

Volume XVII Issue II Version I

Ndarabu¹¹ Universite de Kinshasa.*Received: 8 December 2016 Accepted: 5 January 2017 Published: 15 January 2017***Abstract**

Background: ART had substantially improved the quality of life of PLWHA, but cannot alone eradicate the HIV reservoirs neither fully restoring the impaired immune system functioning. Its life long duration is associated with the risk of resistant strain emerging and metabolic disorders, mainly in resources constrained settings. Objectives: To study the impact of an Immune modulator in PLWHA. Method: In this prospective study, we used a immune modulator, 6,6'-dithiodinicotinic acid (CPDS) in 34 Congolese PLWHA (study group) for a 2 years period opportunely compared to 60 PLWHA who underwent ARV (control group). Data were analyzed using Mixed models using IBM SPSS Statistics v. 20. Results: Both groups were comparable at the starting point. Globally, we observed a similar evolution of weight and the CD4 counts in both groups during the first 12 months period of study with a gradual increase and a peak by the 6th month. Immunotherapy group displayed higher values of CD4, CD8 lymphocytes and the CD4/CD8 ratio. There was no significant difference between risks of dying between both groups. RR: 0.953 0.67; 1.35 neither in the rate of hospitalization. Conclusion: This study suggests the benefit of immunotherapy in treatment of PLWHA and its possible association to ART.

*Index terms—***1 I. Introduction**

In spite of the declaration that the goal of anti retroviral treatment (ART) in 2015/16 is to prolong the patient's life and maintain the best possible quality of health and life 1, only few patients in the Democratic Republic of the Congo (DRC) and other developing countries have wide-scale access to ART.

Treating AIDS in the developing countries means working in a context of limited health-care infrastructures and resources, as well as technical, financial and human resources. The few resources available are mostly concentrated in capital cities. In addition, the ART is not accessible to many patients in Sub-Saharan countries. In DRC, the ART coverage was estimated at only 12% four years ago² and recently, prevalence of HIV infection among general population in the DRC is at 1.2% but only 101,324 HIV patients are under ART, which is an ART coverage of 32%³.

Additionally, when available ART stock runs out, skipping and interruptions of treatment are not uncommon. Adherence to ART is still a big challenge in our setting, with the subsequent risk of resistance and immune system impairment. In a study including Monkole's hospital, 20% of patients were found not adherent to ART mostly because of food insecurity⁴.

Undernutrition also may have short-and longterm effects on HIV-positive children. The short-term effects include impaired immunity, increased risk of opportunistic infections, morbidity, and mortality. The long-term effects include poor cognitive functioning, poor achievement of developmental milestones, and poor levels of education⁵.

Despite great progress globally against food insecurity, it remains a big challenge in Sub-Saharan countries, with a prevalence of 12,9%⁶. In the DRC, 43% of infants aged from 0 to 59 months display chronic malnutrition,

and 14% of women display chronic energy deficiency 3 . Immune functioning is one of the affected parameters in this circumstance 7 .

In Sub-Saharan Africa, HIV diagnosis and treatment are still made very late, leading to early and higher (20-50 times) mortality after the initiation of ART, compared to non-HIV related mortality. Patients who initiate ART at low CD4 counts remain at risk for opportunistic infections for a substantially longer period than patients starting ART at higher CD4 counts, increasing their risk for serious morbidity and death, with tuberculosis (TB) being the most common opportunistic illness. While information on underlying causes of death among people on ART is lacking in sub-Saharan Africa, one study found 86% of deaths in the first year following ART initiation to be HIV-related (CNS infections, TB, Kaposi's sarcoma, pneumonia, and mitochondrial toxicity), with 7% due to immune reconstitution syndrome 8 .

2 Volume XVII Issue II Version I

3 K

Although the treatment of HIV patients with antiretroviral drugs (ARV) has dramatically reduced mortality and morbidity, other studies revealed the need of new strategies with various means of enhancing and /or restoring the host's immune system 9,10,11 .

Cytotoxic T lymphocytes (CTLs) and Natural Killer cells (NK cells) can eradicate virus-infected target cells via the apoptosis process through the perforin/granzyme pathway 12 . In this study an immunomodulator was used to exploit this NK ability 13 , and could be indicative of the broader use of immunotherapy in HIV treatment combined with HAART 14 .

