

# Comune di Cagli

## 2 maggio 2010

33 casi clinici di Terapia Metabolica

Dott. Giuseppe Nacci

# Pazienti attualmente in cura con Terapia Metabolica

- 2 casi clinici di tumore al cervello
- 1 caso clinico di cancro alle ghiandole salivari
- 1 caso clinico di cancro alla tiroide
- 2 casi clinici di cancro ai polmoni
- 10 casi clinici di cancro alla mammella
- 1 caso clinico di cancro alle ovaie
- 1 caso clinico di cancro all'utero
- 2 casi clinici di cancro all'intestino
- 7 casi clinici di cancro alla prostata
- 2 casi clinici di cancro alla vescica
- 1 melanoma
- 1 linfoma
- 1 mieloma multiplo
- 1 leucemia
- TOTALE : 33

# La Terapia Metabolica

- La Terapia Metabolica è stata sempre considerata la base della medicina occidentale
- Questo fin dai tempi di Ippocrate di Kos, fondatore della Medicina Occidentale.
- In merito al Cancro egli affermò:
- *“Il Cancro non si cura con il ferro del chirurgo ma con l’alimentazione corretta e le piante medicinali...”*

# La vitamina B17 (Amigdalina)

- Nell'ambito della Terapia Metabolica, grande importanza rivestono le "VITAMINE".
- Nel caso del Cancro, oggi si conoscono molte vitamine ad azione anti-neoplastica.
- La prima, in assoluto, fu l'AMIGADALINA, o VITAMINA B17, impiegata dal medico russo Inosmetzeff nel 1844, e successivamente da Fishmann nel 1947, Tasca (1958), Morrone, (1962), Rossi e Guidetti (1966)



# Inosemtzeff, 1844

## 2 casi clinici

N° 37.

(TOME XIII. — 1845.)

SAMEDI 13 SEPTEMBRE 1845.

# Gazette Médicale

## DE PARIS.

La Gazette Médicale de Paris (Gazette de Santé et Clinique des Hôpitaux réunies) paraît tous les samedis; chaque numéro est composé de 16 pages in-4°, 32 colonnes, et qui équivaut à 6 feuilles in-8°. — Le prix de l'abonnement est, pour Paris et les Départemens, de 40 fr. par an, 20 fr. pour 6 mois, et 10 fr. pour 3 mois; pour l'Etranger, 44 fr. Les abonnemens ne peuvent dater que du commencement d'un trimestre, 1<sup>er</sup> Janvier, 1<sup>er</sup> Avril, 1<sup>er</sup> Juillet, 1<sup>er</sup> Octobre. — On s'abonne à Paris, au bureau du Journal, rue Neuve-Racine, n° 16, près de l'Odéon, et dans les Départemens, chez tous les Directeurs des postes et des messageries. — On ne reçoit que les lettres affranchies.

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### THÉRAPEUTIQUE.

HISTOIRE DE DEUX CAS DE FONGUS MÉDULLAIRE, TRAITÉS AVEC SUCCÈS PAR L'EMPLOI DES NARCOTIQUES.

pendant. Il est arrivé sans doute à beaucoup de médecins de traiter avec succès des tumeurs fongueuses par l'emploi de remèdes, qui, dans d'autres cas de la même espèce, n'ont apporté aucune amélioration; il y a même des exemples de cas où ces productions morbides ont disparu en partie ou en totalité, uniquement sous l'influence d'un régime et d'une diète convenables qui eurent en même temps pour résultat une amélioration générale de la santé des malades.

C'est sur deux faits de ce genre que je me propose d'appeler l'attention des praticiens.

Obs. I. — Jacques Timoféef, paysan, âgé de 20 ans, non marié, de constitution scrofuleuse, fut reçu à la clinique chirurgicale de l'université de Moscou, le 11 novembre 1843. Un amaigrissement très prononcé annonçait une maladie grave et profonde. L'œil gauche était le siège d'une tumeur du volume d'un fœtus de sept mois environ, inégale, noueuse au toucher; s'étendant depuis la partie inférieure du front et la tempe jusqu'à l'ailé gauche du nez et la joue; et en largeur, depuis le nez jusqu'à la chevelure de la tempe et à l'oreille gauche.

Cette tumeur tirait son origine d'une excroissance silvée sous la paupière supérieure qui avait été énormément distendue et qui avait pris une teinte violente. Sa surface, parsemée de vaisseaux prodigieusement dilatés, offrait plusieurs Alvéoles dont quelques-unes étaient surmontées de papilles. Le nez lui-même

# Fishman, 1947

Fishman 1947

## LETTERS TO THE EDITORS

### THE PRESENCE OF HIGH $\beta$ -GLUCURONIDASE ACTIVITY IN CANCER TISSUE\*

Sirs:

$\beta$ -Glucuronidase is believed to function in the "metabolic conjugation" of the estrogenic hormones.<sup>1</sup> In view of the large amount of experimental

*Distribution of  $\beta$ -Glucuronidase in Adenocarcinoma of Breast with Metastases to Axillary Lymph Nodes*

Organ	Tissue	No. of specimens studied	Glucuronidase activity*
			units
Breast	Edge of lesion	1	450
"	Center of lesion	1	890
"	Uninvolved breast	2	168, 112
"	skin over carcinoma	1	89

ASBMB

Biological Chemistry

# Tasca, 1958, 21 pazienti

1958

GAZZETTA MEDICA ITALIANA

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## Osservazioni cliniche sugli effetti terapeutici di un glicuronoside cianogenetico in casi di neoplasie maligne umane

MARIO TASCA

Primario Reparto Radiologico dell'Ospedale Civile di Sauremo

Le premesse teoriche che hanno condotto alla preparazione per biosintesi, partendo dall'amigdalina, del glicuronoside cianogenetico denominato Laetrile, nella forma di un l-mandelo-nitrile-beta-glicuronoside, derivato dalla lunga serie di studi di biochimica degli

Odell, Burt e Berthea<sup>12</sup>, Homburger e Fishman<sup>7</sup> e altri.

Il glicuronoside cianogenetico può essere scisso in vivo dai due tipi di cellule, per azione della beta-glicuronidasi nei suoi due componenti liberando l'acido

# J. Morrone, 1962, 10 pazienti

## Chemotherapy of Inoperable Cancer

### Preliminary Report of 10 Cases Treated with Laetrile

By JOHN A. MORRONE, M.D., F.I.C.S., A.S.A.S., Jersey City, N.J., Attending Surgeon, Jersey City Medical Center

The hope of the future lies in the chemotherapy of cancer. In view of deep-rooted prejudice against clinical experimentation in this field, a completely objective study and report of 10 cases should be of interest.

The use of Laetrile (1-mandelonitrile-beta-glucuronoside), a beta cyanogenetic glucoside, is based on the unitarian or trophoblastic thesis of cancer. In a review of 17,000 papers on malignant neoplasms and related biological subjects, the trophoblast was described as the *sine qua non* of cancer (1).

#### *Rationale*

The malignant lesion is characterized by a high focal concentration of beta-glucuronidase, which is a beta-glucosidase. Laetrile is a glucoside which is hydrolyzed specifically by beta-glucosidase enzymes, with production of benzaldehyde, glucose, and nascent hydrogen cyanide.

Rhodanese, the cyanide-detoxifying enzyme, is absent or relatively deficient in malignant lesions but present in normal tissues. Nascent hydrocyanic acid is released to the extent of about 10% in the vulnerable carcinomatous areas but not elsewhere in the body.

Laetrile is relatively non-toxic when administered parenterally. Orally it is extremely toxic due to the release of hydrogen cyanide on contact with the hydrochloric acid of the gastric juice.

#### *Previous Reports*

In a group of 14 cases of cancer with metastases treated with Laetrile, there was striking relief of pain with discontinuance of analgesics, disappearance of fetor from ulceration, improved appetite, and regression of the tumor (2).



GUIDETTI 1966

## Clinical Trial of Chemotherapeutic treatment of advanced cancers with Leatril (L-Mandelonitrile-Beta-Diglucoside)

Guidetti Ettore  
Rossi Benedetto  
Deckers Christian

Presented at the 9<sup>th</sup> International Cancer Congress in Tokyo, October 1966

From 1954 to 1966 we gave 150 patients the above-mentioned therapy, chiefly at San Cottolengo Hospital, Turin; Dosio Hospital, Milan; and Louvain University Cancer Institute. All patients were in the terminal stage of the disease, the majority of them prey to cachexia, and all other therapies had failed.

The following table summarizes the cases treated, classified according to the site of the tumor, and showing the number of patients for each degree of reaction to therapy. We use the sign ++ to denote patients who reacted in an objectively favourable manner, by which we mean diminution of volume of the tumor or at least all interruption of its evolution, improvement in the roentgenographic picture, and improvement in laboratory findings. The mark + and + indicates patients who showed a more or less distinct subjective improvement, and the mark - those who reacted negatively to the treatment.

Cases corresponding to ++ represent about 20% of those treated.

We again underline the fact that the majority of these cases were simultaneously subjected to an immunotype therapy, which might have some bearing on the number of positive results observed, grouped under the signs ++ and + totalling about half the number of cases treated.

Cancer Site	No. cases	++	+	±	-
Toruli tactiles	26	5	6	6	9
Breast	25	3	8	7	7
Uterus	24	7	7	4	6
Rectum	20	2	9	2	7
Ovary (with infusion)	10	2	2	2	4
Other types	30	9	7	2	12
Totals	135	28	39	23	45

We have separately considered neoplasms of the pleura with effusion (15 cases), where the product was used direct by injection in the pleural cavity. In these cases we observed our best results, as generally we obtained reduction and then on occasion complete disappearance of the effusion, associated with a distinct improvement in the patients' condition.

### Conclusion:

On the basis of our clinical trial, we are able to state that L-mandelonitrile-beta-diglucoside may be considered an extremely useful chemotherapeutic drug for palliative medical treatment of malign neoplasms, from the standpoint both of its therapeutic effect and its very low toxicity.

# E.Guidetti D.Rossi 1966, 135 casi clinici

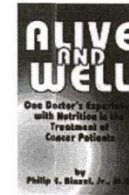
# Importanti lavori ottenuti

- Oggi la Medicina Metabolica è applicata in Cliniche Private.
- Studi famosi sono stati quelli dei 288 pazienti di Binzel (1992, Ohio, USA), dei 153 pazienti di Hildebrand (1995), delle diverse migliaia di casi curati da Contreras, dagli anni 70 a oggi.
- Interessante, il recente approccio del cinese Tan (2000)

# P. Binzel, 1992, 288 pazienti

## ALIVE AND WELL

One Doctor's Experience  
with Nutrition in the  
Treatment of  
Cancer Patients



By  
Philip E. Binzel, Jr., M.D.

ALIVE AND WELL by Philip E. Binzel, Jr., M.D. (split into chapters)

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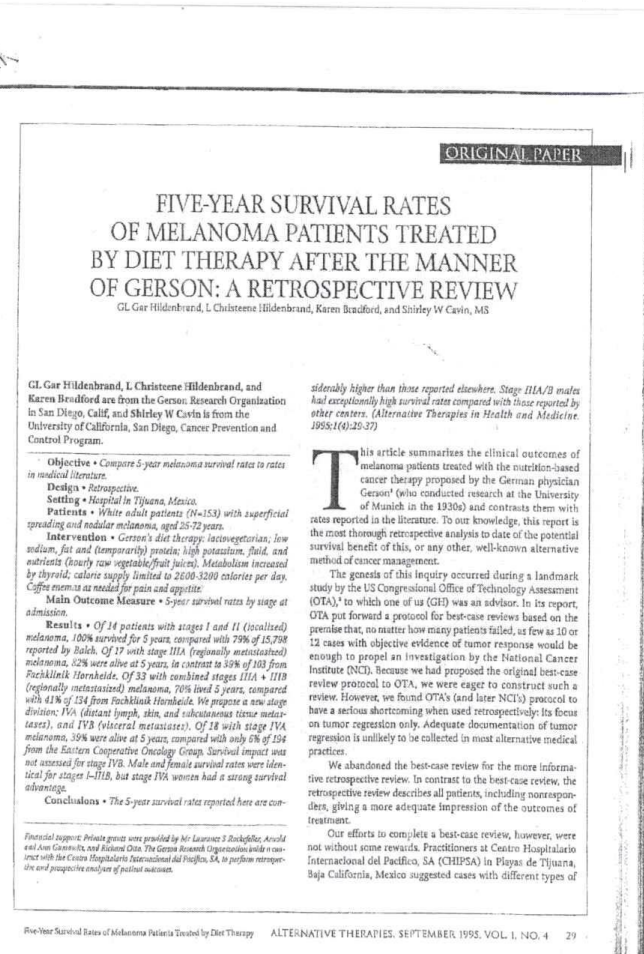
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# Binzel : percentuali

- 288 pazienti trattati fra il 1974-1991
- Su 180 senza metastasi: 131 ancora vivi (72%)
- Di cui 58 seguiti da 2 a 4 anni (44%)
- 80 seguiti per più di 5 (fino a 18 anni) (61%)
- Su 108 con metastasi: 61 ancora vivi (56%)
- Di cui 30 seguiti da 2 a 4 anni
- 31 seguiti per più di 5 anni (fino a 18 anni)



# Hildebrand, 1995, 153 casi clinici



## ORIGINAL PAPER

### FIVE-YEAR SURVIVAL RATES OF MELANOMA PATIENTS TREATED BY DIET THERAPY AFTER THE MANNER OF GERSON: A RETROSPECTIVE REVIEW

GL Gar Hildebrand, L Christene Hildebrand, Karen Bradford, and Shirley W Casin, MS

GL Gar Hildebrand, L Christene Hildebrand, and Karen Bradford are from the Gerson Research Organization in San Diego, Calif, and Shirley W Casin is from the University of California, San Diego, Cancer Prevention and Control Program.

**Objective** • Compare 5-year melanoma survival rates to rates in medical literature.

**Design** • Retrospective.

**Setting** • Hospital in Tijuana, Mexico.

**Patients** • White adult patients (N=153) with superficial spreading and nodular melanoma, aged 25-72 years.

**Intervention** • Gerson's diet therapy: lacto-vegetarian; low sodium, fat and (temporarily) protein; high potassium, fluid, and nutrients (hourly raw vegetable/fruit juices). Metabolism increased by thyroid; calorie supply limited to 2600-3200 calories per day. Coffee restricted as needed for pain and appetite.

**Main Outcome Measure** • 5-year survival rates by stage at admission.

**Results** • Of 14 patients with stages I and II (localized) melanoma, 100% survived for 5 years; compared with 79% of 15,798 reported by Balch. Of 17 with stage IIIA (regionally metastasized) melanoma, 62% were alive at 5 years, in contrast to 38% of 103 from Fachklinik Hornheide. Of 33 with combined stages IIIA + IIIB (regionally metastasized) melanoma, 70% lived 5 years, compared with 41% of 124 from Fachklinik Hornheide. We propose a new stage division: IVA (distant lymph, skin, and subcutaneous tissue metastases), and IVB (visceral metastases). Of 18 with stage IVA melanoma, 39% were alive at 5 years, compared with only 6% of 124 from the Eastern Cooperative Oncology Group. Survival impact was not assessed for stage IVB. Male and female survival rates were identical for stages I-IIIIB, but stage IVA women had a strong survival advantage.

