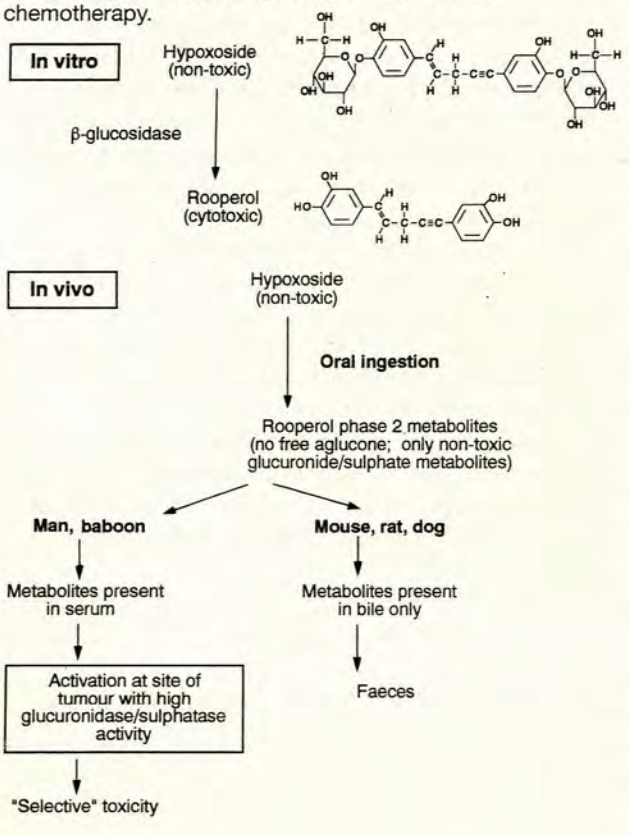

Figure 1

Hypoxoside as a non-toxic prodrug

Fig. 1. Hypoxoside as a non-toxic prodrug for cancer therapy.

The quantitative sequestration of rooperol metabolites in the bile of experimental animals such as the mouse, rat and dog,[1] however, excluded their use as an *in vitro* anticancer model for hypoxoside. We therefore obtained permission from our Ethics Committee and the Medicines Control Council to conduct a phase I clinical trial in 24 lung cancer patients for whom no alternative therapy was available. The first part of the trial included a study of the pharmacokinetic behaviour of the metabolites.[8] A wide interpatient variation in concentration-time relationships was found which can be explained by active enterohepatic recirculation, a lag phase in absorption and saturable conversion of hypoxoside to rooperol in the colon. However, the elimination of the metabolites follows predictable first-order kinetics with acceptable half-life values (20 - 50 hours). In the toxicity part of the study, which we wish to report here, maintenance doses were individualised for patients in order to obtain metabolite levels near 100 μg/ml. This concentration was found to be cytotoxic *in vitro* after enzymatic activation.[1]

Material and methods

Medication and monitoring of serum metabolite levels

Hypoxoside was supplied by Essential Sterolin Products as a standardised plant extract in capsule form, each capsule containing 200 mg of plant extract. Quality control was assured by high-performance liquid chromatography (HPLC) as described earlier.[3,8] The hypoxoside content of the standardised plant extract ranged from 50% to 55%.[8] Routine monitoring of metabolite serum levels was done using the HPLC methodology described by Kruger *et al*.[9]

Patient eligibility

Patients above 21 years of age with histologically proven squamous cell, large-cell or adenocarcinoma of the bronchus were included after informed consent according to the Declaration of Helsinki had been obtained. Patients with impaired renal, hepatic or cardiac function or inadequate performance status (H2 or lower) and those who, in the opinion of the investigator, had less than 3 months to live were excluded. The only concomitant anticancer therapy allowed was palliative radiotherapy.

Study design

The patients were hospitalised at the radiation oncology ward, Karl Bremer Hospital, Bellville, W. Cape, for the duration of the pharmacokinetic studies. During the long-term therapy stage they returned to hospital every 2 weeks and underwent full clinical examinations including radiographs and computed tomography (CT) scanning according to the discretion of the principal investigator.

Laboratory analyses

Biochemical and haematological analyses were performed by the chemical pathology and haematology laboratories at Tygerberg Hospital, Tygerberg, W. Cape.

Results

Patient details

Of the 24 patients who entered the trial 14 were male and 10 female, with ages ranging from 43 to 77 years (mean 56,4 years). Histologically proven diagnoses were adenocarcinoma (9 patients), large-cell carcinoma (9 patients) and squamous cell carcinoma (6 patients). The average survival time for 19 of the patients was 4 months after entering the trial, which agreed with their prognosis without any therapy. Most of them developed metastases.