Even in recent years, in addition to ART, immunomodulatory treatment strategies have been investigated. Although repeatedly discussed as an alternative or supplement, these therapies lack proof of clinical benefit. An important example is the failure of the two large IL-2 studies. Some approaches are nevertheless addressed 14 .

In 1970, Grasseti used an immune-modulator, an analog of Nicotinamide, Dithiodinicotinic Acid (CPDS or Carboxy pyridine disulphyde) on Swiss mice as an alkylating agent in inoculation-induced lung tumor. CPDS treatment resulted in a significant reduction in number and volume of metastatic nodes in the mice. There were no side effects, nor were there any teratogenic or mutagenic effects 15,16 .

In the period from 1977 and 1995, CPDS was used in Italy to treat patients with lung cancer, who had been previously treated by surgery, chemotherapy, or radiotherapy. The results displayed a lack of metastasis or tumor growth, a long survival compared to the control group, and unimpaired biological functions 16,17 .

CPDS also is a potent immunomodulator that significantly increases the number and activity of NK cells and increases the lymphoproliferation of T lymphocytes 15 .

Given the interest of these effects through nonspecific defense in viral infection, we aimed to find out whether CPDS could be useful in HIV infection. The goal of this clinical trial was not to place the immunomodulator in competition with antiretroviral drugs but; on the contrary, to evaluate the clinical and biological value of using the CPDS to People Living With HIV/AIDS (PLWHA) as a supplementary support for protecting their immune capacities. Monkole is a General Referral Hospital located in a semi rural area in Kinshasa and with a good experience in HIV management in a dedicated unit for PLWHA 18 .

Results found comparable clinical and biological indicators between the group of individuals under CPDS and the other under ART. We therefore suggest a trial with a combined use of ART and CPDS in order to augment therapy while patients are receiving HAART and to contain residual virus 19,20,21 .

This study has received the local ethic committee agreement (Approval reference N/Réf: 002/ CEFA-MONKOLE/CE/2002).

4 II. Patients and Methods

This study, a prospective case-control trial, was conducted during a two-year period (October 2002-October 2004) on 34 PLWHA regularly followed at Monkole Hospital, a semi-urban hospital located in a suburb area of Kinshasa, DRC. During the same period, 60 others PLWHA were under ART and were then opportunely checked as a control group.

5 a) Study design

All the individuals enrolled in this study group were PLWHA who met the following inclusion criteria: having a diagnosis of HIV infection confirmed by Elisa test and irrespectively of WHO clinical stage, providing an informed consent for the treatment. The immunomodulator was proposed only to patients who were unable to access ART (ART were to be bought by patients themselves, as this study occurred before the startup of Global Funds Program in our Hospital). Patients could leave the study when they wanted or when they could access ART. All patients received detailed information on the tested drug and on the protocol, and gave a verbal or written consent prior to participating in the study.

The control group included individuals eligible to ART with a WHO clinical stage of 3 or 4 and CD4 count less than 350/ μ l. Pregnant women and young children (aged less than 2-years-old) were excluded from this trial.

We monitored the following parameters: body weight, blood cells count, lymphocytes phenotyping, morbidity and mortality rate. Body weight was assessed at each hospital visit with a medical balance. Morbidity was collected from patient-reported or hospital-documented illness episodes in the medical history. Mortality was collected from the patient medical history.

To monitor the clinical course, we first collected the previous medical history of the participants, focusing on opportunistic diseases encountered at the start of the study and their occurrence over the duration of the study. Diseases encountered were: shingles (zona), tuberculosis, other lung infections, enteritis, prurigo, meningitis, anemia requiring blood replacement, abscess and weight loss.

Phenotyping of lymphocytes were determined on a Cytometer FACS Calibur série (#E5139) (Becton Dickinson) at National Laboratory of Fight against HIV-LNLS and CD4 and CD8 T cells were assessed at the patients' entering in the study, and at three-months intervals thereafter. Viral loads were not available. Blood cell counts were performed with a cytometer "Micro CT 8" (ABX, Horiba) on total blood collected in a EDTA tube. As said above, the immuno-modulator used was a synthetic analog of Nicotinamide Dithiodinicotinic Acid (CPDS). Capsules containing 240 mg of CPDS were administered at doses of 9-12mg/kg/day, two times a day after meal. The ART consisted of a tritherapy combining 1 NNRTI (NVP or EFV) and 2 NRTIs (d4T-3TC or AZT-3TC). No other drugs neither herbal medicines were used during the study period, excepted those related to the HIV complications.