**Conclusions** • The 5-year survival rates reported here are con-

siderably higher than those reported elsewhere. Stage IIIA/IB males had exceptionally high survival rates compared with those reported by other centers. (*Alternative Therapies in Health and Medicine*, 1995;1(4):25-37)

**T**his article summarizes the clinical outcomes of melanoma patients treated with the nutrition-based cancer therapy proposed by the German physician Gerson<sup>1</sup> (who conducted research at the University of Munich in the 1930s) and contrasts them with rates reported in the literature. To our knowledge, this report is the most thorough retrospective analysis to date of the potential survival benefit of this, or any other, well-known alternative method of cancer management.

The genesis of this inquiry occurred during a landmark study by the US Congressional Office of Technology Assessment (OTA),<sup>2</sup> to which one of us (GH) was an advisor. In its report, OTA put forward a protocol for best-case reviews based on the premise that, no matter how many patients failed, as few as 10 or 12 cases with objective evidence of tumor response would be enough to propel an investigation by the National Cancer Institute (NCI), because we had proposed the original best-case review protocol to OTA, we were eager to construct such a review. However, we found OTA's (and later NCI's) protocol to have a serious shortcoming when used retrospectively: its focus on tumor regression only. Adequate documentation of tumor regression is unlikely to be collected in most alternative medical practices.

We abandoned the best-case review for the more informative retrospective review. In contrast to the best-case review, the retrospective review describes all patients, including nonresponders, giving a more adequate impression of the outcomes of treatment.

Our efforts to complete a best-case review, however, were not without some rewards. Practitioners at Centro Hospitalario Intercontinental del Pacifico, SA (CHIPSA) in Playas de Tijuana, Baja California, Mexico suggested cases with different types of

*Financial support: Private grants were provided by Mr. Laurence S. Backgelter, Arnold and Ann Casin-Itz, and Richard Chin. The Gerson Research Organization is in a contract with the Centro Hospitalario Intercontinental del Pacifico, SA, to perform retrospective and prospective analyses of patient outcomes.*

# Classificazione clinica stadiazione melanoma maligno

- Stadio Primo: assenza di metastasi
- Stadio Secondo : metastasi confinate entro un raggio di 3 centimetri dalla lesione primitiva (metastasi localizzate)
- Stadio Terzo : metastasi regionali
- 3 A: metastasi cutanee e sottocutanee ad oltre 3 centimetri di distanza dalla lesione primitiva
- 3 B: metastasi alle linfoghiandole regionali
- Stadio Quarto : metastasi a distanza

# Hildebrand

- 82 pazienti affetti da melanoma
- Sopravvissuti a 5 ANNI :
- Su 14 (Primo e Secondo Stadio ) : 100 % sopravvissuti)
- Su 17 (Terzo stadio A) : 82% sopravvissuti
- Su 33 ( Terzo stadio A+B ) : 71% sopravvissuti
- Su 18 (Quarto Grado) : 39% sopravvissuti

# Tan, 2000

## 40 casi clinici

- Quaranta pazienti con:
- tumore al cervello primitivo (gliomi) o secondario (metastasi da carcinomi)
- Terapia : vit. *Elemene* iniettato in carotide
- Dopo 4 anni : 30 pazienti (75%) ancora vivi, con riduzione significativa (circa 60%) delle masse tumorali pre-esistenti

# Tan, 2000

## · 临床论著 ·

### 中药榄香烯乳注射液治疗恶性脑肿瘤 40 例临床研究

谭平国 钟伟健 蔡望青 邓跃飞 钟志光 林吉惠 陈由芝

**内容摘要** 目的:探讨榄香烯乳注射液治疗恶性脑肿瘤的疗效。方法:总结分析我院 1994 年 1 月—1998 年 5 月间,采用榄香烯乳注射液治疗 40 例恶性脑肿瘤患者(治疗组)的疗效。40 例中原发性脑肿瘤 29 例,脑转移瘤 11 例。采用榄香烯乳注射液 0.4—1.2g/d, 6—12g/疗程,共用 2—6 个疗程,每疗程间隔 1—1.5 个月。经皮动脉穿刺或插管,注射泵注入,静脉滴药或(和)经鼻静脉持续灌注。根据治疗前后肿瘤体积变化、平均生存期、体能状态评分评价疗效。对照组 29 例,原发性脑肿瘤 22 例,脑转移瘤 7 例;采用化疗方法,2—3 个疗程,每疗程间隔 1—1.5 个月。结果:(1)治疗组治疗前后肿瘤平均体积( $\text{cm}^3$ )变化 =  $6.70 - 2.67 = 4.03$  ( $t = 3.02$ ,  $P < 0.01$ ),治疗前后肿瘤体积平均缩小 61%。(2)治疗组 CR 4 例,PR 26 例,CR + PR 30 例,总有效率 75.0% (95% 可信区间  $\pm 13.4\%$ )。对照组 29 例,CR 2 例,PR 10 例,总有效率 41.4% (95% 可信区间  $\pm 17.9\%$ ,  $\chi^2 = 3.867$ ,  $P < 0.05$ )。(3)治疗组治疗前后 KPS 平均记分差为  $94.7 - 88.2 = 6.5$  分 ( $t = 3.5313$ ,  $P < 0.01$ )。(4)治疗组平均生存期 25.4 个月,对照组生存期 17.4 个月,两组比较差异有显著性意义 ( $t = 3.74$ ,  $P < 0.01$ )。结论:榄香烯乳对恶性肿瘤疗效明显,能延长患者高质量生存期,值得进一步探讨。

**关键词** 恶性脑肿瘤 榄香烯乳液注射

Clinical Study on Treatment of 40 Cases of Malignant Brain Tumor by Elemene Emulsion Injection TAN Ping-guo, ZHONG Weijian, CAI Wangqing, et al Department of Neurosurgery, Sun Yat-sen Memorial Hospital of Sun Yat-sen University of Medical Sciences, Guangzhou (510120)

**Objective:** To investigate the effect of elemene emulsion injection (EEI) in treating malignant brain tumor. **Methods:** By conducting a retrospective study of 40 patients with brain tumor, 29 of malignant glioma and 11 metastatic tumor, who were treated with EEI from January 1994 to May 1998. EEI 0.4—1.2g/d was given to each patient by intravenous dripping or/and intravenous infusion by pumps, and directly injected into carotid artery or infused through a carotid artery catheter with pumps. The total dosage of 6—12 g was given in 2—6 therapeutic courses with an interval of 1—1.5 months between courses. The effectiveness of treatment was assessed according to the changes of tumor size, Karnofsky Performance Status (KPS) and survival time of patients. The control group consisted of 29 cases of malignant brain tumor (22 of primary and 7 of metastatic) was treated with chemotherapy 2—3 therapeutic courses with an interval of 1—1.5 months between them. **Results:** (1) In the EEI treated group the mean tumor size was changed from  $6.70 \text{ cm}^3$  (before treatment) to  $2.67 \text{ cm}^3$  (after treatment),  $t = 3.02$ ,  $P < 0.01$ , it was reduced by 61%; (2) In the EEI treated group 4 cases was CR, 26 PR, the total effective rate being 75.0% (95% credibility interval  $\pm 13.4\%$ ), while in the control group, 2 of CR, 10 PR, and the total effective rate 41.4% (95% credibility interval  $\pm 17.9\%$ ), the difference between the two groups was significant,  $\chi^2 = 3.867$ ,  $P < 0.05$ ; (3) KPS decreased in the EEI group from 94.7 scores (before treatment) to 88.2 scores (after treatment), the decrement was 6.5 scores ( $t = 3.5313$ ,  $P < 0.01$ ); (4) The survival time in the EEI treated group was 25.4 months, and that in the control group was 17.4 months ( $t = 3.74$ ,  $P < 0.01$ ). **Conclusion:** Elemene has significant effect on treatment of malignant brain tumor. It could prolong the high quality survival time of patients and is worthy of further investigation.

**Key words** malignant brain tumor, elemene emulsion injection

# F. Contreras, 2000, circa 1.000 casi clinici

In 1981, we conducted a retrospective study to document the five-year survival rates of our cancer patients. It is important to note that **95 percent of these patients came to us with stage IV cancers after conventional therapy had failed to help them. They had been sent home to die. We treated them with our metabolic therapy and the results were encouraging. Our overall five-year survival rate for all types of cancer was 30 percent. We also noted that 86 percent of our patients outlived their prognosis and reported an improvement in their quality of life.**

Malignancies in the lung, breast, colon and prostate are the most prevalent in our experience. For this reason, we designed a prospective study on the efficacy of metabolic therapy focused on these advanced stage IV cancers. In the table below, we compare our results against those from clinical trials with conventional therapies.

Type of cancer	Distant <sup>1</sup> Number of patients	5 yr. survival rate (%)	
		Oasis	Conventional
Lung Cancer	200	30%	2%
Breast Cancer	130	39%	21%
Colón Cancer	150	30%	8%

1. Distant: A malignant cancer that has spread to parts of the body remote from the primary tumor either by direct extension or by discontinuous metastasis to distant organs, tissues, or via the lymphatic system to distant lymph nodes.

2. Source: American Cancer Society Cancer Facts & Figures 2001

# Confronto con la Chemio-Trerapia

- Indiscutibile la notevole differenza di risultati ottenuti della Terapia Metabolica rispetto alla ben nota Chemio-Terapia, di cui riportiamo le percentuali di sopravvivenza a 5 anni, su un campione di 150.000 (CENTOCINQUANTA MILA) di pazienti americani , da un recente lavoro di Morgan, del 2004

# Morgan, 2004

## 150.000 pazienti

*Clinical Oncology* (2004) 16: 549–560  
doi:10.1016/j.clon.2004.06.007

### Overview

## The Contribution of Cytotoxic Chemotherapy to 5-year Survival in Adult Malignancies

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### ABSTRACT:

**Aims:** The debate on the funding and availability of cytotoxic drugs raises questions about the contribution of curative or adjuvant cytotoxic chemotherapy to survival in adult cancer patients.

**Materials and methods:** We undertook a literature search for randomised clinical trials reporting a 5-year survival benefit attributable solely to cytotoxic chemotherapy in adult malignancies. The total number of newly diagnosed cancer patients for 22 major adult malignancies was determined from cancer registry data in Australia and from the Surveillance Epidemiology and End Results data in the USA for 1998. For each malignancy, the absolute number to benefit was the product of (a) the total number of persons with that malignancy; (b) the proportion or subgroup(s) of that malignancy showing a benefit; and (c) the percentage increase in 5-year survival due solely to cytotoxic chemotherapy. The overall contribution was the sum total of the absolute numbers showing a 5-year survival benefit expressed as a percentage of the total number for the 22 malignancies.

**Results:** The overall contribution of curative and adjuvant cytotoxic chemotherapy to 5-year survival in adults was estimated to be 2.3% in Australia and 2.1% in the USA.

**Conclusion:** As the 5-year relative survival rate for cancer in Australia is now over 60%, it is clear that cytotoxic chemotherapy only makes a minor contribution to cancer survival. To justify the continued funding and availability of drugs used in cytotoxic chemotherapy, a rigorous evaluation of the cost-effectiveness and impact on quality of life is urgently required. Morgan, G. et al. (2004). *Clinical Oncology* 16, 549–560

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**Key words:** Chemotherapy, combined modality treatment, palliation, quality of life, radiotherapy, survival

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### Introduction

In adults, cytotoxic chemotherapy became established in the 1970s as a curative treatment in advanced Hodgkin's disease [1], non-Hodgkin's lymphoma [2], teratoma of testis [3] and as an adjuvant treatment for early breast cancer [4].

The initial results suggested the potential use of cytotoxic chemotherapy as a definitive treatment or as an adjuvant therapy in asymptomatic patients with the aim of improving survival. However, as stated by Braverman [5] and others [6–8], the early gains in a few tumour sites have not been seen in the more common cancers. For most patients, the use of cytotoxic chemotherapy is for the palliation of symptoms and to improve quality of life [9], with prolongation of survival being a less important outcome.

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Some practitioners still remain optimistic that cytotoxic chemotherapy will significantly improve cancer survival [10]. However, despite the use of new and expensive single and combination drugs to improve response rates and other agents to allow for dose escalation, there has been no change in some of the regimens used, and there has been little impact from the use of newer regimens. Examples are non-Hodgkin's lymphoma [11] and ovarian cancer [12], in which cyclophosphamide, adriamycin, vincristine and prednisolone (CHOP) and platinum, respectively, (introduced over 20 years ago) are still the 'gold standard' treatment. Similarly, in lung cancer, the median survival has increased by only 2 months during the same time period [13,14], and an overall survival benefit of less than 5% has been achieved in the adjuvant treatment of breast, colon, and head and neck cancers [15–17].

The recent debate on funding of new cytotoxic drugs [18–20] has highlighted the lack of agreement between medical oncologists and funding bodies on the current and

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# Morgan, 2004

## le percentuali

Table 2 — Impact of cytotoxic chemotherapy on 5-year survival in American adults

Malignancy	ICD-9	Number of cancers in people aged > 20 years*	Absolute number of 5-year survivors due to chemotherapy†	Percentage 5-year survivors due to chemotherapy‡
Head and neck	140–149, 160, 161	5139	97	1.9
Oesophagus	150	1521	82	4.9
Stomach	151	3001	20	0.7
Colon	153	13 936	146	1.0
Rectum	154	5533	189	3.4
Pancreas	157	3567	—	—
Lung	162	20 741	410	2.0
Soft tissue sarcoma	171	858	—	—
Melanoma	172	8646	—	—
Breast	174	31 133	446	1.4
Uterus	179–182	4611	—	—
Cervix	180	1825	219	12
Ovary	183	3032	269	8.9
Prostate	185	23 242	—	—
Testis	186	989	373	37.7
Bladder	188	6667	—	—
Kidney	189	3722	—	—
Brain	191	1824	68	3.7
Unknown primary site	195–199	6200	—	—
Non-Hodgkin's lymphoma	200 + 202	6217	653	10.5
Hodgkin's disease	201	846	341	40.3
Multiple myeloma	203	1721	—	—
Total		154 971	3306	2.1%

\*Numbers from Ref. [22].

†Absolute numbers (see text).

‡% for individual malignancy.

