A NEW APPROACH TO CANCER THERAPY[1]

Mirko Beljanski
Centre Oncologique et Biologique de Recherche Appliquée
38370 Saint-Prim - France

INTRODUCTION

Cell function and differentiation are the outcome of multiple and complex events (1) (2) (3) (4) (5). Information contained in the genes is transferred to the enzymes and machinery responsible for protein synthesis via sophisticated biochemical pathways, some of which, despite their intricacy, are now well documented. Conversely, genes receive information which modulates their activity. Many different molecules are able to bind to nucleic acids (deoxyribonucleic acid, DNA, and ribonucleic acid, RNA), thereby modifying gene activity as well as that of various enzymes connected with it (6) (7) (8). It is well established that the effect of endogenous or exogenous molecules on such fundamental processes of cell life as DNA duplication, transcription and translation may dramatically affect other biochemical processes both downstream and upstream (7). Binding of any molecule to DNA may influence cell life "for better or for worse".

We have devoted a great many years of fundamental research to a thorough investigation of the mechanisms involved in these crucial steps, with the aim of selecting molecules capable of specifically correcting or arresting cell disturbances at the nucleic acid level and of enhancing beneficial gene expression.

The challenge of the cancer cell must be met on three different levels. First, cancer cell multiplication must be selectively arrested without injury to normal cells. Second, competence of the immune system must be protected and/or restored for an active local and whole body defence. The third necessity concerns enzymes whose activity escapes normal biological regulation (this may be a cause or a consequence of cancer) and contributes to disease development.

With these targets in mind, we defined a non toxic, selective strategy, encompassing the multiple aspects of the fight against cancer. New, unexpected processes were discovered which are now directly applicable not only to cancerology, but to other medical disciplines as well.

---

[1] In Biswapati Mukherjee (ed. in chief) *Proceedings of the international seminar : Traditional Medicine: a Challenge of the twentyfirst Century*, 7-9 Nov. 1992, Calcutta. Oxford & IBH Publishing CO. PVT. LTD., , 1993, 403 p.

RESULTS

I. PROTECTION AND RESTORATION OF IMMUNE DEFENCES

We shall start with this because it was chronologically our first achievement in the field of cancerology.

Everyone is aware of the high cell toxicity of drugs and ionising radiation commonly used for conventional cancer therapy (6)(9)(10). The two main immediate dangers are chromosome breakage and destruction of the bone marrow cells which give rise to blood cells : leukocytes, erythrocytes and platelets. As a result, immune defences are highly impaired or even destroyed (11) (12). Lymphocytes (which make up about a third of total leukocyte count) are choice victims. In addition, severe imbalance is induced among lymphocyte subsets, each of which is assigned a specific task and alloted a certain number of cells so that it reaches a certain size with regard to other subsets. These well-defined properties enable all these groups of cells to work efficiently together under the close control of physiological regulation. T4 lymphocytes are often compared to orchestra conductors, activating and directing other T lymphocytes and the antibody-producing B lymphocytes. If immune defences are to be preserved, it is imperative to restore not only a normal total lymphocyte count, but also a proper subset ratio.

Destruction of the immune system may be very largely avoided if blood stem cells in the bone marrow are successfully protected before or during toxic cancer therapy. To this end, molecules must be provided which are able to activate the necessary genes in the cells at risk so as to maintain their normal physiological count without inducing either subset imbalance or toxic effects and without enhancing cancer cell multiplication. The difficulty lies in finding such highly specific molecules, capable of "knowing" precisely where to go and act in the body. Such a feat could only be expected of biological molecules.

BLRs AND BLOOD CELLS

When we undertook this research, it had just been previously discovered that specific oligoribonucleotide primers were necessary for DNA replication, the initial step of cell multiplication (1) (13). We discovered short RNA fragments which act as specific primers for the replication of bone marrow and spleen DNAs, that is, DNAs of tissues in which cells of the immune system, but also platelets and erythrocytes originally form. The size of these RNA fragments, their purine/pyrimidine ratio and their selective activity on blood cell genesis were fully determined and reported (14) (15) (16). Using an original, biological technique, we were able to produce these RNA fragments in large amounts and this allowed extensive experimentation. We found that they promote in a physiological manner the formation of leukocytes and platelets, rapidly restoring their normal counts and, most importantly, also restoring normal ratios of lymphocyte subsets.

Experiments in rabbits demonstrated that this activity is in no way impaired by continuous administration of cyclophosphamide (Fig. 1). Use of our fragments does not induce tolerance and does not lead to stem cell exhaustion; on the contrary, stem cell activity is highly, rapidly and specifically enhanced, but always within physiological limits (17) (18) (19). We named these RNA fragments BLRs (for "Beljanski Leukocyte Restorers"; in French RLB). Their selectivity for normal blood stem cell DNA is so marked that they can be given to leukemic patients, because they never prime cancer DNA replication (Fig. 2A).

In cancer patients, BLRs have an optimal effect when they are given from the inception of chemotherapy or radiation therapy, or, at least, when patients still possess a sufficient number of intact bone marrow and spleen blood stem cells (15). When damage to these cells has become too extensive (inducing, for instance, chromosome breakage), rapid leukocyte and platelet genesis cannot always be achieved, but at least cell count collapse is generally arrested.