Clinical and biological evaluations were conducted every three months and at any other time, if required by the participant health state.

6 III. Statistical Analysis

7 Data were analyzed with IBM SPSS Statistics version 20

To analyze the evolution of parameters within each group over time and to compare the mean values between the two groups, we used a Mixed models allowing random intercept after adjustment of age and gender in order to neutralize the confusion effect. Frequency was evaluated in a 2X2 dichotomic table and comparison made by using the Fischer's exact test. The results were statistically significant at p-value less than 0.05. Given the lack of advantage of one group to another at the starting point, statistic decision making was made bilaterally.

8 IV. Results

9 a) Socio demographic data on population

Globally, 94 individuals were registered in this study: 34 who underwent CPDS (Immunotherapy group or Group 1) and 60 ART (Control group or Group 2). The Pillai test allowed a multivariate comparison of the two groups at the beginning of the study ($p=0.09$) as none of them displayed any kind of advantage apart the weight: 46.13kgs in CPDS versus 53.52 in ARV ($p=0.024$).

There was no difference in mean age: 35.53 yrs in CPDS versus 37.47 yrs in ARV group.

The gender distribution was globally comparable within the two groups, and showed a high prevalence of female individuals: 61.76% (21/34 cases) and 66.67% (40/60 cases) in Group 1 and Group 2, respectively. There was no significant difference in mean age between females and males in both groups: In Group 1, 36.33 yrs old versus 34.23 ($p=0.71$) and in Group 2: 36.25 yrs old versus 39.9 yrs ($p=0.66$).

According to the marital status in Group 1 vs Group 2, 38% vs 34% were unmarried and 32% vs 26% were widows; 30% vs 37% were married and 0% vs 3% were separated. Professionally, Group 1 and Group 2, respectively, displayed 56% and 35% housewives, 20% and 12% jobless, 15% and 10 % of schoolchildren, 8% and 15% employed, and 0% and 20% managerial.

10 b) Previous medical and biological data

The main medical data collected in patients at the beginning of the trial are summarized in Table ???. Weight loss was one of the main indicators observed in a same proportion in both groups (78.6 % versus 84.2%) followed by tuberculosis and zona infections. Histories of other form of pneumopathies, prurigo and skin abscesses were more frequent in Group 1 patients at the start of the trial.

The mean weight at the entry in the trials was 47.54 kgs (SD: 14.5) and 55.67 (SD: 15.5) in Group 1 and Group 2, respectively. ($p=0.137$) No significant differences were noticed in CD4 count: 393.42 in Group 1 versus 175.70 in Group 2 ($p=0.055$); CD8: 1114.08 in Group 1 versus 882.7 in Group 2 ($p=0.439$). c) Clinical and biological evolution Globally, we observed a similar evolution of weight and the CD4 counts in both groups during the first 12 months period of study with a gradual increase and a peak by the 6 th month and a slope down by the 12th month. Immunotherapy group displayed higher values of CD4, CD8 lymphocytes and the CD4/CD8 ratio. (Figure ??).

We also observed a similar increase in lymphocytes and platelets count and Hb level (Figure 2). Morphological study of the slides showed large platelets in many of the HIV patients in both groups.

12 months after the beginning, no significant difference in mean weight between groups (54.07 kgs in the ARV group and 53.21 kgs in CPDS ($p = 0.891$)).

11 patients under CPDS were hospitalized versus 0 in the ARV group in 24 months.

We observed no significant difference in death rate: 8/34 patients (23.5%) and 14/60 patients (23.3%) died in the immunotherapy and ARV group, respectively. There was no significant difference between risks of dying between both groups. RR: 0.991 and OR: 0.989 ($p = 0.983$).

In course of this study, 6 patients (17.6%) from Group 1 were submitted to ARV and then joined the group 2 in the second year of the study.

Administration of CPDS was well tolerated and no side effects were reported.

V. Discussion

In this study, we examined the effect of an immunomodulator on the clinical course (weight gain, morbidity and mortality rate) and biological (CD4 and CD8 lymphocytes, Blood cell count) parameters in HIV patients.