# L'immuno-Terapia

- La Terapia Metabolica, oltre che sull'utilizzo di particolari vitamine, fa anche largo uso di sostanze capaci di stimolare la Risposta Immunitaria dei globuli bianchi contro le cellule tumorali. In questo , assomiglia molto alla Immuno-Terapia, oggi ottenuta con estrazione di globuli bianchi dal tumore, loro moltiplicazione in laboratorio, e successiva loro re-iniezione nel paziente (Pizza, 2001)

# G. Pizza, 2001

## 122 pazienti

Int. J. Cancer: 94, 109–120 (2001)  
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Publication of the International Union Against Cancer

### IMMUNOTHERAPY OF METASTATIC KIDNEY CANCER

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From April 1986 to September 2000, 122 MRCC patients were treated by monthly intralymphatic injections (containing a mean of 573 IL-2 U and  $26 \times 10^6$  LAK cells) and i.m. administration of IFN and TF; 71 patients also received a 3-day cycle of monthly IL-2 inhalations with a mean of 998 daily U. MRCC cases not treated by immunotherapy ( $n = 89$ ) represent our historical controls. Adverse clinical side effects related to treatment were negligible. CR ( $n = 11$ ) and PR ( $n = 13$ ) were noticed in 24/122 patients. Of 24 responding patients, 17 resumed progression, whereas 7 remain in remission 11–69 months later. The overall median survival of treated patients (28 months) was 3.5-fold higher than the median survival of historical controls (7.5 months), and a Kaplan-Meier curve showed 25% survival 11 years after the beginning of immunotherapy. Apparently, the addition of IL-2 by inhalation improved survival. The present immunotherapy protocol appears to be efficacious, safe, devoid of adverse side effects, far less costly than others and able to offer a good quality of life to MRCC patients; if confirmed in a multicenter trial, it could set the basis for developing low-dose immunomodulatory treatments.

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**Key words:** immunotherapy; IL-2; lymphokine-activated killer cells; metastasis; transfer factor; interferon; renal cancer

The most promising systemic therapy for MRCC is immunotherapy with IL-2.<sup>1</sup> The original observations by Rosenberg *et al.*,<sup>2</sup> using i.v. administration of high-dose rIL-2 and LAK in MRCC patients showed encouraging clinical response rates and long-lasting remissions. These results have prompted many clinical trials using various administration routes: i.v. both in bolus and continuous or s.c. in combination or not with IFN and/or chemotherapeutic agents. In 1,411 MRCC patients, the overall rate of response was 20%.<sup>3,4</sup>

Nonetheless, despite the lowering of the IL-2 dosage and the decrease of the 4% fatal outcome of the initial studies, thanks to the improved selection of patients and the clinical experience acquired,<sup>5,6</sup> the persistence of more often than not severely adverse clinical side effects remains the most important hindrance for the wide use of IL-2.

Hence, being aware of the risk from a high IL-2 dose and of its ability to activate, at low doses, tumor mass rejection,<sup>7–9</sup> we attempted to develop alternative protocols. An observation made in 1 MRCC patient, in whom injection of a metastasis with IL-2 and LAK cells was followed by regression not only of the treated tumor but also of several other abdominal lymph node metastases, prompted us to further investigate the low-dose effect using the intralymphatic route. Following intratumor low-dose injections of IL-2 into infiltrating bladder cancers or lymph node metastases of MRCC patients, both the injected and the non-injected distant metastases often disappeared.<sup>7,8</sup> Thus, by administering very low doses of IL-2 and LAK cells into the lymphatic vessels of the foot, we observed encouraging clinical results with minimal side effects.<sup>10,11</sup>

To boost the overall immune response<sup>12,13</sup> and prevent opportunistic infections,<sup>14</sup> patients also received i.m. TF injections. In our institution, TF is currently used as an adjuvant treatment of

certain tumors, *i.e.*, non-small-cell lung cancer<sup>15</sup> and hormone-unresponsive prostate cancer,<sup>15</sup> with promising clinical results.

Following our preliminary observations, we report here results obtained in 122 MRCC patients treated with intralymphatic administration of non-recombinant IL-2 and LAK cells, IFN and TF.

#### MATERIAL AND METHODS

##### Patient population

From April 1986 to September 2000, 122 MRCC patients were treated. Their sex, age, appearance of metastasis from nephrectomy and organ involvement are reported in Table I. One hundred showed a performance status, according Karnofsky, of between 80 and 100, with 22 between 50 and 79. We further identified a subgroup of 99 patients by excluding 23 patients according to the following criteria: 8 because they were treated for <2 months; 1 because he was suffering from a known non-responsive histology (transitional-cell carcinoma), 4 because they were not nephrectomized and 10 because they were previously treated with rIL-2 elsewhere and, because of the adverse side effects, asked to enter into our protocol. These 10 patients are identified as group IL-2-R. Statistical analyses were carried out considering all groups. Four patients were not nephrectomized because of high surgical risk.

Only 118 patients underwent nephrectomy: 7 showed histological grade 4, 56 grade 3 and 20 grade 2. For 39 patients, we were unable to obtain grading and had only the pathologist's report stating "carcinoma of the kidney;" these nephrectomies were performed outside our hospital years before and slides were not available. All patients gave written informed consent for IL-2 treatment. The ethics committee of our hospital approved the written protocol. After evaluation of our results, the Emilia-Romagna Regional Therapeutic Committee, responsible for treatments, included, "intralymphatic administration of therapeutic drugs" in the list of approved therapies.

**Abbreviations:** CD, cluster designation; CR, complete response; EBRT, external-beam radiotherapy; LAK cells, lymphokine-activated killer cells; MRCC, metastatic renal-cell cancer; MU, mega-unit; PB, peripheral blood; PBL, peripheral blood lymphocyte; PR, partial response; PROG, tumor progression; rIL-2, recombinant IL-2; TF, transfer factor; TNM, tumor, node, metastasis.

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# G. Pizza, 2001

## Polmoni e Fegato

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PIZZA ET AL

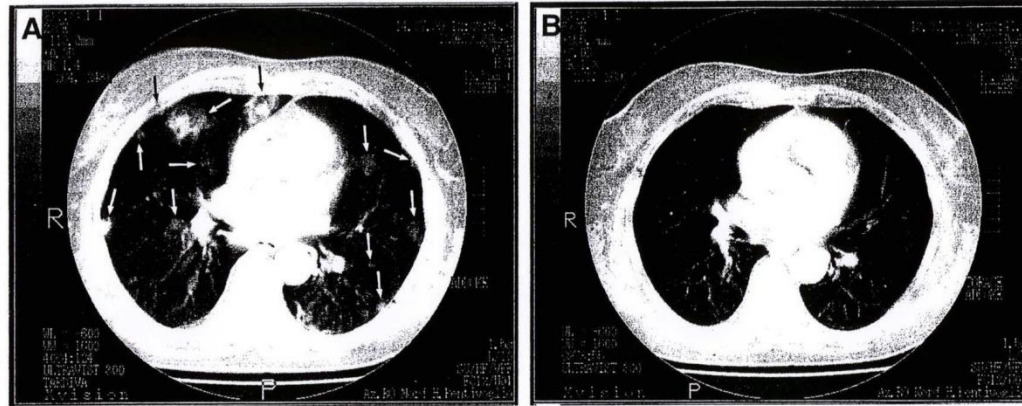
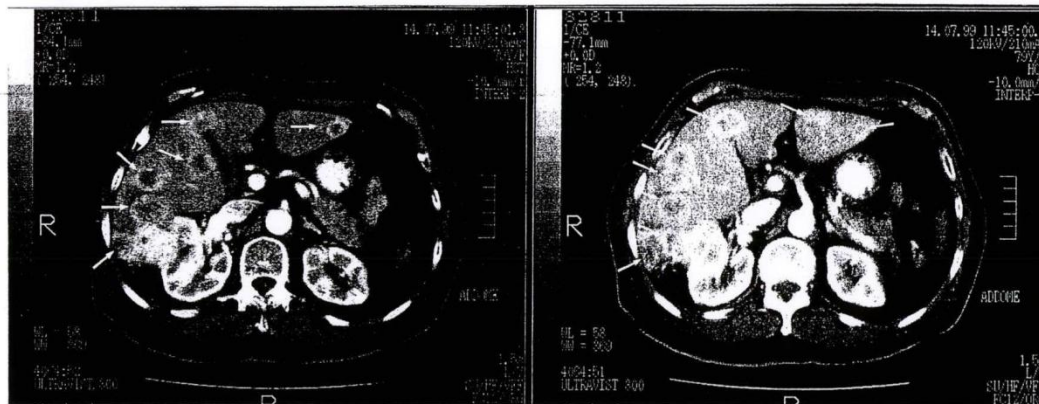


FIGURE 6 – An example of an important tumor load regression is the case of this 80-year-old female patient (code 67,188) who experienced 1 year after starting immunotherapy CR of more than 15 lung metastases (arrows) measuring 1 to 4 cm in diameter. PR was also observed in liver metastases.



# Principi della Terapia Metabolica

- Togliere al tumore i principi nutritivi necessari alla replicazione della cellula (Proteine, Glucosio, vit. B12, acido folico)
- Fare suicidare le cellule tumorali (APOPTOSI)
- Attivare i globuli bianchi contro le cellule malate
- Aiutare il fegato a smaltire le sostanze tossiche rilasciate dal tumore “sotto attacco”

# Le sostanze proibite (1)

- Nove aminoacidi essenziali
  - Leucina
  - Valina
  - Isoleucina
  - Lisina
  - Metionina
  - Triptofano
  - Treonina
  - Fenilalanina
  - Istidina

# Cibi che contengono i NOVE aminoacidi essenziali

- Carne
- Uova
- Latte (carne liquida) e quindi TUTTI i suoi derivati (formaggio, burro, panna....)
- Lievito (Pane, Pizza)
- Cereali OGM (Peter Day : Am.J.Clin.Nutr., 1996, 63, pp:651S-656S)
- Legumi OGM (Peter Day: Am.J.Clin.Nutr., 1996, 63: pp:651S-656S)





## Genetic modification of plants: significant issues and hurdles to success<sup>1-3</sup>

Peter R Day

**ABSTRACT** Transformation and regeneration is routine for many crop plants. A genetically engineered tomato with a longer shelf life at full ripeness was introduced in the United States in 1994, and other soon-to-be-released products, both foods and fibers, incorporate genes for resistance to pests, diseases, and environmentally benign herbicides. Other possibilities are altered plant fats and oils, methionine- and lysine-enhanced grain and legume proteins, plant foods that can deliver immunizing antigens, and other ways of controlling fruit ripening. Food safety concerns include the inadvertent production of toxicants and allergens. Foreign DNA can be introduced into plants by bacterial vectors, direct uptake by protoplasts, and mechanical introduction on metal particles or other materials. Limitations include little or no control of copy number or site of integration of the introduced DNA, dependence on selectable markers for recovery of traits, and inadequate knowledge of how to control key metabolic steps to maximize desirable traits. Directed genetic change still requires conventional crop breeding to deliver benefits to farmers and consumers. *Am J Clin Nutr* 1996;63:651S-6S.

**KEY WORDS** Plant transformation, genetically engineered foods, allergens, toxicants, plant breeding, fatty acids, phytase

### INTRODUCTION

Ever since the discoveries of Mendel, geneticists have been interested in the prospect of directed genetic change. Conventional plant breeding always requires progeny large enough to recover forms that recombine the desired features from the parents of a cross. Recombination results from random segregation due to chromosome reassortment and from crossing-over during gamete formation at meiosis. The gametes, pollen and egg cells, fuse randomly to form fertilized eggs, or zygotes, that develop into seeds. Hundreds of different crosses are made each year in large-scale crop-breeding programs, and individual segregating ( $F_2$ ) populations of  $\geq 1000$  of each cross are grown in successive years for selecting recombinants in later generations. In a major program of breeding winter wheat in the United Kingdom, several miles of single-row segregating families are grown each year to recover desirable forms for selection and evaluation. The number of families is progressively reduced by selection in each succeeding generation and the number of the plants of each family increases so that after 3 or 4 y yield trials are performed, first in small plots and then in larger plots in different locations.

After World War II there was some interest in producing mutations by exposing seeds to ionizing radiation. This was followed by treatment with chemical mutagens. Much time and effort was spent in searching for useful variants but few were found. Most of the mutants were inferior in vigor and in other respects compared with the original parental material.

### PLANT TRANSFORMATION

The discovery of DNA transformation in bacteria by Avery et al (1) in 1944 and the realization that genetic information is encoded in the nucleotide base sequence of DNA suggested that it might be possible to transform higher organisms with DNA-coding sequences to effect directed genetic changes. One of the first successful methods harnessed a circular DNA molecule, or plasmid, carried by the pathogenic plant bacterium *Agrobacterium tumefaciens*. The bacterium invades wounds in plant tissue, where it stimulates rapid host cell growth, resulting in the production of a plant tumor or gall. This occurs because a tumor-inducing (Ti) segment of the plasmid DNA becomes integrated in a chromosome of the host cell nucleus. Deleting the genes that control the production of tumor cells from the plasmid leaves right and left border fragments, each 24 nucleotides long, that mark the ends of the integrating Ti segment. This segment of bacterial plasmid DNA can be used as a vector to introduce foreign DNA. If a bacteriophage lambda *cos* site is included in the vector, up to  $\sim 40$  kb of DNA can be packaged in vitro between the borders. Transformation vectors include marker genes that can be detected in bacterial and plant host cells and controlling elements, or promoters, to drive the expression of the desired gene in plant tissue at the appropriate phase of development.

A drawback of the Ti vector system is that it is restricted to plants that are hosts for *Ag. tumefaciens*. Another method uses protoplasts that are prepared by treating plant cells with enzymes that dissolve cell walls. Held in a suitable osmoticum, such as a 4-mol/L solution of mannitol, they are stable and do not burst. The protoplasts are treated with a solution of transforming DNA, some of which is taken up and may become

<sup>1</sup> From the Center for Agricultural Molecular Biology, Rutgers, The State University of New Jersey, New Brunswick.

<sup>2</sup> Supported by the New Jersey Commission on Science and Technology.

<sup>3</sup> Address reprint requests to PR Day, Center for Agricultural Molecular Biology, Rutgers, The State University of New Jersey, New Brunswick, NJ 08902.

# Cereali e Legumi OGM P.Day, Am.J.Clin.Nutr., 1996,63,651S



# Concetto di VITAMINA

- Qualsiasi molecola biologica, prodotta in natura, generalmente da piante, che viene assorbita dall'organismo umano all'unico scopo di essere utilizzata per il corretto funzionamento dei complessi meccanismi cellulari biochimici di base che permettono la corretta e normale funzionalità vitale di organi, sistemi e apparati del corpo umano.

# Vitamina (2)

- La mancanza di queste particolari sostanze (“vitamine”) determina “malattia a partenza biochimico-cellulare”, a causa dell’impossibilità da parte dell’organismo umano di sintetizzare ex-novo tali sostanze.