Special mention must be made of BLR activity on platelet formation. Depletion of these cells by toxic cancer therapy causes haemorrhage. BLRs rapidly restore normal platelet counts (19) (Fig. 2B). We observed moreover that after several weeks of BLR treatment, reticulocyte formation is also enhanced. Patients who took BLR during conventional cancer therapy were able to lead a normal life and continue working, while their cancer treatment was more efficient because it did not suffer either from nausea or hair loss. BLRs have also been used successfully to treat medullar aplasia and various immune deficiencies.

II. SPECIFIC ANTICANCER DRUGS

Rapid and uncontrolled multiplication of cancer cells, as well as synthesis of molecules which they normally should not produce, exactly parallel the biological behaviour of their DNA, which undergoes enhanced replication and spurious gene activation. Our first initiative was to compare the physical and biological characteristics of DNAs isolated and purified from normal and from malignant cells.

At the time we undertook this study, the scientific community at large considered that the difference between normal and cancer DNAs was caused by mutation, that is to say, by alterations of DNA primary structure. This explanation is still held to be true: though interest now focuses on oncogenes, their harmful activities considered to derive from mutations which have occurred in normal proto-oncogenes.

In contrast, we demonstrated that the fundamental difference between normal and cancer DNAs really lies in their secondary structure: the cancer DNA double helix is permanently relaxed over large areas, whereas, in normal DNA, relaxation only occurs locally and temporarily for replication or for gene expression.

We came to this conclusion after observing that molecular absorption of 260 nm UV radiation was steadily higher for cancer DNAs than for normal DNAs[2]. This UV absorption, which is maximum for fully denatured DNA, is known to relate to relaxation induced by breakage of hydrogen bonds which hold together the two strands of the molecule. Another way of evaluating this relaxation is to measure the decrease of the denaturation temperature (20), but the UV technique is simpler and easier (21). Hyperchromicity, i .e. higher UV absorption compared to a control, means that more H-bonds are broken, so that the molecule becomes less stable. Hyperchromicity of cancer DNA compared to normal DNA means that the malignant molecule has lost normal stability. Cancer DNA is a destabilized molecule.

Cancer DNA hyperchromicity is specially marked when UV absorption is measured in the presence of carcinogens. This too may be accounted for. We showed that cancer DNA is more receptive to carcinogens than normal DNA. We can visualise the cancer molecule as having in many places broken H-bonds inducing strand separation, and isolated strands are an easy prey for all sorts of molecules, which come and bind to them. As our experiments indicated, a number of those molecules, i.e. carcinogens, have the property of enhancing DNA destabilization and strand separation (22). This in turn makes the DNA molecule more receptive to carcinogens, so that it reacts ever faster and more extensively to their presence, with destabilization spreading over increasingly extensive areas and eventually reaching a point where so many H-bonds are broken that the strands come apart and severe damages are free to occur. Our initial findings concerning the mechanism of DNA destabilization were later supported by data reported by several authors who studied DNA chain relaxation in the presence of various chemical agents (23) (24) (25) (26) (27).

DNA chain opening and the ensuing strand separation are fundamental conditions for DNA replication and for gene expression. In normal DNA, precisely controlled strand separation exposes at one time a limited number of initiation sites, which so become accessible to replication or transcription enzymes. Facilitated strand separation in malignant DNA may be expected to induce extensive uncontrolled exposure of initiation sites, leading to accelerated replication and spurious gene transcritpion. But when such regions of the helix become distended by H-bond breakage, other regions on either side become more tightly wound. Genes there situated cannot be transcribed anymore and must remain silent. Gene expression becomes aberrant.

THE ONCOTEST

Measurement of 3H-labeled DNA synthesis using normal and cancer DNA templates not only shows that cancer DNA replicates more actively than normal DNA, but also demonstrates that the

[2] "Normal DNA" will be used throughout for "non cancer DNA", as opposed to "cancer DNA".

amount of newly synthesised labelled DNA is clearly correlated to template DNA destabilization (22) (Fig. 5). The causal relationship between DNA destabilization and cancer holds true not only for mammalian but also for plant DNA (28) (29).

Conversely, any compound which may be shown to significantly increase cancer DNA replication and hyperchromicity should be considered to have carcinogenic properties. This is the basis of our ONCOTEST (22) (30) (31) (32), which combines both these in vitro measurement techniques. We were able to demonstrate the additive effect of several destabilizing compounds (Fig. 4) and to detect a number of yet unrecognised carcinogens. We must remember that non mutagenic carcinogens are not detected by conventional tests; a case in point is that of steroid hormones, which are revealed as potent carcinogens by the Oncotest, a property which had been clinically observed from the time of Lacassagne's pioneer work but was only recently fully documented (33) (34).

In addition, many usual anti mitotic drugs, which, in high therapeutic doses, are designed to destroy cancer cell activity, prove, in low doses, to be carcinogens themselves; this is true for such drugs as cyclophosphamide, daunorubicin, mitomycin, DMBA, CCNU... (Fig.3). When treatment must be interrupted due to harmful side effects, these drugs are progressively excreted by the body until the small amounts that remain boost cancer cell activity anew.

In the Oncotest, carcinogens are detected by their ability to strongly enhance DNA replication and hyperchromicity while affecting normal DNA five to ten times less (Fig. 3). This striking difference makes Oncotest results unequivocal. In vivo, normal DNA is preserved from damage by a number of repair enzymes. However, if destabilizing compounds, especially potent ones, are persistently present is a cell, they can succeed in overwhelming natural biochemical defences and in progressively inducing H-bond breakage and destabilization of normal DNA; from then on, malignant transformation becomes possible.