Table I lists 5 patients who survived longer than expected. Two of them (patients 23 and 24) showed significant arrest of their tumour sizes. Their clinical status will be discussed in more detail (*vide infra*).

Long-term therapy

Because the serum metabolite concentrations showed considerable interpatient variation[8] owing to apparent zero-order formation of rooperol, the maintenance dose was adjusted when necessary for each patient during hospital visits in order to achieve combined metabolite blood levels near 100 µg/ml. According to *in vitro* experiments[1] this concentration was considered adequate for activation of the metabolites to tumouricidal rooperol concentrations. The minimum and maximum maintenance doses required were 1 200 and 3 200 mg standardised plant extract per day divided into 3 equal doses every 8 hours. For most patients a daily dose of 2 400 mg plant extract (4 capsules 3 times a day) was sufficient.

Biochemical and haematological data

Figs 2 - 4 provide summaries of all the biochemical and haematological data collected from the patients while they were on long-term therapy. The vertical bars in the figures represent the mean ± 1 standard deviation (SD) of the value measured during intervals of approximately 30 days.

Since 19 of the 24 patients survived less than 1 year it must be realised that the data presented in Figs 2 - 4 are the trends of the mean values in a decreasing population. Table II shows the distribution of surviving patients at monthly intervals and hence the sample sizes at each time.

Table II. Number of patients in the trial at various time intervals (refer to Figs 2 - 4)

Time (d)	No.
30	20
60	16
90	15
120	13
150	10
180	7
210	5
400	4
600	3

Values of the liver enzymes alanine transaminase (ALT), aspartate transaminase (AST) and lactate dehydrogenase (LD) stayed within normal limits, while mean values of enzymes sensitive to metastases were above normal. Several patients also abused alcohol periodically (Fig. 2).

Table I. Details of patients entered in the trial who survived longer than expected

Patient no.	Age (yrs)	Sex	Diagnosis	Survival time (mo.) From diagnosis	Survival time (mo.) On trial	Medical treatment before entering trial	Major clinical events in course of trial
20	47	F	Large-cell carcinoma	22	19,5	50 Gy ^{60}Co	Partial collapse of lung with small pleural effusion. Clinically well after 5 mo. Developed widespread metastases
21	54	M	Large-cell carcinoma	15	13,3	Partial resection	Clinically well except for alcohol abuse as reflected in liver enzyme levels. Elected to leave trial
22	57	M	Squamous carcinoma	15,5	12	Inoperable tumour	Gradual progression of tumour. 20 Gy ^{60}Co on days 330 - 335 resulted in a marked reduction of tumor size. Developed liver metastases
23	77	M	Squamous carcinoma	42	35	50 Gy ^{60}Co	Clinically well except for dyspnoea on exertion. Death due to cerebrovascular haemorrhage
24	49	M	Adeno-carcinoma	63	60	25 Gy ^{60}Co	Large tumour with destruction of 3 - 5 ribs. Clinically well except for sporadic alcohol abuse. CT scan showed reversal of rib destruction after 2 yrs. Died of TB pneumonia. Histological examination of autopsy material showed absence of cancer