The baseline characteristics were identical between the two groups at the entry in the study (Tab I) except the WBC and lymphocyte count that was higher in the Group 1. (Tab II) Globally, our results showed some curative effects of the immunomodulator in inducing and maintaining immune response and increasing weight.

Volume XVII Issue II Version I

The evolution of patient weights during the study period showed no statistical difference between the two groups, although the mean weight was less in immunomodulator group (52,7 kg) comparing to the ART group (62,1 kg). In fact, most of the Group 1 patients belonged to a low socio-economic stratum as shown in professional occupation and had developed more tuberculosis infections and pneumonia (Tab II).

We observed an increase of the mean weight at 3-6 months interval in course of the therapy concomitantly with the increase of CD4 cell counts. Despite of fluctuations of mean weight observed in course of the study, there was no statistical difference in the weight evolution between the two groups. (Figure ??).

Weight gain could be considered as an indirect indicator of immunity rescue. Additionally, weight gain or stable weight is considered as one of the positive effects of treatment and care of PLWHA, and has an important impact on the psychological status of the patients and the drug compliance.

One of the striking results we observed in the Group 1 was the increase of CD4 and CD8 cell counts, and total lymphocytes numbers, especially in the first 6 months of administration of CPDS. The successive fluctuations we observed could be due to the various clinical events and the immunoresponse of each patient.

It's known that the improvement in immune function in HIV patients is biphasic: there is an initial increase in B lymphocytes, and CD4 and CD8 cells, followed by a second phase of increased thymic cell turnover and production. Furthermore, the restoration of cell immunity in patients submitted to ART depends on the phase disease 23 The impact of CPDS on mortality is obvious as we observed no difference between the two groups (21% and 23% in Groups 1 and 2 respectively) in a twoyear interval of follow-up. Other studies on mortality rate in Africa showed data varying from 10-15% but in a short period of follow-up (6-12 months) 8 .

Immunotherapy is actually considered as a complementary strategy in HIV patient management 23,24 . Different forms of immunotherapy have been proposed, including cytokines, growth factors and virus-specific therapeutic vaccines. 1 Most of these approaches have been aimed at correcting defective elements of adaptive immunity and in recovering virus-specific responses. However, it is known that HIV-1 infection also causes functional defects in natural killer (NK) cells and in monocytes/macrophages.

Although ART can improve limited functions of certain sub-populations of NK cells and antigenpresenting cells (APCs), some authors think that cells of the innate immune system act as ARV drug -resistant virus reservoirs, contribute to virus dissemination and are believed to be the origin of defective HIV-specific lymphocyte responses in infected patients. It's then necessary to correct innate immune dysfunctions in order to restore global immunity and more efficacious long-term control of HIV-1. Murabutide, a synthetic immunomodulator, has displayed such a capacity 24 .

CPDS is a powerful immune modulator which effects are targeted in increasing the number of NK cells, inducing the lymphoproliferative function of T lymphocytes 15 . The mechanism of the CPDS in increasing the CD4 and lymphocytes count is not clearly known but it probably involves cytokines production and chemokines. Moreover, the increasing number of platelets could be explained by the releasing of cytokines, including IL 6, with potential implication in megakariopoiesis 25 .

Limits of our study

The lack of viral load out of our indicators deprives the result with one of the most valuable information on the impact of CPDS to the immune system in case of HIV infection. Viral load should be one of main indicator for any prospective study for this purpose.

After the first six-twelve months of the study, clinical and biological indicators were analyzed only globally at the end of the study, without indicating the values over the time. A better systematic comparison of data between the two groups should be made quarterly, as planned in the protocol. Comprehensive collection of data over the time should be a key point in a prospective study.

We used a single immunomodulator in this study even if hypothesis suggest the combination of multiple immune-based intervention strategies in order to achieve effective immune-mediated antiviral effects 14,24,25, ??6, ??? .

VI. Conclusion

This study suggests the benefit of immunotherapy in treatment of PLWHA. The immunomodulator CPDS used alone in 34 patients resulted in an increase of mean body weight and mean CD4 mostly during the first six months of treatment. Weight gain and CD4 increase indicates a recovering immune system.

In addition, our result showed no difference between the CPDS group vs ART group in mortality rate during the same 2-year period of follow up.

Despite the positive impact of ART in PLWHA in terms of quality of health and life, and given the limit of ART to overcome the immune system impairment lead by HIV, we suggest the simultaneous use of CPDS with ART.