# VITAMINE (3)

## meccanismi cellulari di base

- Metabolismo cellulare di base
- Funzioni specifiche di organo o di apparato
- Stimolazione o inibizione della MITOSI
- APOPTOSI

# APOPTOSI

- Morte programmata o suicidio delle cellule, se caratterizzate da danni genetici irreversibili, come ad esempio

le ABERRAZIONI CROMOSOMICHE

(rotture e cambiamenti del DNA),

e tali quindi da rendere irrecuperabile la funzionalità corretta della cellula

# APOPTOSI (specifico)

- Attivazione di *Endonucleasi* (CASPASI)  
che frammentano il DNA,  
agendo a livello di  
siti nucleosomiali

costituenti l'unità strutturale primaria della  
cromatina nucleare della cellula.

# CASPASI (1)

- Caspasi 1 (ICE)
- Caspasi 2 (ICH-1)
- Caspasi 3 (CPP32, Yama, Apopainina)
- Caspasi 4 (4TX, ICH-2, ICE-II)
- Caspasi 5( TY, ICE-III)
- Caspasi 6 (Mch 2)
- Caspasi 7 (Mch3, ICE-LAP3, CMH-1)
- Caspasi 8 (MACH, FLICE, Mch6)
- Caspasi 9 (ICE-LAP6, Mch6)
- Caspasi 10 (Mch4)

# CASPASI (2)

Tab. 4.3 : Substrati delle CASPASI umane (Tratto da : Porter N.: *Death substrates come alive*, Bioessays, 19, pp.: 501-507, 1997) modificata.

Substrato	CASPASI	Localizzazione cellulare	Conseguenza del taglio nell'apoptosi <u>Inattivazione di funzione</u>
D4-GDI	CASPASI 3	citoplasma	non noto
Gas2	non noto	microfilamenti	Cambiamenti morfologici
Huntingtina	CASPASI 3 (?)	citoplasma	Morte di un <i>subset</i> di neuroni
Alfa-fodrina	CASPASI 3 - like	citoscheletro	Cambiamenti morfologici(?)
Actina ?	non noto	varie	non noto
PARP	CASPASI 1, 3, 4, 6, 7	nucleo	Accelera la frammentazione del DNA e ne impedisce la riparazione
DNA-PK	CASPASI 3	nucleo	Inibisce la riparazione del DNA
U1-70 kD	CASPASI 3	nucleo	Inibisce lo <i>splicing</i> del RNA
Rb	CASPASI 3 - like	nucleo	Perdita dell'effetto anti-apoptotico (?)
PITSLRE	CASPASI 3 - like	nucleo	non noto
NuMA	non noto	nucleo	non noto
Laminine A, B, C	CASPASI 6	Impalcatura nucleare	Perdita di integrità della matrice cellulare
DNA topo I, II	non noto	Impalcatura nucleare	non noto
HnRNP C1, C2	CASPASI 3 - like	nucleo	non noto

D4-GDI : *GDP dissociation inhibitor type 4* (un inibitore delle GTPasi della famiglia Rho)

Gas2 : proteina codificata da un gene appartenente alla famiglia dei *Growth Arrest Specific Genes*

PARP : *poli(ADP)riboso polimerasi*

DNA- PK: *DNA-dependent protein kinase*

U1-70 Kd : componente di 70 KDalton della *U1 Small Nuclear Ribonucleoprotein*

PITSLRE : superfamiglia di chinasi *cdc2-like*

NuMA : *Nuclear Matrix and mitotic apparatus protein*

DNA topo I, II : *DNA topoisomerasi I e II*

HnRNP C1 e C2 : *heteronuclear ribonucleoprotein C1 e C2*

# APOPTOSI (specifico 2)

- Le molecole d'induzione (in genere VITAMINE) inducono l'APOPTOSI mediante l'attivazione di enzimi proteolitici intracellulari (CASPASI) che provocano degradazione per proteolisi di sequenze vitali del DNA, provocando così la morte della cellula



# APOPTOSI (specifico 3)

- La sequenza di APOPTOSI è caratterizzata da un alto consumo di ATP (energia biochimica) che ben la differenzia dai *processi infiammatori* propri della NECROSI

# Esempio 1 di APOPTOSI

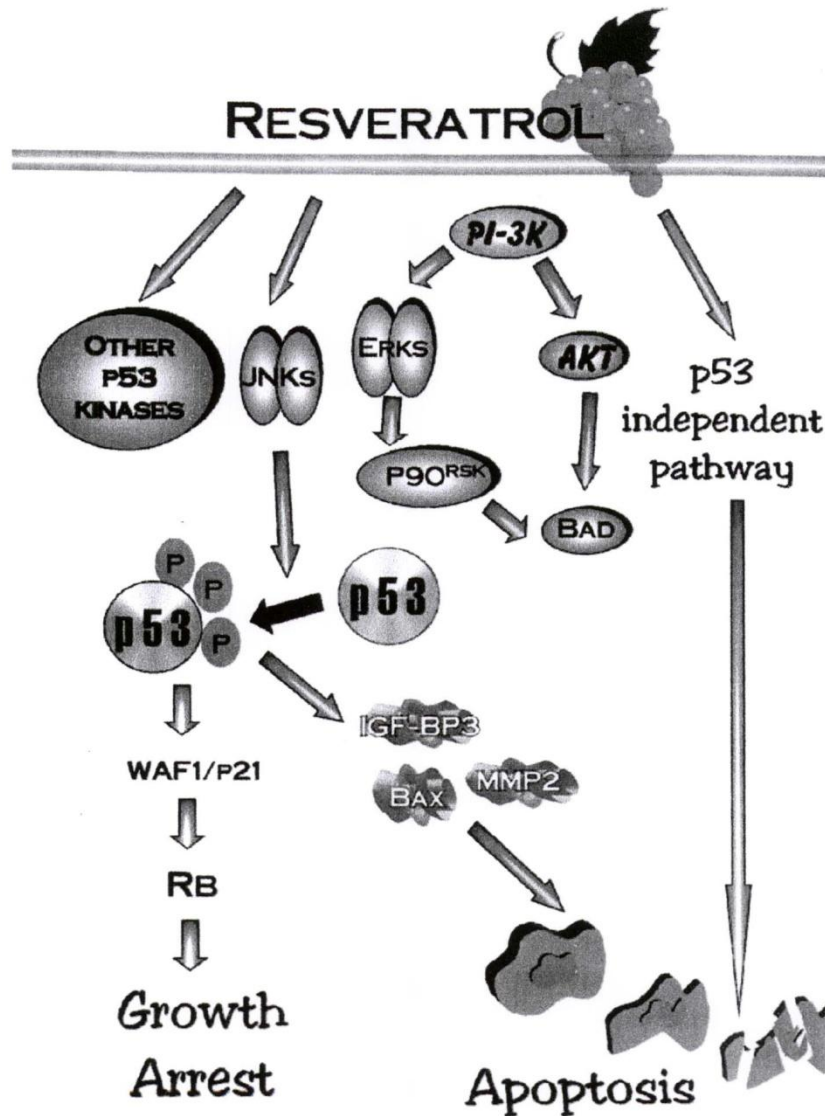
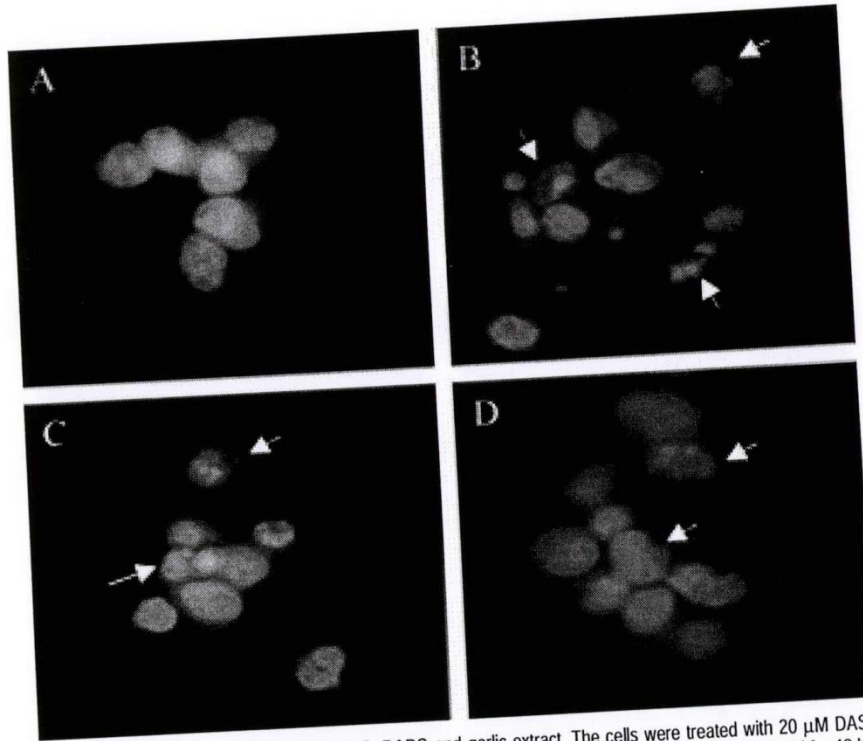


Fig. 1. Resveratrol induces pathways leading to apoptosis.

# Esempio 2 APOPTOSI

## estratto d'aglio su cancro polmone (1)

132 *Exp. Mol. Med. Vol. 32(3), 127-134, 2000*



**Figure 7.** Morphological analysis of H1299 cells treated with DAS, DADS and garlic extract. The cells were treated with 20  $\mu$ M DAS, 5  $\mu$ M DADS and 100  $\mu$ g/ml garlic extracts for 1 h. After incubation, new RPMI-1640 medium supplemented with 10% FBS was added and incubated for 48 h. At 48 h, the cells were stained with acridine orange and analyzed under a fluorescence microscope. **A**, control; **B**, DAS; **C**, DADS; **D**, garlic extract.

# Esempio 3 APOPTOSI

## estratto d'aglio su cancro prostata (2)

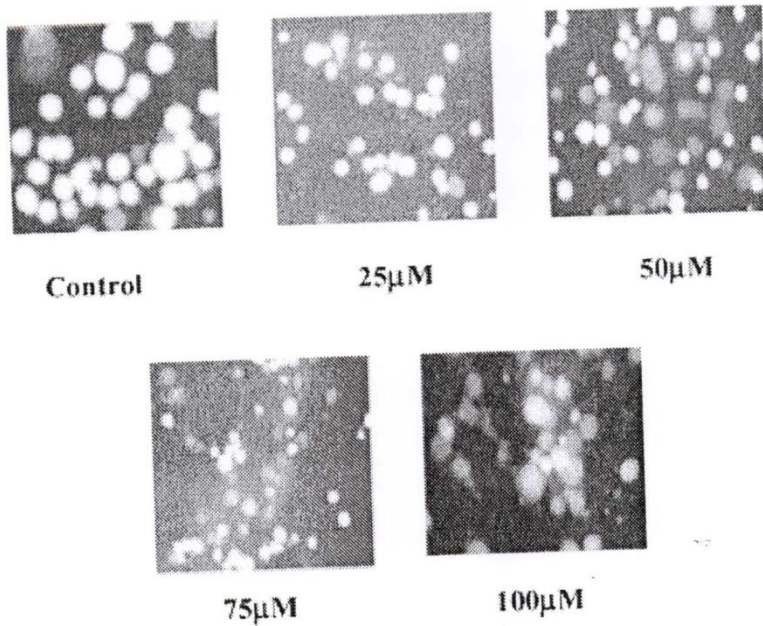
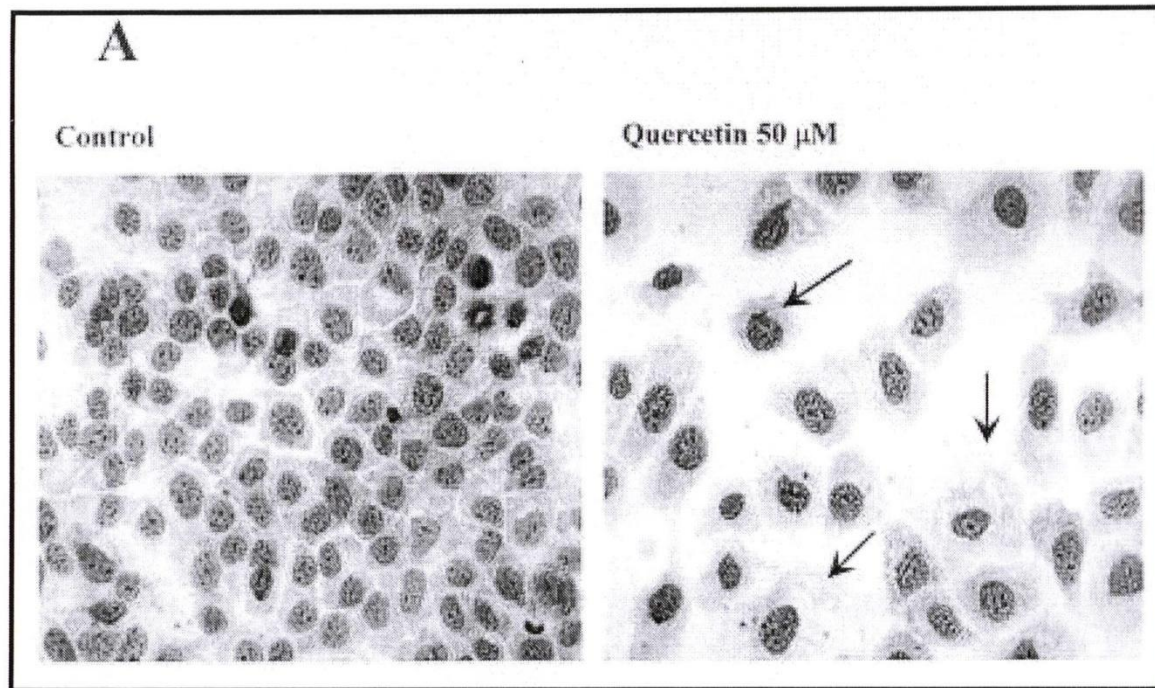


Figure 7. Ethidium bromide and acridine orange staining of LNCaP cells treated with different concentrations of diallyl disulfide for 24 h. The viable cells are shown as bright light colour, early apoptotic cells as grey colour and late apoptotic cells are shown as dark colour

# APOPTOSI esempio 4

## Quercetina su cancro della bocca

Nutrition and Cancer,53,220-231,2005





# APOPTOSI esempio 5

(J.Ethnopharmacology,98,2005,163-70)