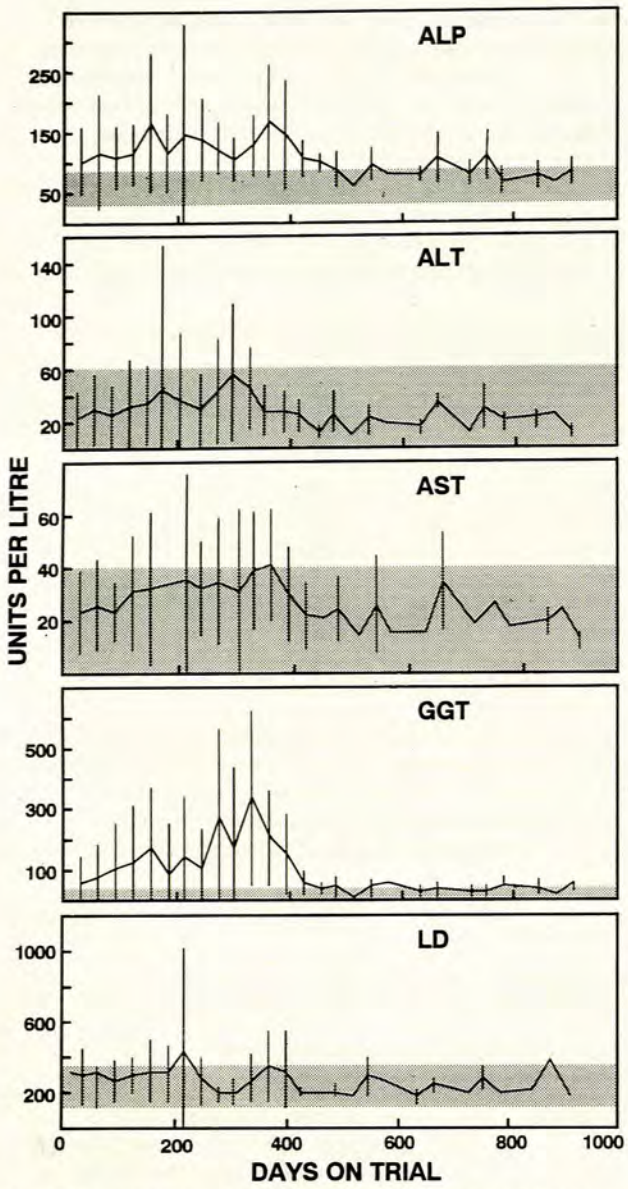

Fig. 2. Liver enzyme activity of all the patients in the trial. Each point represents the average of all values obtained on a monthly basis with 1 SD shown as a vertical bar. The shaded areas depict the upper and lower normal limits for the population.

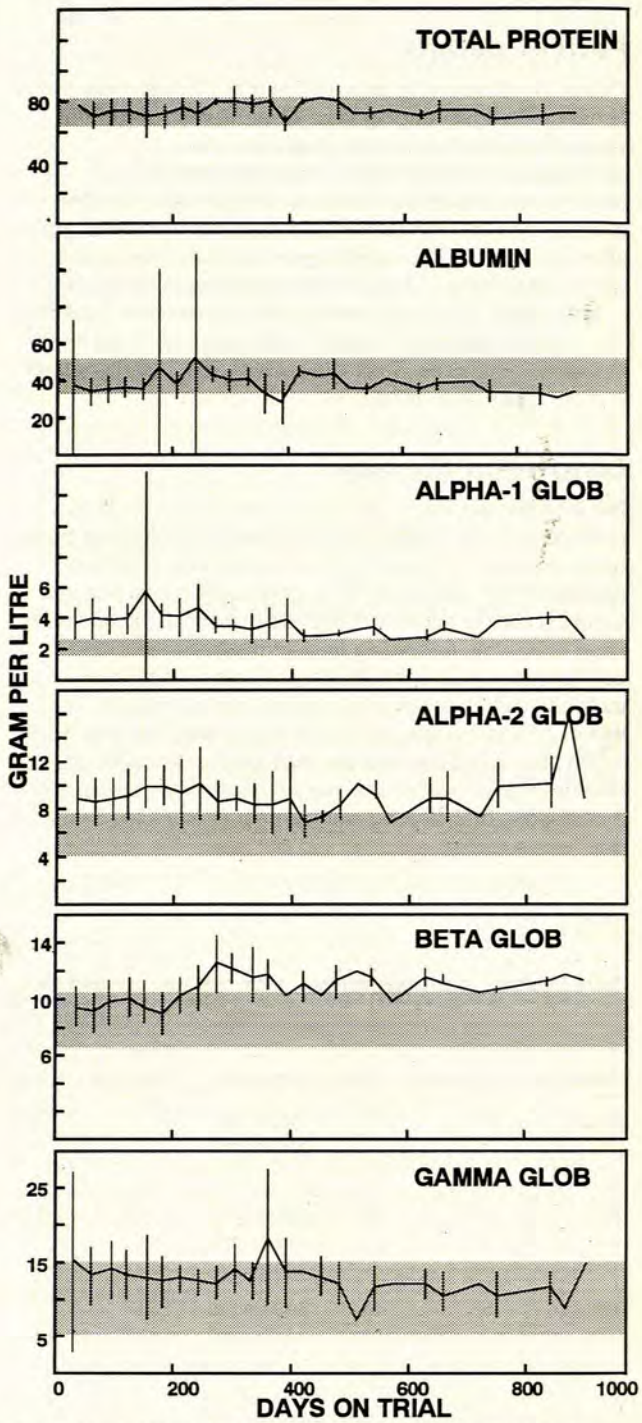

Fig. 3. Serum protein concentrations of all the patients in the trial, plotted as in Fig. 2.