It should be noted that the Oncotest also detects purely toxic compounds, which interfere with polymerase activity but do not destabilize DNA, and neutral substances, which show no effect on DNA secondary structure and replication[3].

[3] Experimental data obtained in vitro and in vivo have shown that various agents may favor the destabilization of DNA chains in the helical structure, thus creating conditions which permit DNA template activity to occur (6) (7) (35) (36).

In order to achieve DNA destabilization and/or strand separation, hydrogen bonds which hold together DNA complementary chains have to be somehow broken. This can be accomplished at high temperature or alkaline pH, or by the action of various substances, such as enzymes, carcinogens, hormones, antimitotic drugs, etc. Free purine and pyrimidine bases which interact with DNA destabilize the secondary structure of DNA, and this results in a drop of several degrees in the denaturation temperature (35)(37). DNA unwinding by organic solvents (non intercalating agents) indicates that dehydration leads to structural perturbation and that water activity in the micro environment of DNA is related to the stability of the double-stranded DNA (37).

This structural transformation may result in a change of binding efficiency as well as binding specificity of various agents including carcinogens. Other agents, such as acridines, daunorubicin, adriamycin or psoralen, bind to DNA with non-covalent linkages (they may also intercalate), thus modifying the physicochemical structure of DNA. By using absorption spectra or X-ray diffraction diagrams, evidence may be obtained that under the influence of the above agents DNA molecules become substantially elongated (38).

Destabilization of DNA may be controlled either by a decrease in the denaturation temperature of DNA (21) or by an increase in UV absorbance (hyperchromicity) at 260 nm. The UV absorbance technique is a rather simple one and easily accessible.

Because cancer DNA so easily responded to destabilizing agents, it could also be expected to respond to molecules which would act in the opposite way and restabilize it by bringing separated strands back together, closing the double chain.

We took advantage of the Oncotest to search for compounds which would "bolt" DNA strands together, so that secondary structure returns to normal. To this day, we found over half a dozen specific anticancer compounds, mostly plant-derived beta-carboline alkaloids, flavonones and flavonoids. We must insist on the term "specific": experiments demonstrated that these "bolt-molecules", which close up destabilized chains and hold the strands strongly together, selectively restabilize cancer DNA, yet have no effect on normal DNA. Due to their unique properties, they do not prevent normal DNA replication and expression, while checking those of cancer DNA. In the latter, some of these anticancer drugs bind to replication initiation sites (39), others prevent chain elongation. They incapacitate highly malignant cells, causing their death, but they can revert to normal the cells in which malignant transformation has not gone too far. In no way do they perturb normal cell life. They have proved active both in mammalian and in plant cells, in vitro and in vivo (40) (41) (42) (Fig. 6).

One of these molecules is alstonine. In vitro, this alkaloid binds to purified DNA from cancer cells: an alstonine-cancer DNA complex was isolated (39). In contrast, alstonine "ignores" DNA from normal cells. Alstonine inhibits growth of cancer cell cultures (43) (Fig. 7). It also exhibits pronounced activity in vivo. We carried out a very large number of experiments in mice to investigate inhibition of cancer cell multiplication by alstonine and other selective anticancer alkaloids. Assays not only confirmed their efficacy, evidenced by complete disappearance of tumours, but also demonstrated their total lack of toxicity, even in amounts largely exceeding therapeutic doses (this was to be expected from Oncotest results). In particular, hemopoietic cells were in no way damaged. Cured mice survived in perfect condition (44).

We should like to mention here a series of experiments on mice which were designed to test the hypothesis that low doses of antimitotics or radiation could enhance the effect of our anticancer compounds. As mentioned above, we showed over ten years ago, using the Oncotest, that a number of conventional anticancer drugs, when present in very small amounts, become carcinogens; this was recently demonstrated by other workers too. Then we thought that this unwanted DNA destabilizing effect could be put to good use: if given in very low doses, unable to cause noxious side effects, conventional anticancer drugs, as well as radiation, would enhance cancer DNA strand separation just enough to facilitate binding of our non toxic anticancer compounds. This proved indeed to be the case. For instance, when 24 to 48 h after cancer cell inoculation, mice were given suboptimal doses of one of our compounds, together with low doses of an antimitotic, results were very positive (Fig. 8).

Once this synergy had been demonstrated, combined therapy was successfully used, this time with optimal therapeutic doses of our compounds, to accelerate and/or improve cancer treatment and increase percentage of recoveries. An additional benefit is that our novel therapy is more easily accepted by physicians, who feel more secure when there is no need to discontinue classic therapies.

The flavanone (JO-1)[4] prevents in vitro multiplication of human cancer cell lines (colon, ovary, breast, leukaemia); under the same experimental conditions, normal bone marrow cell multiplication is not affected (Fig. 9). Naringin enters into our "JO-1". We must underline that two cancer cell lines resistant to conventional anticancer agents prove sensitive to this JO-1, which strongly inhibits their proliferation in vitro and in vivo. In addition, combination of JO-1 with classic antimitotics or radiation proved beneficial when tested in mice, yielding a high percentage of survivals (Fig.10), with animals remaining in good condition.