Since many efforts are made to facilitate the adherence to treatment of PLWHA by the use of daily single dose pill, the adjunction of a CPDS pill would not The most critical challenge -as for ART coverage-should be the access to this treatment tool for all individuals in need, mostly in Sub-Saharan Africa.

VII. Acknowledgment

Authors thank the Laboratory Moro Color and M. Camillo Moro for have provided the Immuno-Modulator used in this study.

Legends

1

		GROUPS									
Year	Meningitis	CPDS	(n34)	n	(%)	1	ARV	(n60)	n	(%)	1
2017			(2.94)					(1.67)			
	Zona Infection	2	(5.89)				2	(3.33)			
	Tuberculosis	4	(11.76)				5	(8.33)			
Volume	Other pneumopathies	2	(5.89)	1	(2.94)	4	(11.76)	1	(1.67)	3	(5.00)
XVII	Enteritis Prurigo Weight	11	(32.35)	2	(5.89)	4	(5.00)	16	(26.67)	1	
Issue	loss Blood transfusion	(11.76)	Mean (SD)	47.54			(1.67)	0	Mean (SD)		
II	Skin abscess Weight (kg)	(14.5)	393.42	(344.5)			55.67	(15.5)	175.7		
Ver-	CD4 (cells/mm3)	1114.08	(946.8)				(109.1)	882.7	(415.2)		
sion	(cells/mm3)										
I											
D D	CD3 (cells/mm3)	1724.40	(1264				1059.24	(499.9)			
D D											
)											
(CD4/CD8	0.45	(0.32)				0.32	(0.21)			
	WBC (cells/mm3)	4918.18	(1672.6)				3400	(1112.0)			
	Lymphocytes (cells/mm3)	2169.75	(780.4)				1323.9	(428.9)			
	Hemoglobin (g/%ml)	11.03	(0.97)				10.93	(2.2)			
	Platelets (cells/mm3)	2.08E5	(4.2E4)				2.2E5	(4.2E4)			
	VS (mm/h)	8	9.33	(40.2)			82.5	(50.9)			

[Note: Kstudies,]

Figure 1: Table 1 :

1 2

¹© 2017 Global Journals Inc. (US)

²Trial of Immunotherapy in HIV Patients: Our Experience with the Immuno-Modulator Dithiodinitrocinic Acid (CPDS) in 34 Congolese Patients

[Rockville et al.] , Maryland Rockville , Usa : Mpsmrm , Msp .

[Msf and De Plaidoyer (2012)] , Unité Msf , De Plaidoyer . *Juillet* 2012. Jan 2016.

[Kalichman et al. ()] *Alcohol Use and Food Insecurity in HIV Disease Management, in Health of HIV Infected People*, S C Kalichman , J A Pellowski , D Hernandez . 2015. Elsevier. p. .

[Bahr1 et al. ()] ‘Clinical and immunological effects of a 6 week immunotherapy cycle with murabutide in HIV-1 patients with unsuccessful long-term antiretroviral treatment’. G M Bahr1 , X De La Tribonnière , E Darcissac . *JAC* 2003. 51 p. .

[Grasseti ()] ‘Effect of 6,6-ditiodinicotinic acid on the dissemination of Ehrlich ascites tumor’. D R Grasseti . *Nature* 1970. 5258 (228) p. .

[Gontran et al. ()] ‘Effects of IM28 on HIV-1 and Metabolic Disorders-induced Highly Active Antiretroviral Therapy in Gabonese Patients’. M Gontran , L T Jerome , A M Yvonne . *J Antivir Antiretrovir* 2009. 1 p. .

[Gontran et al. ()] ‘Effects of IM28 on HIV-1 and Metabolic Disorders-induced Highly Active Antiretroviral Therapy in Gabonese Patients’. M Gontran , L T Jerome , A M Yvonne . 10.4172/jaa.1000011. *J Antivir Antiretrovir* 2009. 1 p. .

[Ndungu et al. ()] ‘Efficacy of amaranth grain consumption on CD4 count and morbidity patterns among adults living with HIV in Nyeri’. A Ndungu , Zw , E N Kuria , N K Gikonyo , D K Mbithe . *Kenya. J Aids Hiv Res* 2017. 9 (4) p. .