Fig. 3 shows the trend observed with serum proteins. The relatively low albumin and increased acute-phase protein concentrations (notably α-globulins) are clearly linked to the disease state of the patients. Serum electrolytes (Na^+, K^+, Cl^-, Ca^{2+}) and serum creatinine and urea concentrations were mostly within normal limits except for sporadic deviations during terminal phases.

Fig. 4 summarises the haematological profiles of the patients. These values are usually grossly affected by chemotherapeutic agents used in the treatment of cancer, but it is clear that hypoxoside had no negative effect on them. The haemoglobin concentration and the white cell count stayed constant, while the neutrophil count probably increased marginally and the lymphocyte count decreased slightly. The platelet count stayed remarkedly constant.

The absence of any adverse effect of hypoxoside on the values reported above has been verified by specialists in the Departments of Chemical Pathology and Haematology at Tygerberg Hospital.

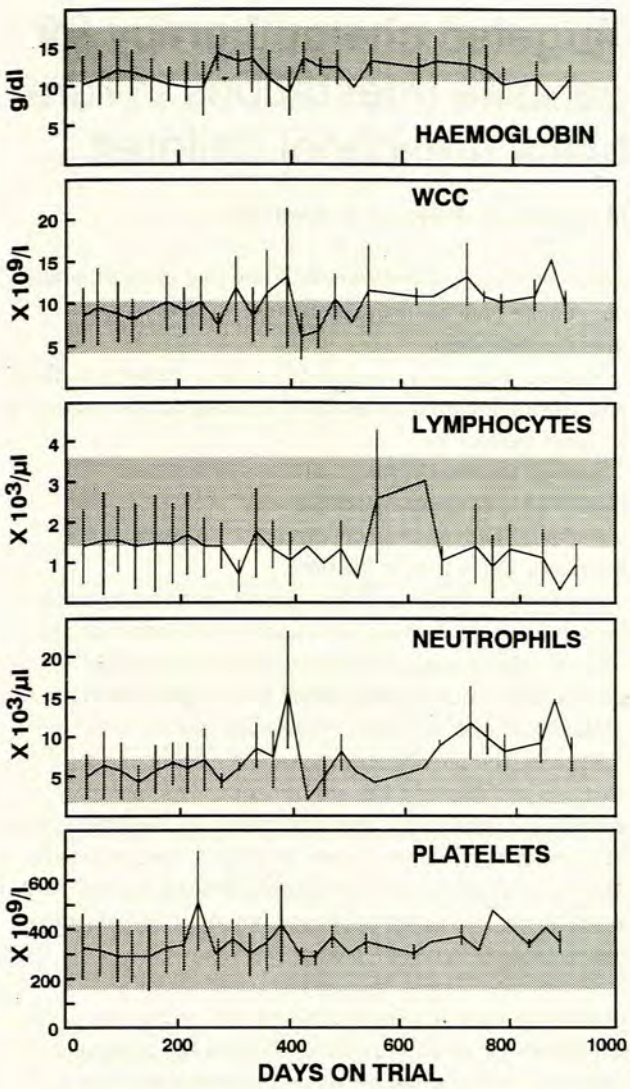

Fig. 4. Haematological profile of all the patients in the trial, plotted as in Fig. 2.

Other measurements

Regular measurements of blood pressure and temperature showed no abnormalities. Body mass also stayed constant except when patients developed cachexia in the terminal phase. Serial electrocardiograms also failed to demonstrate any evidence of cardiotoxicity of hypoxoside as confirmed by the Cardiology Department at Tygerberg Hospital.

Side-effects

One patient experienced possible drug intolerance on day 171 of the trial when the serum concentration of hypoxoside metabolites rose to 163 µg/ml. Anxiety, nausea, vomiting, diarrhoea, dyspnoea and rigors were associated with a doubling in the LD and alkaline phosphatase values. The patient stopped taking the drug and the symptoms subsided after 4 - 6 hours. The dose was then reduced from 2 400 mg to 1 200 mg per day and the drug was tolerated without further incidents for another 36 days, after which the patient died of cardiorespiratory failure.

Clinical status of patients 23 and 24

Since these 2 patients survived the longest while on hypoxoside they underwent a full neurological examination together with the normal regular clinical examinations during the 30th month. No evidence of neurotoxicity resulting from hypoxoside ingestion was found after clinical evaluation by the Neurology Department at Tygerberg Hospital.

Fig. 5 shows chronological CT scans of the tumour in case 24. It is clear that the initial lesions of the ribs reversed to a large extent, together with a reduction in original tumour mass. During this period the alkaline phosphatase levels fell to normal. The patient died of TB pneumonia resulting from tuberculosis after 5 years on hypoxoside therapy (approximately 1 g per day). Histological examination of tissue taken at autopsy showed that all organs (kidney, liver, bone marrow, colon, intestine, brain, spleen) were normal, and surprisingly no cancer could be detected in the fibrotic, cystic lesion in the lung.