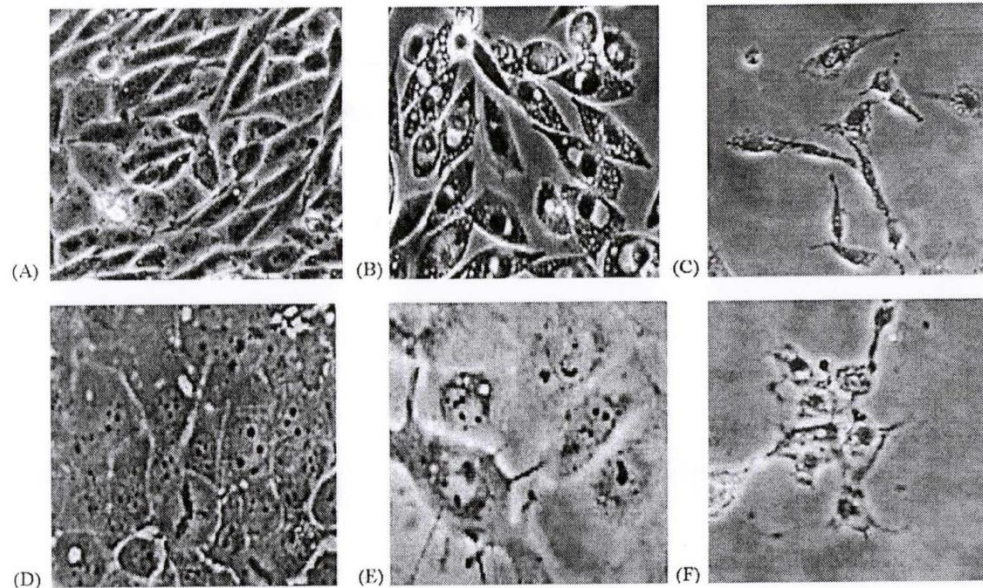
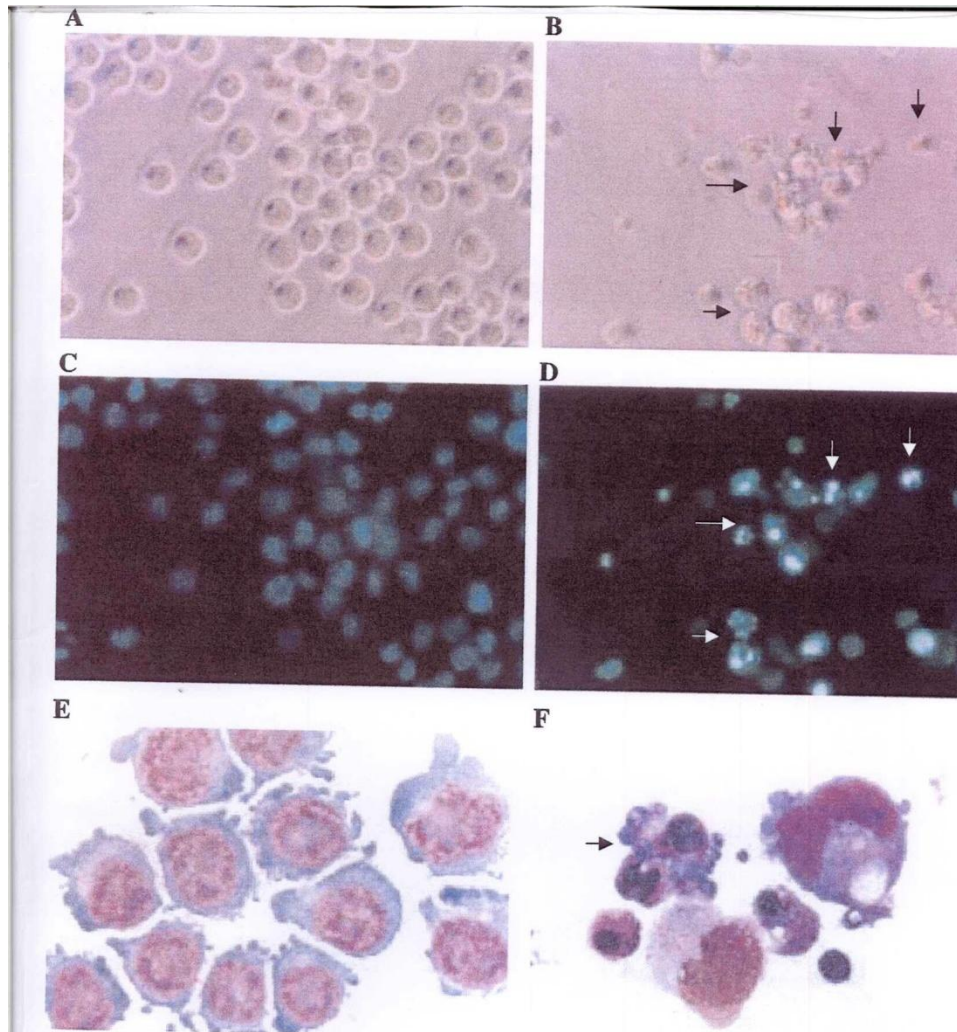


Fig. 1. Morphological observation of *Sutherlandia frutescens* WCP extract tested on CHO and cervical carcinoma (Caski) cells at different exposure times. CHO cells and Caski cells ( $2.5 \times 10^6$ ) were grown in Hams F12 and RPMI media respectively. (A) Untreated control of CHO cells; (B and C) CHO cells induced with 3.5 mg/mL with *Sutherlandia frutescens* WCP extract and treated for 8 and 24 h respectively; (D) Untreated control of Caski cells; (E and F) are Caski cells treated with 3.5 mg/mL of *Sutherlandia frutescens* WCP extract and treated for 8 and 24 h, respectively. The cell morphology was observed using an inverted microscope at 200 $\times$  magnification (Nikon). Cell death was indicated by cell shrinkage, cell disintegration and reduction in cell number.

# APOPTOSI esempio 6

## (Toxicology Applied Pharm.194,2004



Morphological changes of K562 cells after exposure to woodfordin I (15  $\mu$ M) for 48 h. (A and B) Phase-contrast microscopic view of the same visual field of (A) and (B); (A and C) untreated cells; (B and D) cells treated with woodfordin I. Apoptosomes are seen as spots in the nucleus by DAPI staining; apoptotic cells are shown with arrowheads, magnification  $\times 400$ . (E and F) View of untreated and woodfordin I-treated cells ( $\rightarrow$  the cell with distinct apoptotic bodies), magnification  $\times 1000$ .



# APOPTOSI esempio 8

J.Cellular Biochem.Suppl.27, 106, 1997

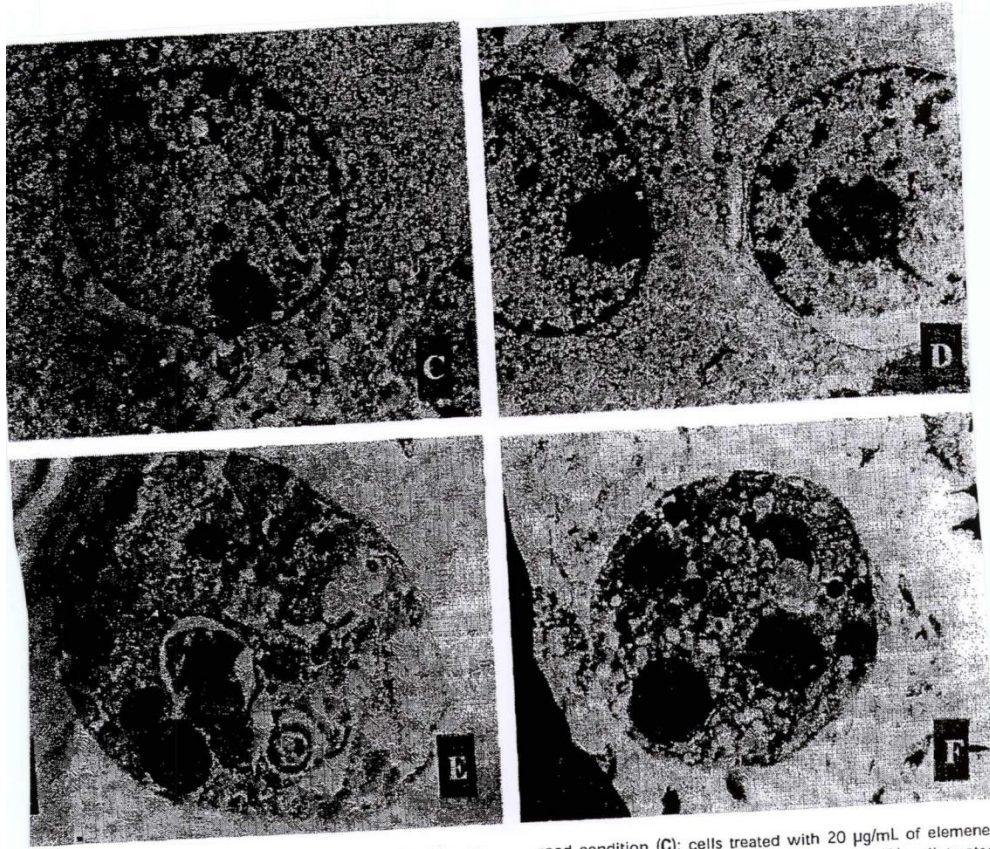


Fig. 3. Analysis of morphological changes of HL-60 cells induced by elemene. Control of HL-60 leukemia cells (A); cells treated with 10 µg/mL of elemene, structure of nucleus still in good condition (B); cells treated with 20 µg/mL of elemene, chromatin anchored to inner side of nucleus membrane, numbers of endoplasmic reticulum (ER) increased, mitochondrion in good condition (C); cells treated with 20 µg/mL of elemene, local lesion of nucleus membrane demonstrated (D); cells treated with 30 µg/mL of elemene, chromosome condensation, rudimentary nucleus membrane, and vacuolization of degenerative cells (E); cells treated with 10 µg/mL of CDDP, karyopyknosis, and apoptotic body surrounded by nucleus membrane (F).



# APOPTOSI

## esempio 9

Leukemia Research,  
29,2005,573-1

search 29 (2005) 573-581

575

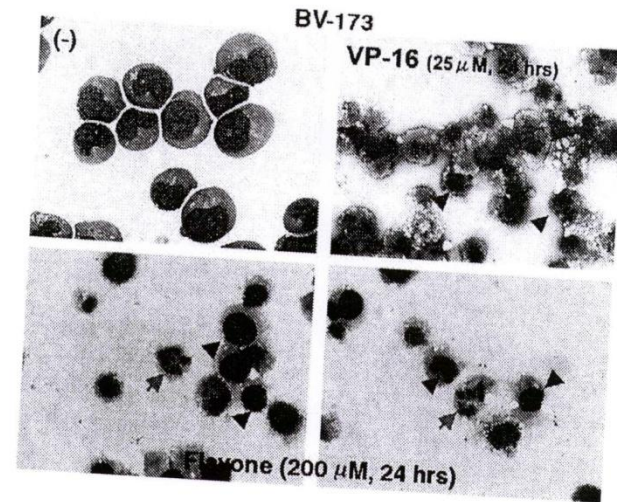


Fig. 2. Morphological examination of Flavone-treated BV-173 cells. BV-173 cells were cultured in the presence or absence of either Flavone or VP-16, as a positive control of apoptosis induction, for 24 h as indicated in the figure, then cytocentrifuged on the slide glasses. After Giemsa-staining, the morphological appearance of the cells was examined using light microscopy. The arrowheads indicate nuclear condensation. Typical apoptotic cells, characterized by cleaved nuclei, are indicated by the arrows. Magnification 400 $\times$ .

# APOPTOSI esempio 10

## J.Ethnopharmacology,96,2005,287-94

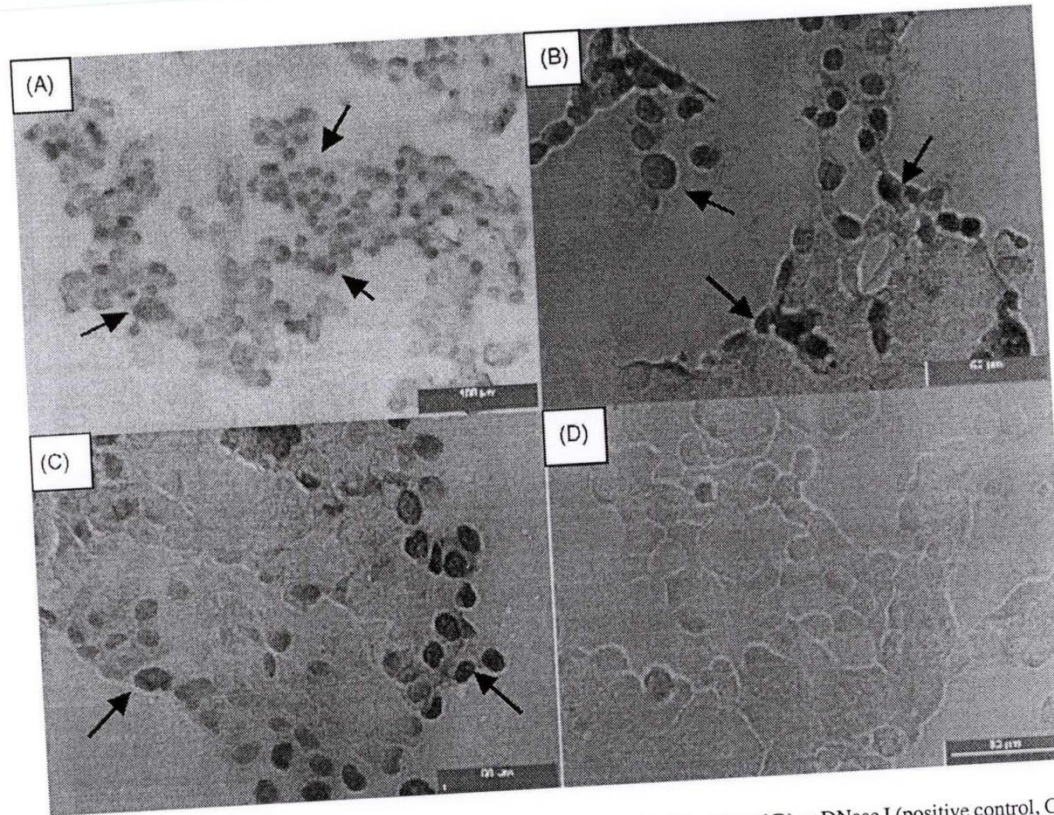


Fig. 2. Breast carcinoma T-47 cells were treated with either the methanol extract of *Pereskia bleo* (A and B) or DNase I (positive control, C) or DMSO (negative control, D) for 24 h and subjected to Deadend Apoptosis Detection System (Promega). Dark stained nuclei (arrows) of the T-47D cells were observed after cell treatment with the extract and DNase I whereas no stained nucleus in cells treated with DMSO was detected. A: 100 $\times$  magnification; B–D: 200 $\times$  magnification.