It should be noted however that a few types of cancer cells respond less favourably to inhibition by our selective anticancer drugs. Though mouse and human melanoma cells respond well in vitro, treatment in vivo seems primarily hampered by poor penetration of the drug into melanoma cells, due to the presence of excessive amounts of ferritin, which competes with the drug for DNA binding sites. A number of years ago, we showed that this protein, like other cancer markers, is a carcinogen (45) (46) ; iron too should be watched in as much as it enters into ferritin and amounts of both iron and ferritin should be closely monitored in cancer patients.

III. BIOLOGICAL REGULATORS

A by-product of DNA destabilization is the formation of enzymes, particularly nucleases, which differ in some measure from their normal counterparts; they are specific of malignancy and contribute to its maintenance. It is imperative to check their formation and restore that of normal enzymes using purified plant extracts. We were able to prepare biological regulators which, while stemming from the same principle as our anticancer drugs, have no true anticancer activity, but succeed in bringing progressively back to normal gene activation and related enzyme activity in cells that have dysregulated DNA. One of these targeted biological regulators has become a valuable component of our specific cancer therapy. Its applications also extend to other diseases (unpublished results).

DISCUSSION AND CONCLUSION

There is presently a patent need for a new approach to cancer therapy, because conventional anticancer treatment has failed to meet its objectives: most agents have been revealed as mutagenic and/or carcinogenic (6) ; they are highly toxic, not only for cancer but also for normal cells, and this is fundamentally due to the fact that they make no difference between a normal and a cancer cell. At present, cancer therapy faces a deadlock: about 80% of solid malignant tumours in adults are intrinsically resistant to a variety of anticancer drugs or else become rapidly resistant to mono- and even polychemotherapy (47) (48).

[4] JO-1 contains Naringin in a large amount in addition to some not yet identified substances.

Almost fifteen years ago, we proposed a totally novel approach to cancer therapy, based on the use of highly selective drugs specifically targeted to malignant cells and inactive in normal cells. By measuring UV absorption of normal and cancer DNAs, which was always considerably higher in the latter, we were able to discover, in the mid-seventies, that what makes the fundamental and steadily observed difference between normal and cancer DNAs is not the existence of mutations (which are not consistently found), but a difference in the secondary structure of their molecule. The cancer DNA helix contains extensive permanently relaxed areas which are not found in normal DNA (22) (31). In these regions H-bond breakage causes strand separation, while on either side the helix turns are tighter. Cancer DNA is a destabilized molecule.

Unprogrammed strand separation, exposure of new initiation sites for replication and for gene expression account for the characteristic properties of the cancer cell, namely enhanced multiplication and spurious protein synthesis. We were able to demonstrate that a clear correlation exists between cancer DNA persistent strand separation, accelerated DNA synthesis in vitro and accelerated cancer cell multiplication in vivo (22).

We then thought that restabilization of DNA secondary structure would lead to inhibition of uncontrolled DNA replication and cell multiplication. This led us to search for specific compounds which would selectively bind to the relaxed strands of cancer DNA and restore its normal secondary structure, while not binding to normal DNA.

In view of their interesting properties, we turned to plant-derived substances such as beta-carboline alkaloids, flavanones and flavonoids, and, using our in vitro Oncotest, we selected compounds possessing the following characteristics:

a) they "recognised" and selectively bound to the destabilized cancer DNAs;
b) this specificity ensured that they would confine their activity to malignant cells;
c) they induced contraction of the relaxed cancer DNA double chain, leading to restabilization of the molecule;
d) they were in no way toxic to normal cells, largely due to the fact that they did not bind to normal DNA.

A long series of in vivo experiments fully demonstrated that the compounds we had selected for our anticancer drugs fully met their requirements. The availability of such substances, which selectively inhibit multiplication of cancer cells and also can revert to normal the cells just starting to undergo malignant transformation, without affecting normal cells, represent a truly positive breakthrough in the fight against cancer. We were also able to apply this selective action principle to viral diseases and other pathologies (due for publication).

Whereas the search for new anticancer agents has long focused on highly toxic drugs or rapidly destructive radiation, it has been our opinion for over twenty years, and it has now dawned on the scientific community, that the future of cancer therapy lies in the possibility of using compounds devoid of general toxicity and highly selective for malignant cells.

Paradoxically, toxic present-day antimitotics prove beneficial when used in the low doses at which they acquire carcinogenic properties: this is because by further destabilizing cancer DNA they

facilitate binding of our anticancer drugs. Conventional therapies, in doses too small to induce toxic side effects, have thus been used in synergy with our compounds, leading to excellent results in animals and in man.

Derived from other sources, BLRs, which are small RNA molecules specifically prime blood stem cell DNA replication; they induce genesis of leukocytes and platelets in such a way that cell counts are kept within physiological limits and the ratios of lymphocyte subsets are preserved. BLRs solely and selectively prime replication of normal DNA, never that of cancer DNA. They can be used during conventional cancer therapy because they do not interfere with it and protect blood cells from its harmful side effects (49) so that there is no need to suspend the treatment, which can thus reach its maximum efficiency. Patients who start taking BLRs (by the oral route) early enough during conventional cancer therapy do not experience its usual side effects and are routinely able to pursue their normal activities.