[Musumari et al. ()] ‘Food Insecurity Is Associated with Increased Risk of Non-Adherence to Antiretroviral Therapy among HIV-Infected Adults in the Democratic Republic of Congo: A Cross-Sectional Study’. P M Musumari , E Wouters , P K Kayembe . 10.1371/journal.pone.0085327. *PLoS ONE* 2014. 9 (1) p. e85327.

[Ndarabu et al. ()] ‘HIV status of Children Born to Mothers with HIV infection in a semi urban area in DR Congo: an experience from Monkole Hospital Center’. A Ndarabu , D Shada , T Katako , D Mbuyi , A Lukusa , R Ngalamulume , L Tshilolo . *Women’s Health Gynecol* 2017. 3 (2) .

[Burini and Moreto ()] *HIV-Positive Patients Respond to Dietary Supplementation with Cysteine or Glutamine, in Health of HIV Infected People*, R C Burini , F Moreto , Yong-Ming , Y . 2015. Elsevier. p. .

[Hoffman and Rockstroh (2016)] C Hoffman , J K Rockstroh . *HIV 2015/16*, *www.hivbook.com* [Access on, Jan 2016.

[Bart and Pantaleo] *Immune-based interventions in HIV infection: doing the right*, P A Bart , G Pantaleo .

[Fao ()] ‘L’état de l’insécurité alimentaire dans le monde’. Fida Fao , Pam . *Objectifs internationaux 2015 de réduction de la faim: des progrès inégaux*, (Rome, FAO) 2015. 2015.

[Grasseti and Moro ()] *Method of immunomodulation using thione-forming disulfides. US patent Application Serial No 10/044*, D R Grasseti , C Moro . 2002. 463.

[Ministère de la Santé Publique (MSP) et ICF International ()] *Ministère de la Santé Publique (MSP) et ICF International*, 2014. Ministère du Plan et Suivi de la Mise en oeuvre de la Révolution de la Modernité (MPSMRM) (Enquête Démographique et de Santé en République Démocratique du Congo 2013-2014)

[Ca?yniuk et al. ()] *Nutrition and Food in AIDS Patients, Health of HIV Infected People*, B Ca?yniuk , T Kokot , E Nowakowska-Zajdel . 2015. Elsevier. 2 p. .

[Conolly et al. ()] ‘Proinflammatory cytokines in HIV disease-a review and rationale for new therapeutic approaches’. N C Conolly , S A Ridler , C R Rinaldo . *AIDS Rev* 2005. 7 (3) p. .

[Sunguya et al. ()] ‘Role of Nutrition Training for Health Workers in Addressing Poor Feeding Practices and Undernutrition Among HIV-Positive Children’. B F Sunguya , D P Urassa , J Yasuoka . *Health of HIV Infected People*, 2015. Elsevier. p. .

[Chang et al. (2005)] ‘Sustained expansion of NKT cells and antigen-specific T cells after injection of alpha-galactosyl-ceramide loaded mature dendritic cells in cancer patients’. Dh1 Chang , K Osman , J Connolly . *J Exp Med* 2005 May 2. 201 (9) p. .

[Grasseti ()] *The antimetastatic and tumor growth retarding effects of sulfur containing analogs of nicotinamide. Cancer letters*, D R Grasseti . 1986. 31 p. .

[Saag ()] ‘The impact of highly active antiretroviral therapy on HIV-specific immune function’. M S Saag . *AIDS* 2001. 15 (2) p. .

[Lahuerta et al. ()] ‘The Problem of Late ART initiation in Sub-Saharan Africa: A Transient Aspect of Scale-up or a Longterm Phenomenon?’. M Lahuerta , F Ue , S Hoffman , B Elul . *J Health Care Poor Underserved* 2013. 24 (1) p. .

[Saag ()] ‘The role of immunotherapy in the treatment of HIV’. Michael S Saag . *AIDS* 2001. 15 (2) p. .

[Smith ()] *To cure chronic HIV infection, a new therapeutic strategy is needed, COI*, K A Smith . 2001. 13 p. .

[D’ancona et al. (1984)] *variations of growth, adhesion, and nucleotide pool in cancer cells treated with CPDS(a pyridine disulfide). XVII Symposium of the Italian Cancerology Society*, S D’ancona , G Magnolfi , G Toffoli . December 1984. Abano, Italy.