# APOPTOSI esempio 11

## Life Sciences 74, 2004, 1013-26

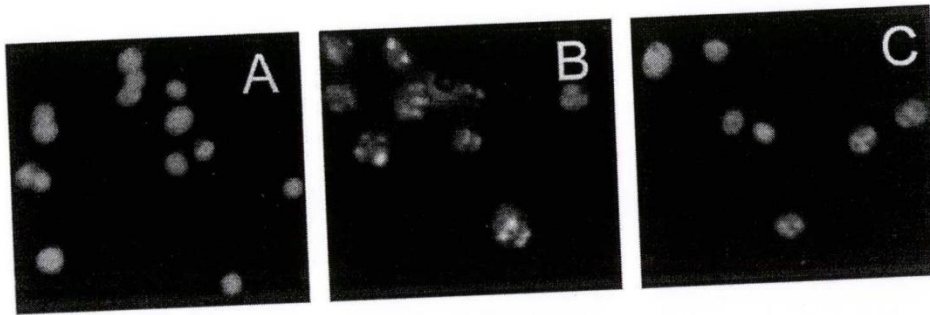


Fig. 2. *Betula platyphlla* var. *japonica* extract reduces  $H_2O_2$ -induced apoptosis in V79-4 cells. Cells were treated with *B. platyphlla* var. *japonica* extract for 1 hr prior to  $H_2O_2$  treatment and cellular morphological changes were observed using a fluorescence microscope at the magnitude of  $200\times$ . Representative photomicrographs of V79-4 cells treated with (A) vehicle only (B)  $100\text{ }\mu\text{M}$  of  $H_2O_2$  and (C)  $100\text{ }\mu\text{g/ml}$  of *B. platyphlla* var. *japonica* extract for 1 hr prior to the addition of  $H_2O_2$  ( $100\text{ }\mu\text{M}$ ) are shown.



T-H. Tseng *et al.*

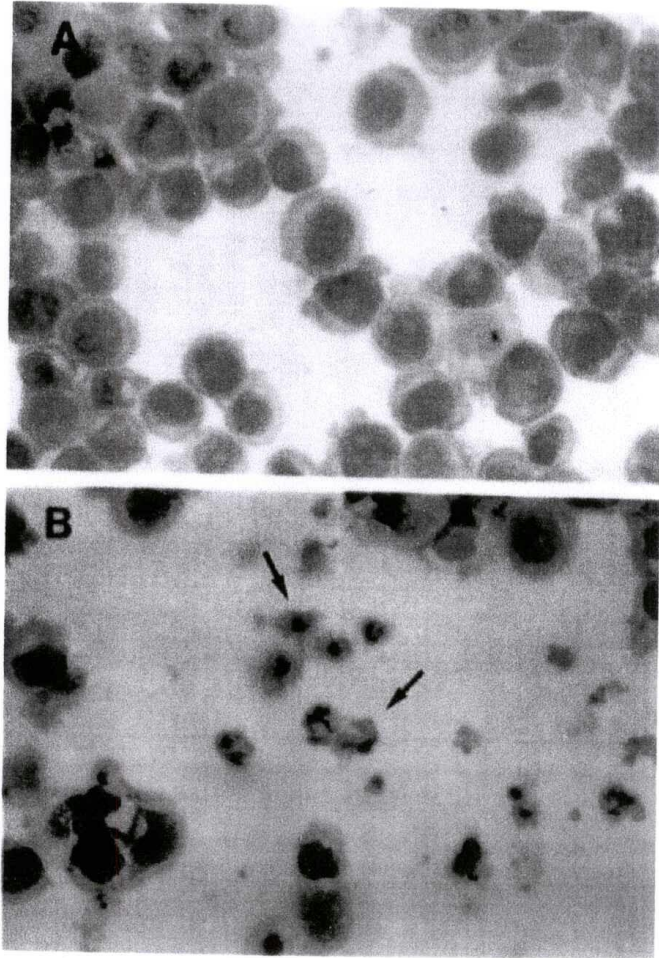


FIG. 3. Microscopic appearance of hematoxylin-stained nuclei of *Hibiscus* BCA treated HL 60 cells

APOPTOSI  
esempio 12  
Biochem.Pharmacol.  
60,2000,307-12

# APOPTOSI esempio 13

## European Journal Pharmaceutical Sc.

406

C.C. Chou et al. / *European Journal of Pharmaceutical Sciences* 19 (2003) 403–412

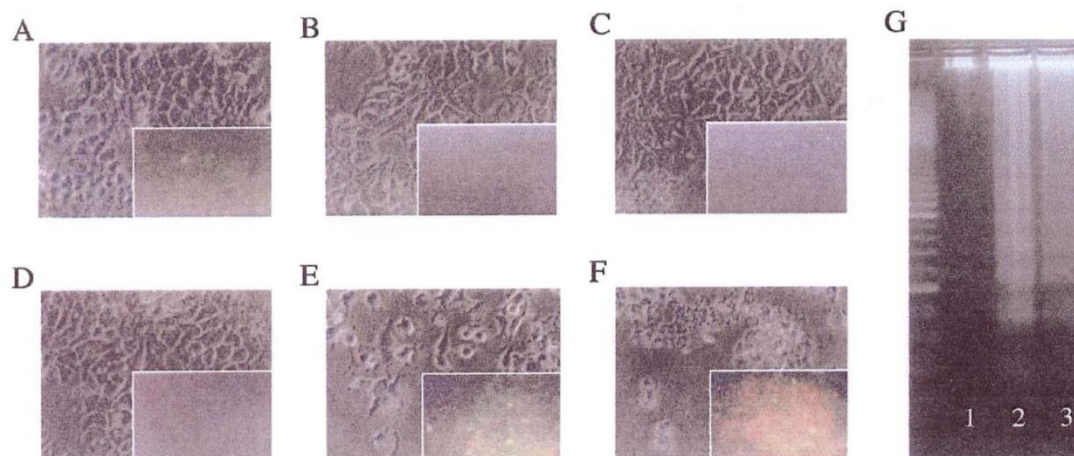


Fig. 1. Effect of the major ingredients of Long-dan-tan on apoptosis in Hep3B cells. Cells were exposed to vehicle (A), gentiopicroside (300 μM, B), baicalein (100 μM, C), geniposide (300 μM, D), alisol B acetate (50 μM, E) or saikosaponin-d (5 μM, F) for 24 h. Then, the cell morphology or double immunostaining (inset) with annexin V (green fluorescence) and propidium iodide (orange fluorescence) was detected as described in the Materials and methods section. Furthermore, cells were treated with vehicle (lane 1), alisol B acetate (50 μM, lane 2) or saikosaponin-d (5 μM, lane 3) for 24 h, and then the cells were harvested for the detection of DNA ladder using DNA fragmentation assays as described in the Materials and methods section.

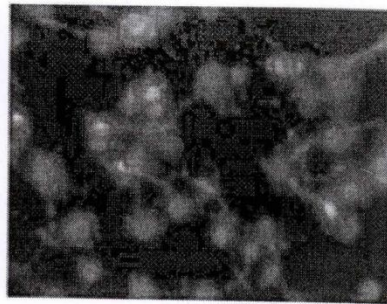
ingredients on cytotoxicity were also examined using FACScan flow cytometric analysis. As demonstrated in Fig. 2, gentiopicroside, baicalein, and geniposide had little influence on the phase distribution of the cell cycle progression. However, alisol B acetate and saikosaponin-d

significantly induced an increase in sub-G1 phase, indicating the induction of apoptosis in Hep3B cells. The concentration-dependent responses of alisol B acetate- and saikosaponin-d-induced effects were analysed. The data showed that both agents induced cell apoptosis in a

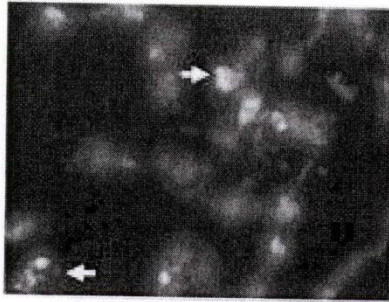
(B)



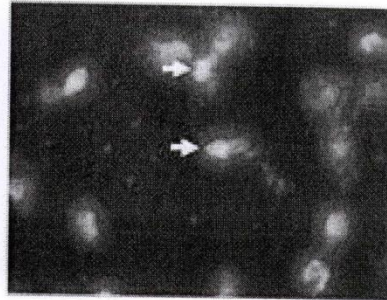
peonidin 3-glucoside 0  $\mu$ M



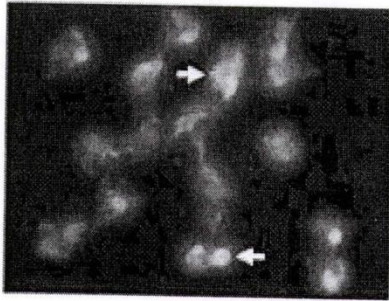
cyanidin 3-glucoside 0  $\mu$ M



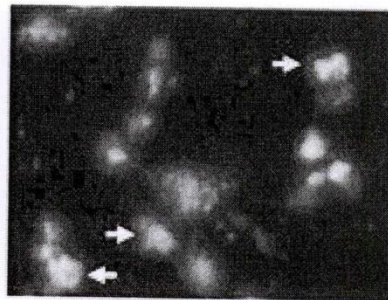
peonidin 3-glucoside 30  $\mu$ M



cyanidin 3-glucoside 10  $\mu$ M



peonidin 3-glucoside 50  $\mu$ M



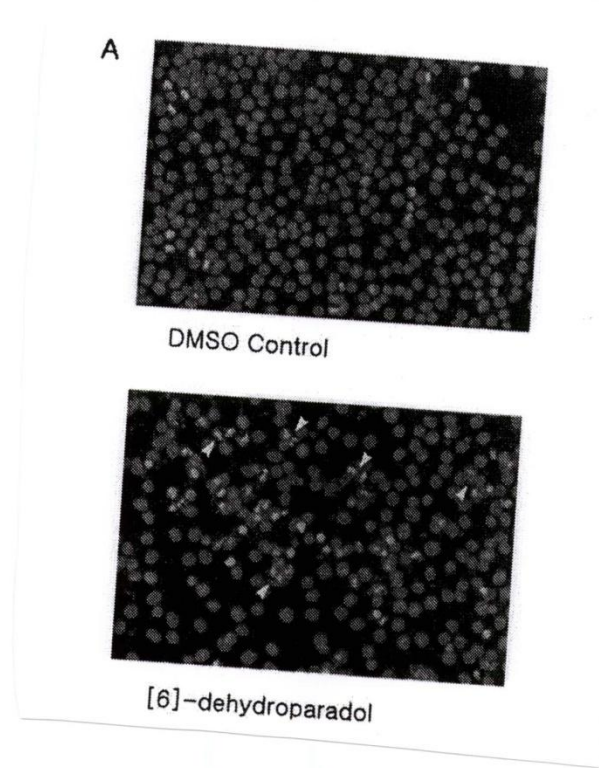
cyanidin 3-glucoside 30  $\mu$ M

APOPTOSI  
esempio 14  
Nutrition and Cancer,  
53,232-43, 2005



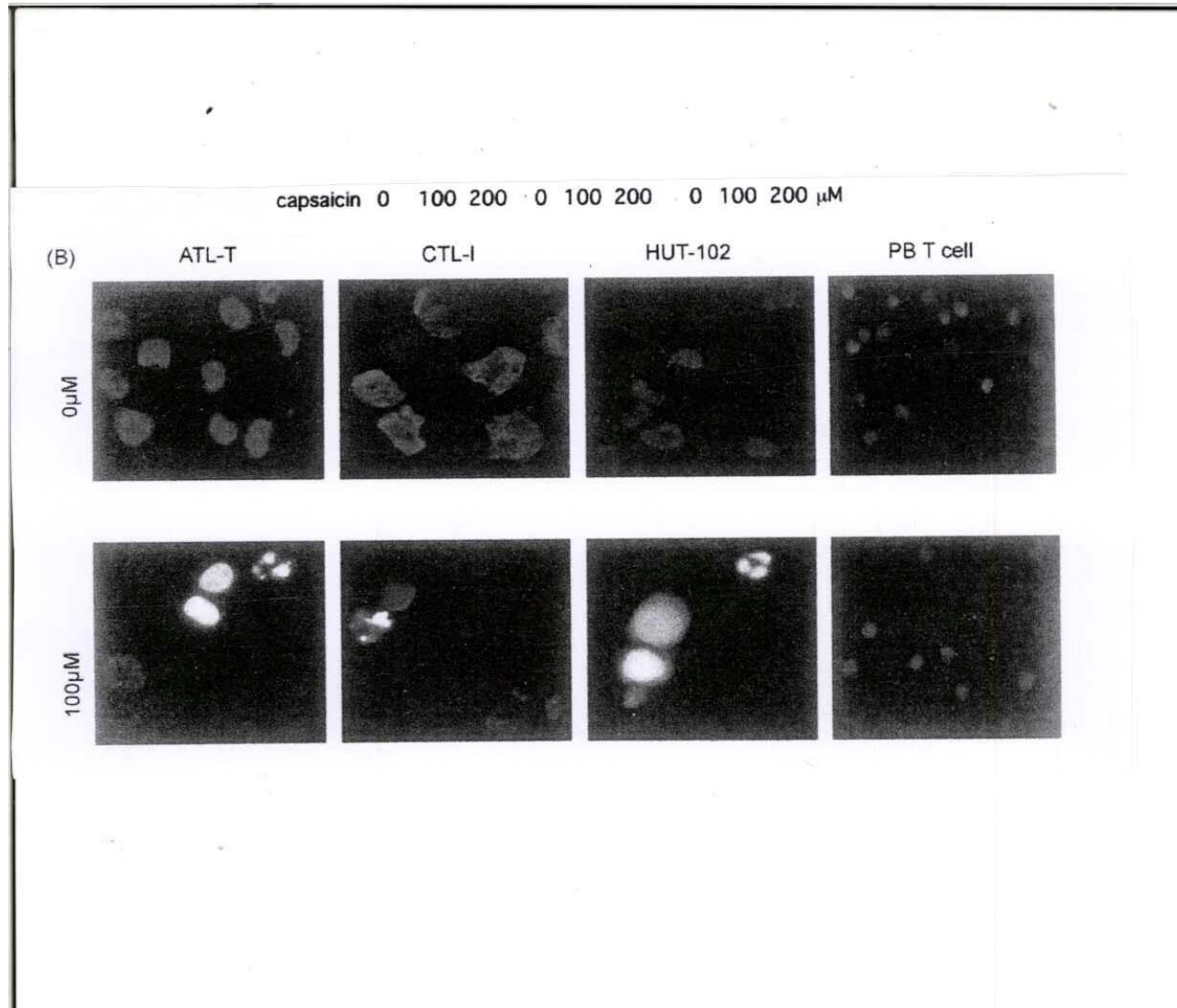
# APOPTOSI esempio 15

Cancer Letters,  
177, 2002, 41-7



# APOPTOSI esempio 16

Leukemia Research, 27, 2003, 275-283





# Elenco di Vitamine

## Gruppo A

- Circa 600 tipi di vitamine di gruppo A (isoprenoidi)
- **Caroteni** ( Alfa – carotene, Beta- Carotene, Gamma-carotene, Licopene.....)
- **Xantofille** (Luteina, Asta-xantina, Cripto-xantina, Canta-xantina, Zea-xantina.....)