Finally, we were able to propose biological regulators which, while also stemming from the principle of DNA restabilization, are not anticancer drugs, but contribute to cancer eradication by normalizing enzyme formation at gene level. These molecules, derived from different plants, have no toxic side effects. They prove useful in other pathologies such as viral infections and autoimmune diseases, where many enzymes are dysregulated and exhibit abnormally increased or decreased activity.

Our original approach to cancer therapy aims to restore homeostasis, first, at the very root of malignancy, the destabilized cancer DNA molecule, then at the level of essential enzymes such as nucleases and finally in the immune system. The validity of this strategy was demonstrated many years ago and it has proved operational and efficient as its use started to spread at the hands of physicians who understood its interest and were willing to take the risk of novelty; to this day, many patient shave benefited from it and survive in good condition. Our wish is to see our line of work develop and hopefully become adopted by others, as should also develop the search for new selective and non toxic natural substances derived from the flora of different countries. In this way a new generation of anticancer drugs could be at mankind's disposal in the coming years.

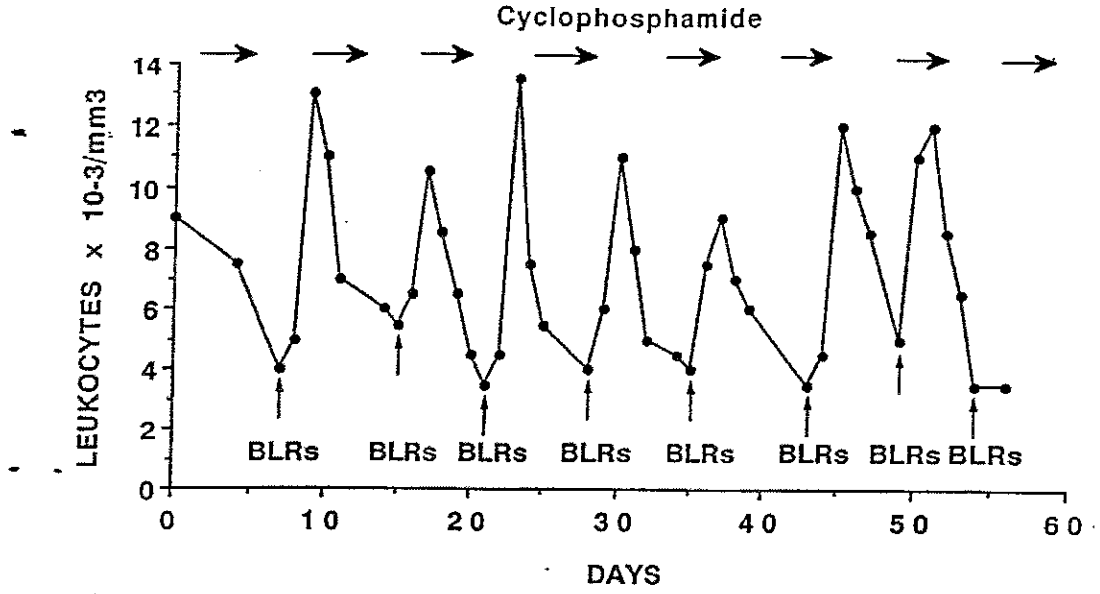

Fig.1 : Effect of BLRs on leukocyte formation in cyclophosphamide-treated rabbits. After leukocyte count has been strongly decreased by cyclophosphamide (100 mg/day) in a 3.5 kg rabbit, doses of RLBs ranging from 1 to 6 mg are given by the I.V. route every second day as shown by the arrows. Circulating leukocytes are counted daily with a Coulter counter. The results given in this figure represent an average obtained with 10 rabbits. The mean increase in leukocyte count was 172 % ± 17 % (standard error). Confidence interval calculated using paired sample Student's test : $p < 0.001$.