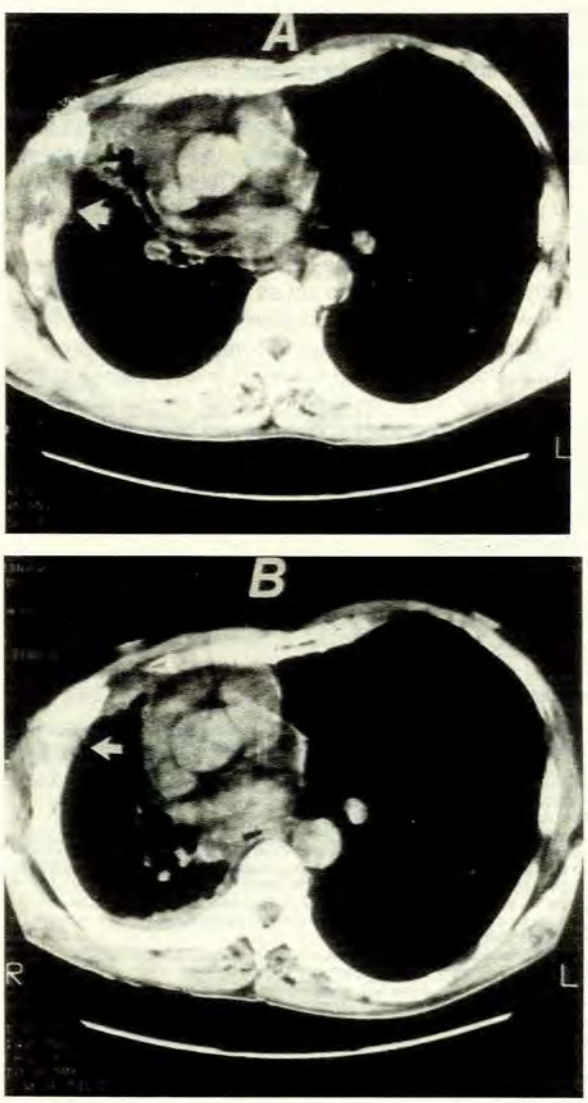

Fig. 5. Chronological CT scans of the tumour in case 24 (Table I), at 2 months (A) and 29 months (B) after institution of hypoxoside therapy. Destruction of ribs 3 - 5 by adenocarcinoma (thick arrow) **was reversed during therapy. Unique opacities were used as markers to select comparable scans.**

Discussion

Several reports published in the mid-1970s focused attention on high β-glucuronidase and sulphatase activities in experimental tumours.[4-7] The idea of designing a prodrug that would be activated by these enzymes at its site of action is therefore not new. However, a successful therapeutic prodrug has yet to be developed for human use. It has been shown that a mouse tumour with high β-glucuronidase activity was curable with glucuronide conjugates of aniline mustard formed *in vivo*.[4] However, the drug produced significant anticancer activity in only 5 of 78 patients studied.[7]

It is not the objective of a phase I clinical trial to reach a conclusion with regard to efficacy. In this trial 19 out of 24 patients survived as long as their estimated prognosis (4 months), while 5 survived longer than expected (12 months to 5 years). The patient who survived 5 years had no detectable metastases. It therefore seems possible that cancer patients who might benefit from hypoxoside are those with relatively slow-growing necrotising tumours that are inoperable and have high concentrations of β-glucuronidase and sulphatase as well as a high sensitivity for rooperol, as was found for the H552 human adenocarcinoma cell line.[1] Other types of cancer that merit investigation are tumours of the pancreas and bile ducts. There is a verifiable anecdotal case in which a patient with cancer of the pancreas on hypoxis plant extract is still alive 10 years after diagnosis. It could be argued that cancer of the pancreas might result in significant deconjugating enzyme release to activate the hypoxoside metabolites sufficiently for the aglucone to act as an effective cytotoxic agent. However, the major conclusion of this trial is that short- and long-term therapy (up to 5 years) with relatively high hypoxoside dosages did not result in any obvious toxic effect. This is not only important for further clinical studies on the anticancer potential of hypoxoside; we have also found that the sulphated metabolites of rooperol show significant *in vitro* and *in vivo* anti-HIV activity. A report on its stabilising effect on CD4 cell numbers in HIV-positive patients will be presented separately.

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