# Elenco di VITAMINE

## gruppo B

- B1 (Tiamina)
- B2 (Riboflavina)
- B3 (Niacina )
- B4 (Adenina)
- B5(Acido pantotenico)
- **B6 (Piridossina)**
- B7 (Colina)
- B8 (Biotina)
- B9 (acido folico)
- B 10 (acido para-aminobenzoico)
- B11 (Carnicina)
- B12 (Cobalamina)
- **B13 (acido orotico)**
- B1 4 (Xantopterina)
- **B15 (acido pangamico)**
- **B17 (Laetrile)**

# Elenco vitamine gruppo C

- Circa 50 sottotipi.....
- Vitamina C 1 (acido ascorbico)
- Vitamina C 2 (Esculoside)
- ...acido deidro-ascorbico

# Elenco vitamine gruppo D

- Vitamina D 1 (Calciferolo)
- Vitamina D 2 (Ergo-calciferolo)
- Vitamina D 3 ( Cole-calciferolo )

# Elenco vitamine gruppo E

- Alfa- Tocoferolo
- Beta-Tocoferolo
- Gamma-Tocoferolo
- Delta-Tocoferolo
- Epsilon-Tocoferolo
- Zeta-Tocoferolo
- Eta-Tocoferolo

# Elenco vitamine gruppo F

(parziale)

- Acido alfa-linolenico (ALA) *omega-3*
- Acido eicosapenta-enoico (EPA) *omega 3*
- Acido docosapenta-enoico (DPA-3) *omega 3*
- Acido gamma-linolenico (GLA) *omega 6*
- Acido linoleico (LA) *omega 6*
- Acido arachidonico (AA) *omega 6*
- Acido di omo-gamma-linoleico (DGLA) *omega 6*

# Altre vitamine

- Vitamina K (Menadione)
- Vitamina I (Inositolo)
- Vitamina M (Stigmasterolo)
- Vitamina N (acido tiotico o lipoico)

# Polifenoli

- Circa 5.000.....
- Polifenoli Bioflavonoidi
- Polifenoli NON Bioflavonoidi



# Polifenoli NON Bioflavoidi

- **Stilbeni** (Resveratrolo.....)
- **Lignani** (Pino-resinolo....)
- **Acidi fenil-acetici** (idrossi-fenil-acetico)
- **Idrossi-cinammati** (acidi fenolici: acido gallico, acido ellagico, acido dsalicilico....)
- **Idrossi-benzoici** (acidi fenolici: acido caffeico, acido clorogenico, curcumina, acido cumarinico, acido ferulico.....)
- **Fenoli semplici** : catecolo, idrochinone, resorcinolo

# Polifenoli bioflavonoidi

- Antocianine / Antocianidine (Nasunina...)
- Flavoni (Luteolina, Apigenina...)
- Flavanoli (Catechine, Teaflavina, Tearubigina...)
- Flavonoli (Quercitina, Kampferolo, Miricetina, Rutina, ....)
- Flavanoni (Narigenina, Tangeretina, Esperidina, ...)
- Isoflavoni (Genisteina, Daidzeina....)
- Pro-antocianidine (Tannini condensati)

# Isoprenoidi

- Circa 180 (centottanta ) tipi diversi
- Acorenone
- Bergamotene
- Bisabolene
- Carvone
- Cedrina
- Cineolo
- Cresolo
- Cimene
- Elemene
- Estragolo
- Eugenolo
- Umulene
- Limonene
- Mentolo
- Ocimene
- Pinene
- Timololo

# Glucosinolati

- Circa 90 (novanta) tipi diversi
- Sulforafano
- Indolo-3-carbinolo (I3C)
- Di-idoilmetano (DIM)
- Ascorbigeno
- Glucobrassicina

# Organo-solfuri (Tio-allili)

- Alliina (S-allylcisteina solfossido)
- Allicina (diallil-disolfuro ossido o diallil-tio-sulfinato)
- Ajoene
- Diallil-solfato (DAS)
- Diallil-disolfuro (DADS)
- Allil-metil-disolfuro (AMD)
- Diallil-trisolfuro (DAT)
- Allil-metil-trisolfuro (AMT)
- S-allil-cisteina (SAC)
- S-allil-mercapto-cisteina (SAMC)
- S-metil-cisteina

# Saponine

- Ginsenoidi
- Saikosaponina D.....

# Terpeni

- Alisol A
- Bisabololo
- Partenolide
- Timoquinone
- Acido betulinico
- Acido boswellico
- Acido carnosico
- Acido pomolico

# Vitamine proteolitiche

- Bromelina (Ananas)
- Papaina (Papaia)
- Actinidina (Kiwi)



# Minerali organici

- Vengono assorbiti dalle piante in forma colloidale, che ne permetterà poi, in seguito, l'assorbimento a livello gastro-intestinale umano.
- Boro, Calcio, Cromo, Ferro, Germanio, Iodio, Magnesio, Manganese, Molibdeno, Selenio, Silicio, Vanadio, Zinco,

# Penetrazione delle vitamine nella parete cellulare

- E' stato ipotizzato che l'importanza degli OMEGA 3 nella Terapia Metabolica sia da attribuire alla loro capacità di rendere le pareti cellulari in grado di assorbire meglio le vitamine, determinando così una più facile APOPTOSI (dati non dimostrati)

# Omega 3 in terapia metabolica

## Pardini:Nutrition and Cancer,2005

NUTRITION AND CANCER, 52(2), 121-129  
Copyright © 2005, Lawrence Erlbaum Associates, Inc.

### Nutritional Intervention With Omega-3 Fatty Acids in a Case of Malignant Fibrous Histiocytoma of the Lungs

Ronald S. Pardini, David Wilson, Steven Schiff, Stephen A. Bajo, and Randall Pierce

**Abstract:** We present a case of a 78-yr-old man with malignant fibrous histiocytoma with multiple lesions in both lungs. Following diagnosis, he declined conventional chemotherapy and elected nutritional intervention by increasing intake of omega-3 fatty acids and lowering intake of omega-6 fatty acids. We estimated that he consumed 15 g of the long-chain omega-3 fatty acids eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) per day, and the ratio of linoleic acid/long-chain omega-3 fatty acids in his diet was 0.81. Serial computed tomography scans and pulmonary x-rays revealed remarkably a slow and steady decrease in the size and number of bilateral nodules. He has no apparent side effects from consuming large quantities of fish and algae oils rich in DHA and EPA and he remains asymptomatic.

#### Introduction

Malignant fibrous histiocytoma (MFH) is the most common soft tissue sarcoma of the elderly. MFH arising from the lungs is rare, although the lungs are the primary sites of metastasis (1-3). Lung MFH has a poor prognosis, and early diagnosis with timely surgical resection is the most common treatment resulting in long-term survival (3). We

a case-controlled study in women, the consumption of fish oil protected against the development of colorectal cancer (8), and epidemiological studies support the hypothesis that consumption of a diet rich in omega-3 fatty acids reduces the risk of breast and prostate cancer (9-11). Similarly, a population-based prospective study with 5,885 residents concluded that frequent consumption of fresh fish reduced the risk of lung cancer (12).

In laboratory animal models, nutritional intervention with high levels of dietary fat rich in omega-3 fatty acids resulted in decreased growth of a variety of mammary, prostate, and colon tumors (13-26). In a series of studies employing human mammary, colon, prostate, and ovarian carcinomas grown in athymic "nude" mice, consumption of diets rich in fish oil containing the long-chain polyunsaturated omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) resulted in decreased rates of tumor growth from 50 to 75% (16,18,27). Feeding diets rich in golden algae oil containing only one omega-3 fatty acid, DHA, suppressed human prostate and colon tumor growth in athymic mice by 75 and 90%, respectively (18,27), and was preferentially inhibitory to mammary carcinoma (19,20), suggesting that DHA was the primary tumor-suppressing long-chain omega-3 fatty acid. This conclusion was verified

studies to demonstrate loss of muscle of all autonomic lesions. Fig. 1E. The two arguments observed in the posterior segment were 18 and 21% in July 21, 2000 (Fig. 1C) and decreased to 2 and 4.8% respectively by April 2, 2001 (Fig. 1E), representing a decrease of over 95% of both muscle. The muscle mass observed in the anterior segment was 12.8 mm in November 2, 2000 (Fig. 1C) and decreased to 0.6 mm by April 1, 2004 (Fig. 1E), representing a decrease of 95% during the time period. Hyaline cast was also 0.4–2.4 mm in diameter without claspings. Interestingly, the cast diameter was 0.25 mm and 0.6 mm in the anterior and posterior segments, respectively.

In fact, the modern Western diet is considered to be deficient in omega-3 fatty acids and contains excessive amounts of omega-6 fatty acids, resulting in an omega-6:omega-3 essential fatty acid ratio of 15:1 (10,11).

Several reports suggest that humans possess an enzyme converted to a delta-5 fatty acid synthase, and a study of 24 Japanese men (all of whom were vegetarians) reported that the delta-5 fatty acid synthase gene was present in 100% of the subjects (12). The delta-5 fatty acid synthase gene was also present in 100% of the subjects in a study of 100 healthy men (13). The delta-5 fatty acid synthase gene was also present in 100% of the subjects in a study of 100 healthy women (14).

# Pardini, Nutrition and Cancer, 52,121-129, 2005

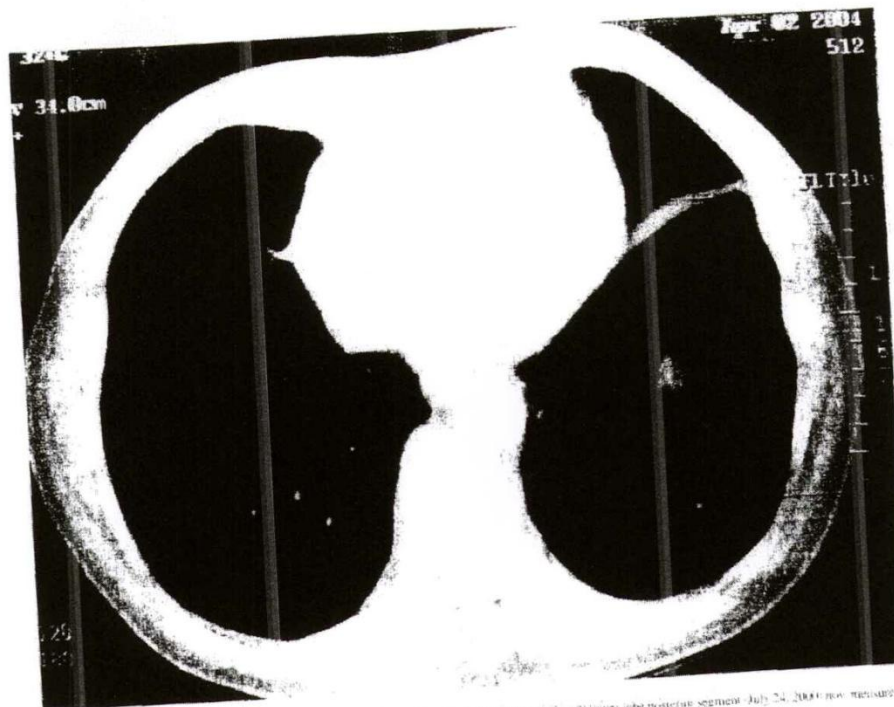


Figure 1. B. The thin-section CT scan was performed on April 2, 2004. The two masses shown on the left lower lobe posterior segment (July 24, 2000) now measure 1.4 x 1.4 cm (right) and 6.7 x 4.0 cm (left).

ratio of total omega-6 fatty acids to long-chain omega-3 fatty acids in adipose tissue was related to breast cancer risk in the EURAMIC multicenter study (11), a case-control study in Lyon, France, that concluded that breast tissue

The observation that the single resistant lesion was sensitive to a higher dose of long-chain omega-3 PUFAs is suggestive of an omega-3 PUFA dose-response relationship. This supports the conclusion that omega-3 PUFA intake is associated with the shrinkage of the lung lesions

# Risposta immunitaria

- *Inflammatio lymphonodis* (Linfociti Natural Killer)
- *Inflammatio tumoris* (Linfociti Killer, Monociti)
- *Detossificatio tumoris* (Transaminasi epatiche)
- *Deproteinatio tumoris* (Proteine TOTALI)
- *Reliquatio tumoris* (Granulociti)
- *Expurgatio tumoris* (Granulociti)
- *Resolutio ad integrum* (VES torna normale)

# Pancreas

- *“Pancreatic proteolytic enzymes are the body’s main defense against cancer and would be useful as a cancer treatment.....”*
- John Beard, M.D.
- British Medical Journal, 1906

# Natural Killer (1)

## Come agiscono le cellule «killer»

*Riconoscuta una cellula bersaglio, queste componenti del sistema immunitario aderiscono a essa e secernono una proteina letale che ne perfora la membrana provocando così la fuoriuscita del citoplasma*

di John Ding-E Young e Zarell A. Cohn

**L**o sistema immunitario viene da molto tempo considerato un esercito e i suoi soldati di cellule che si compiono in un'ora e si moltiplicano a dismisura, si sono sempre più riconosciuti nel caso delle cosiddette cellule «killer», o cellule assassine, che hanno il compito di dare la caccia alle cellule dell'organismo che funzionano male e non distinguono, in questo modo, tra i tumori, le cellule infettate e quelle difettate da virus e forse anche da altri agenti estranei. Lo scatenano a sé e le uccidono, che le cellule «killer» svolgono il loro compito con grande efficacia. Sperimenti sono stati fatti per capire come funzionano.

Oggi sappiamo che, se un bersaglio è colpito, le cellule «killer» si muovono verso la superficie della vittima e si attaccano. Più precisamente, si copano con le loro membrane di una proteina letale che trasporta la membrana del bersaglio e lo rompe. Poi, a meno di un minuto, la vittima si è distrutta.

Ricerche compiute in questi anni hanno dimostrato che le cellule «killer» sono attivate da una proteina letale che fa parte dell'immunità. Si chiama il tipo di cellule «killer» i «killer T» (cioè le cellule assassine) e si chiama «killer T» (cioè le cellule assassine) e si chiama «killer T» (cioè le cellule assassine). Una proteina di una sola catena e fatta da una molecola che si chiama «killer T» (cioè le cellule assassine) e si chiama «killer T» (cioè le cellule assassine). Una proteina di una sola catena e fatta da una molecola che si chiama «killer T» (cioè le cellule assassine) e si chiama «killer T» (cioè le cellule assassine).



In questa serie di micrografie elettroniche si vede una cellula killer che distrugge una cellula bersaglio. Qui sopra un filamento di citosol (in alto) entra in contatto con una cellula bersaglio. Nella pagina a fianco, a sinistra, il danno è completo: la cellula bersaglio, con la membrana perforata da una proteina secreta dalla cellula killer, si è svuotata: un flusso



## Come le cellule elaborano gli antigeni

Victor H. Engelhard

per un gran numero di patologie, dalle infezioni al cancro.