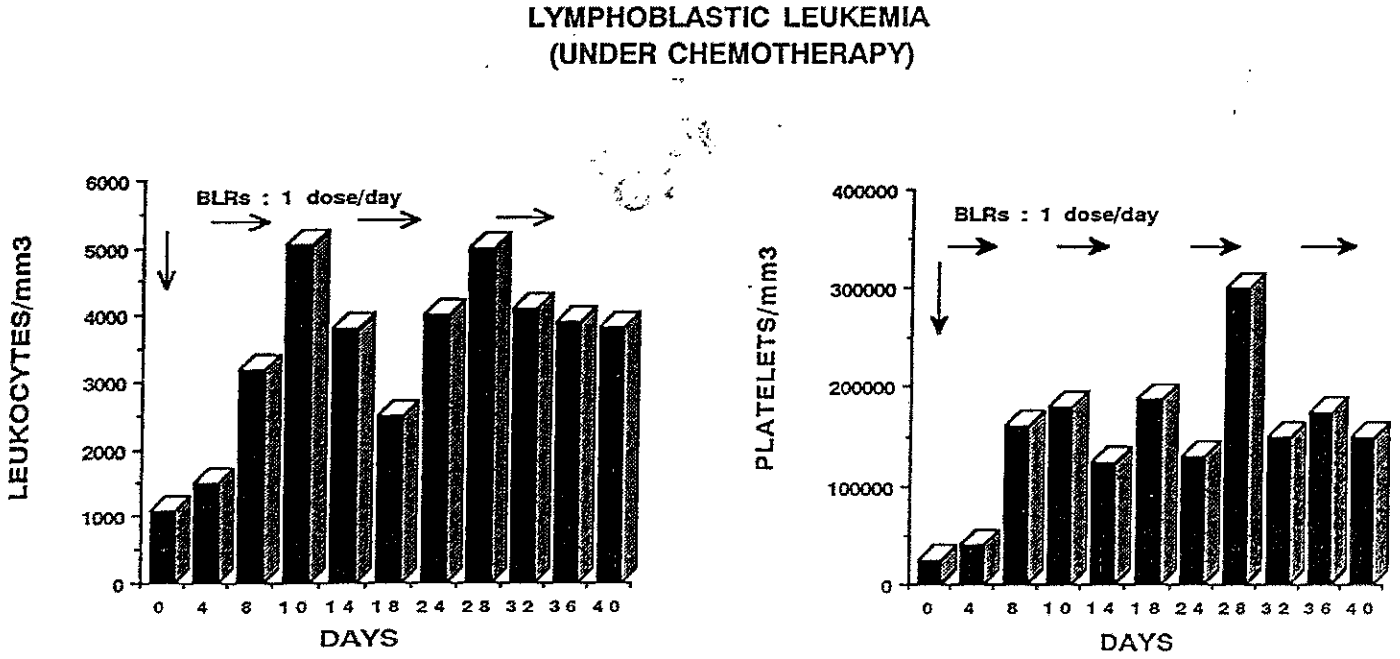

Fig. 2A : Effect of BLRs in human lymphoblastic leukaemia treated by chemotherapy. This twenty year old female patient was treated by conventional chemotherapy. After long term aplasia had occurred, BLRs were administered by the per lingual route as indicated in the figure (1 dose = 15 mg of BLRs).

Fig. 2B : Effect of BLRs on platelet count.

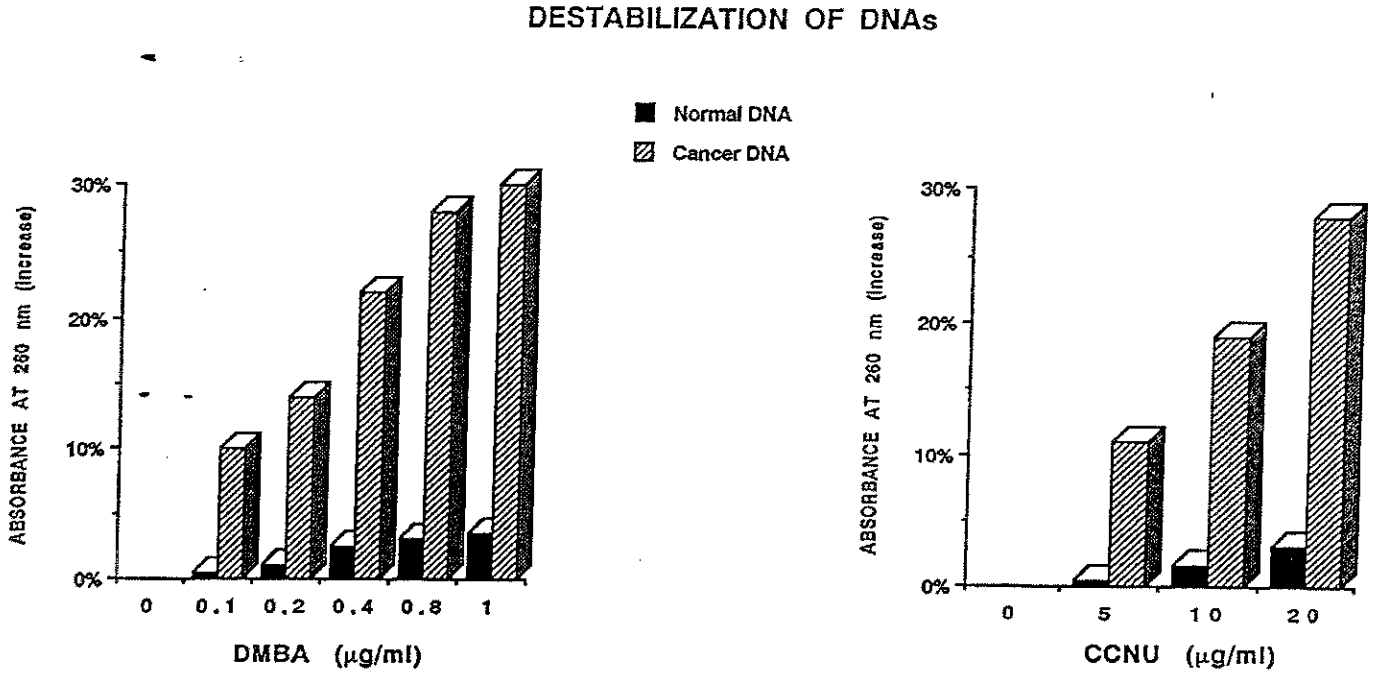

Fig. 3 : Effects of DMBA and CCNU on normal and cancer cell DNA chain opening. Purified DNA was dissolved in 0.01 M Tris-HCL buffer solution (pH 7.5). The substances to be tested were dissolved in 10 μl of the same buffer and were then added to the blank and the DNA solution. The mixture was gently agitated at 20°C and its UV spectrum was determined. Concentrations inducing maximal increase of UV absorbance at 260 nm were determined (%).