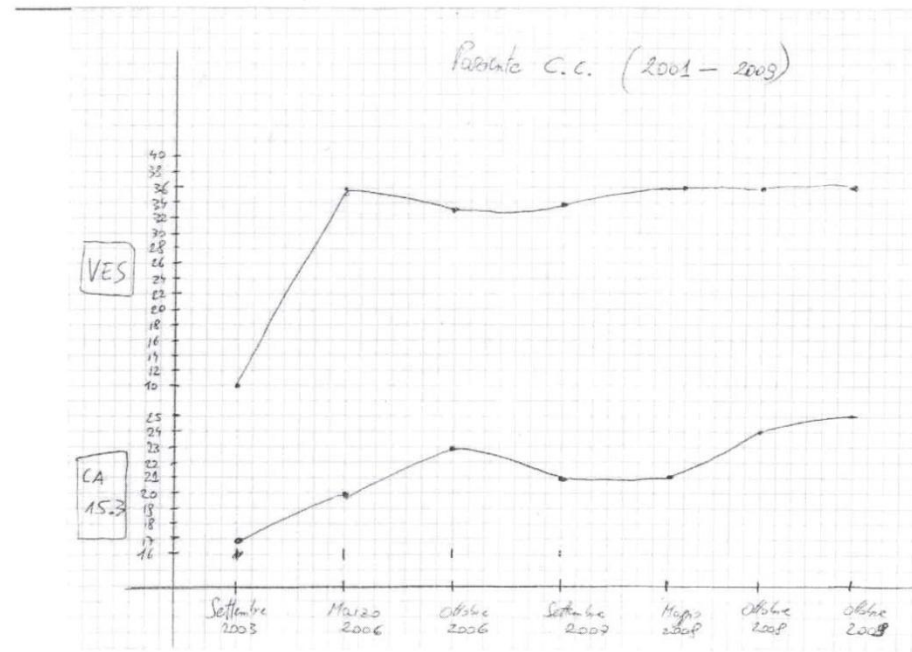
erogabano, deve entrare in azione un altro sistema presente nel sistema immunitario. Tutte le cellule del sangue portano molecole MHC in superficie. Nella cellula infettata, le molecole MHC si legono a piccoli peptidi, o frammenti di proteine, che provengono dal parassita, e li espongono alla superficie cellulare. I complessi di peptidi del parassita e molecole MHC dell'ospite formano gli antigeni che possono essere riconosciuti dai recettori presenti sui linfociti T citotossici (killer). I linfociti T possono così identificare e distruggere selettivamente le cellule infette, risparmiando quelle sane. La funzione dei complessi peptidi-MHC è dunque quella di segnalare

I complessi peptide-MHC sono importanti anche nella regolazione della risposta immunitaria. Cellule specializzate, come i macrofagi, presentano l'antigene ingerendo i materiali extracellulari che incontrano, determinando così

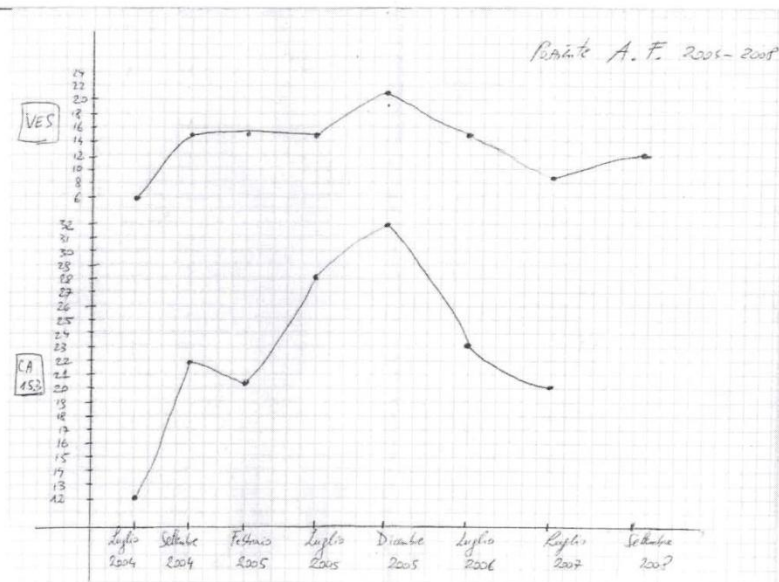
produrre peptidi e presentando questi ultimi come antigeni. Le cellule che espongono gli antigeni si spostano dai siti di infezione ai linfonodi, dove facilitano l'infetto per la risposta immunitaria; in effetti le cellule che presentano gli antigeni sono come messaggeri provenienti dalla prima linea di una battaglia. Quando i linfociti T helper riconoscono un complesso peptide-MHC su queste cellule, soccorrono le cellule aiutate a ornare (le infestano) con i primari, il differenziamento delle cellule del sistema immunitario.

presso le cellule della nostra superficie di una cellula è un evento fondamentale nell'affollamento di tutte le risposte immunitarie e, in particolare, nell'eliminazione dei parassiti intracellulari. Negli ultimi 20 anni, immunologi di tutto il mondo hanno cercato di scoprire come si forma il complesso fra una molecola

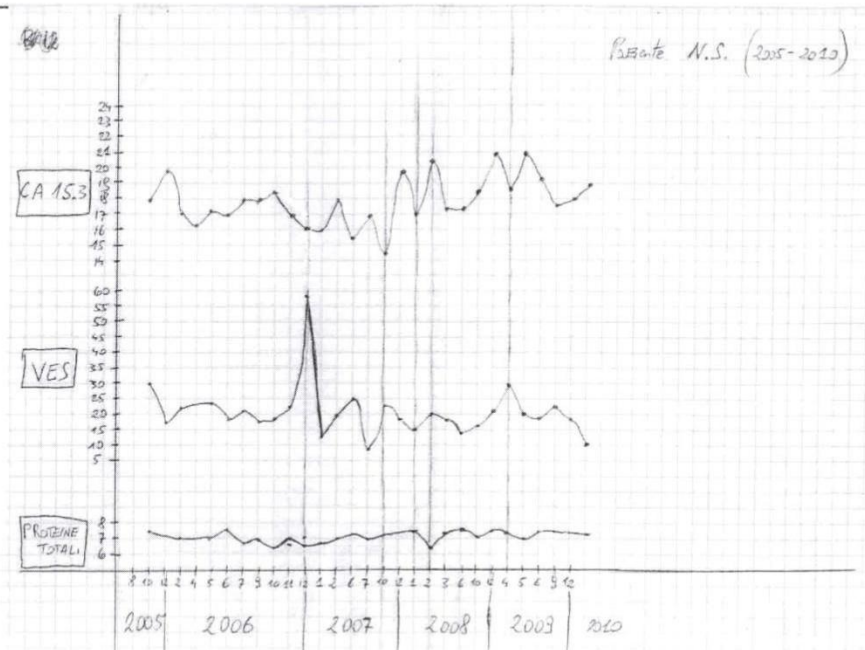
# Primo caso clinico



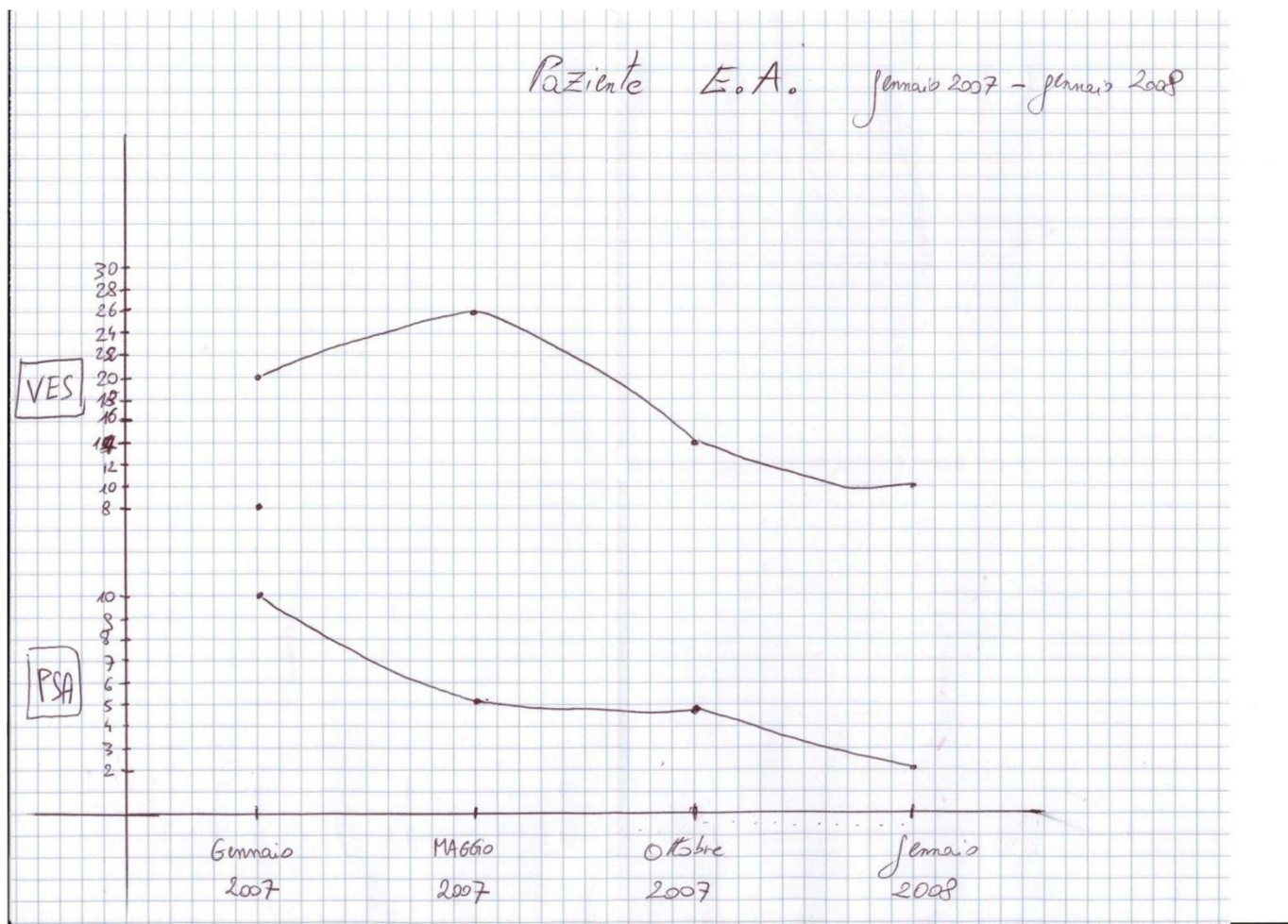
# Grafico 2



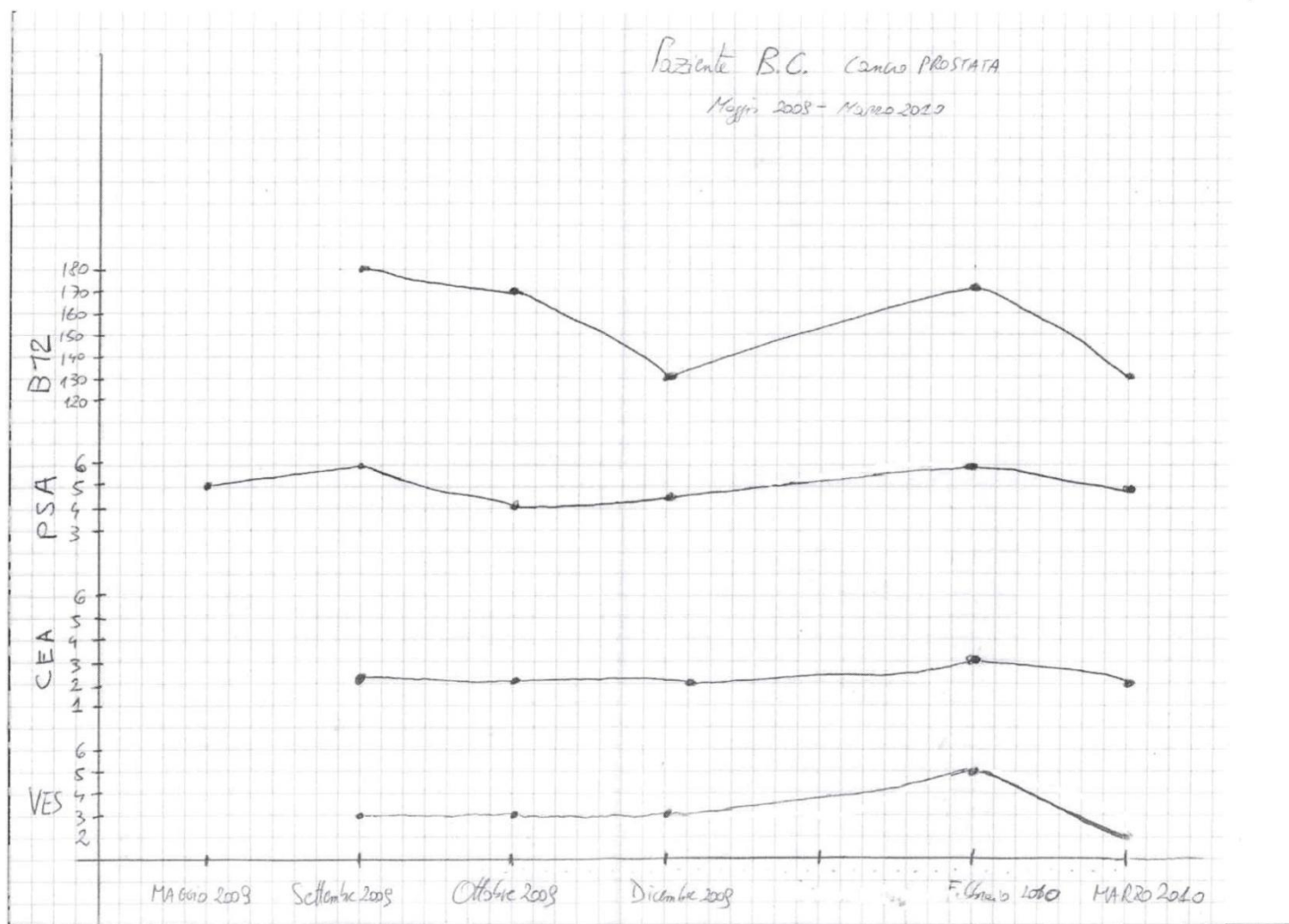
# Grafico 3



# Grafico 4



# Grafico 5



# CONCLUSIONE

- *The legal climate for alternative medicine remains repressive, the power and authority of conventional medicine, despite its well documented and rather glaring limitations, is formidable. However, I am gratified by the success of patients, and the others like them in our practice, who were able to avoid all aggressive surgery as well as toxic drug and radiation treatments. They still have their breasts, their lives, and their health.*

Nicholas Gonzales, New York