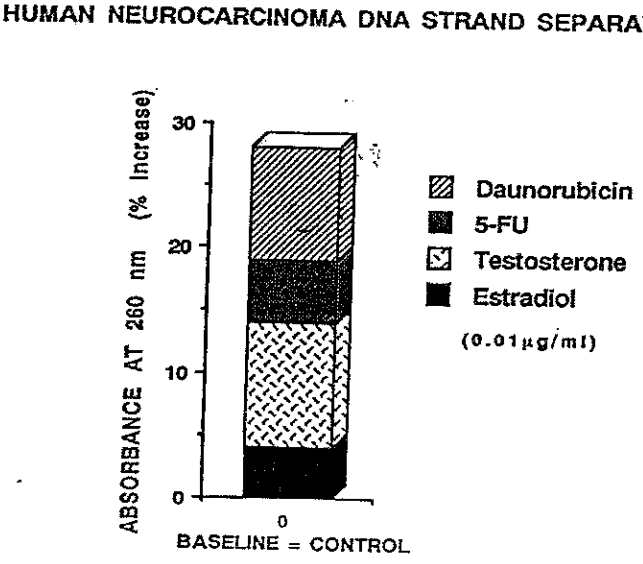

Fig. 4 : Additive effect of 4 different substances on cancer DNA strand separation (here, human neurocarcinoma DNA) (see also legend to Fig. 3).

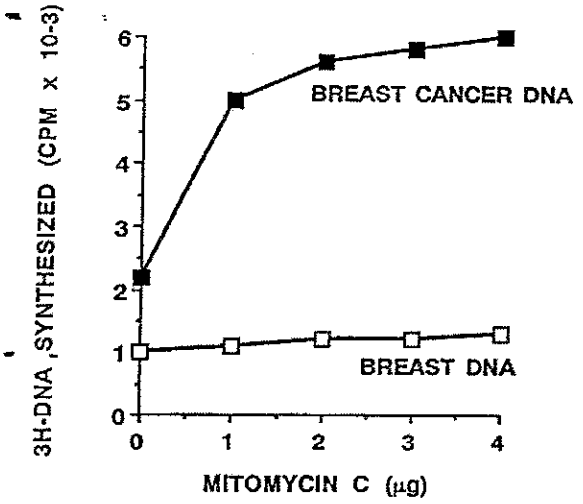

Fig. 5 : Effect of mitomycin on in vitro synthesis of human breast normal and cancer DNA. It can be seen that in low amounts this antimitotic behaves like a carcinogen. Incubation mixture contained per 0.15 ml : Tris-HCl buffer pH 7.65, 25 μM ; MgCl2 : 2 μM ; four d-XTP : 5 nM each (+ 3H-TTP, 50,000 CPM) ; DNA : 0,2 μg. ; DNA dependant DNA polymerase 1,80 μg. Incubation 10 min. at 36°C. TCA (trichloroacetic acid)-precipitable material was filtered on GF/C glass filter, washed, dried and radioactivity measured with a Packard liquid spectrometer (Prias). Analyses were carried out in triplicate for each incubation time.

SYNTHESIS OF NORMAL AND CANCER CELL DNA +ANTICANCER DRUG (ALSTONINE)

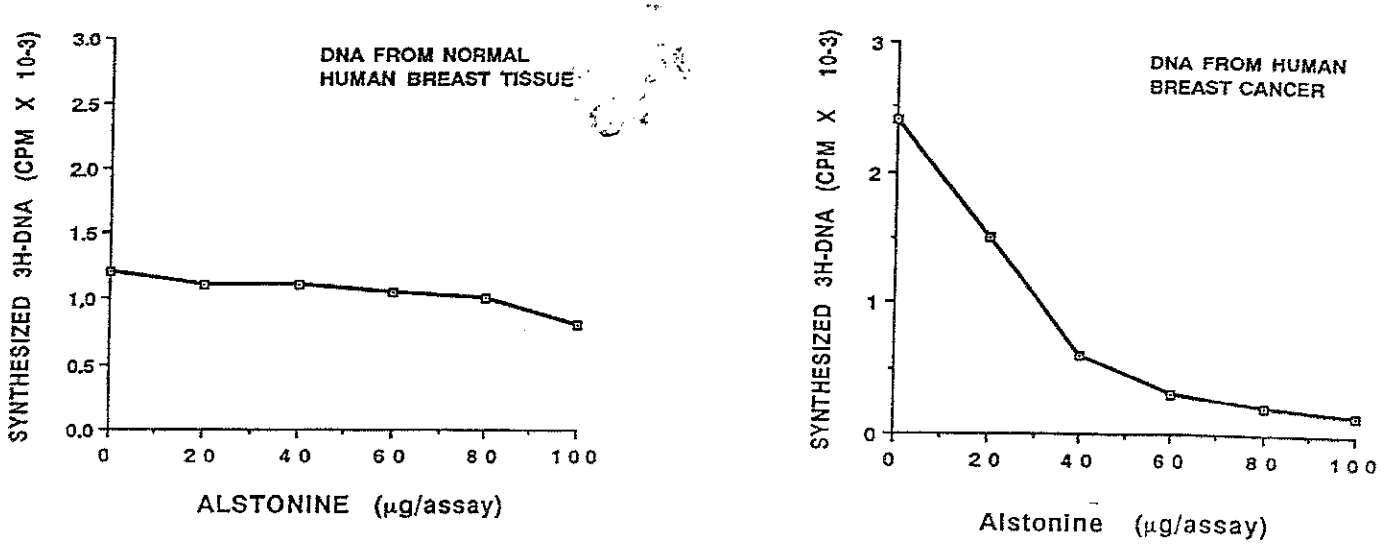

Fig. 6 : Effect of Alstonine on in vitro synthesis of human breast normal and cancer DNA (see also legend to Fig.5).

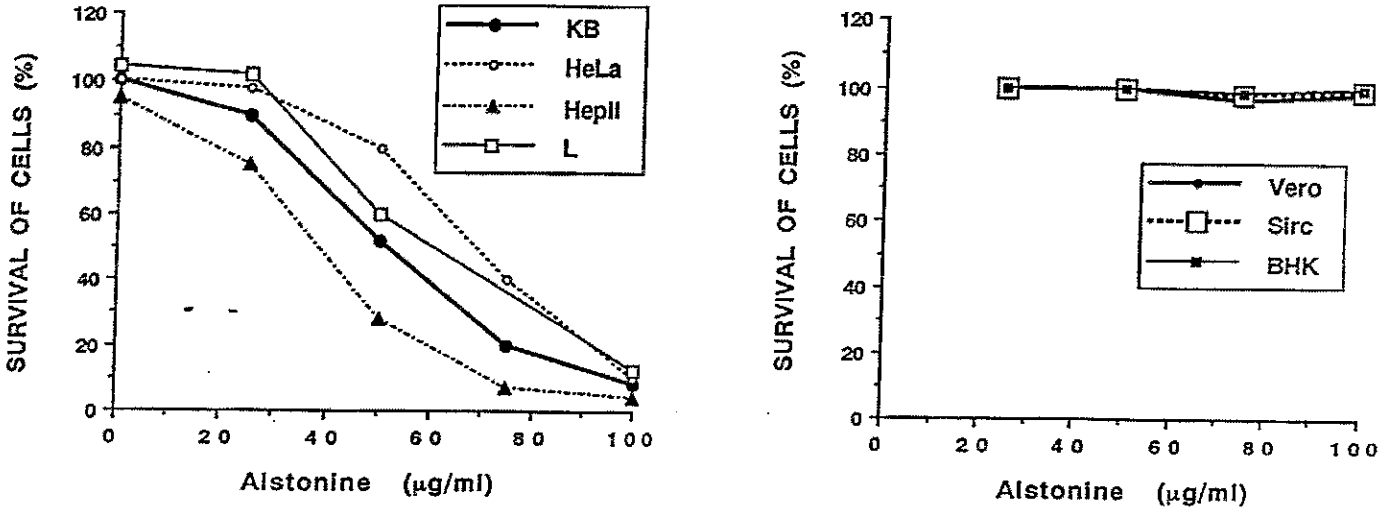

Fig. 7 : Percent survival of different normal and cancer cell lines in the presence of alstonine (for details, see ref. 44).

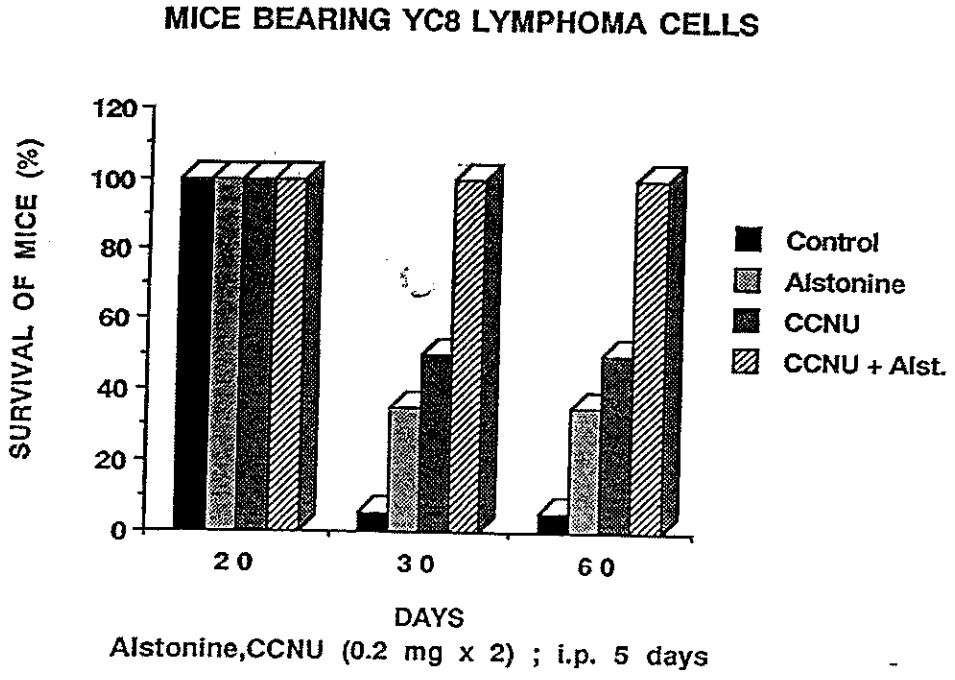

Fig. 8 : Synergistic effect of alstonine with classic anticancer drug (CCNU) in Balb C mice infected with YC8 lymphoma cells (5 x10^3 cells/mouse) ; both alstonine and CCNU were used in amounts well below therapeutic doses.

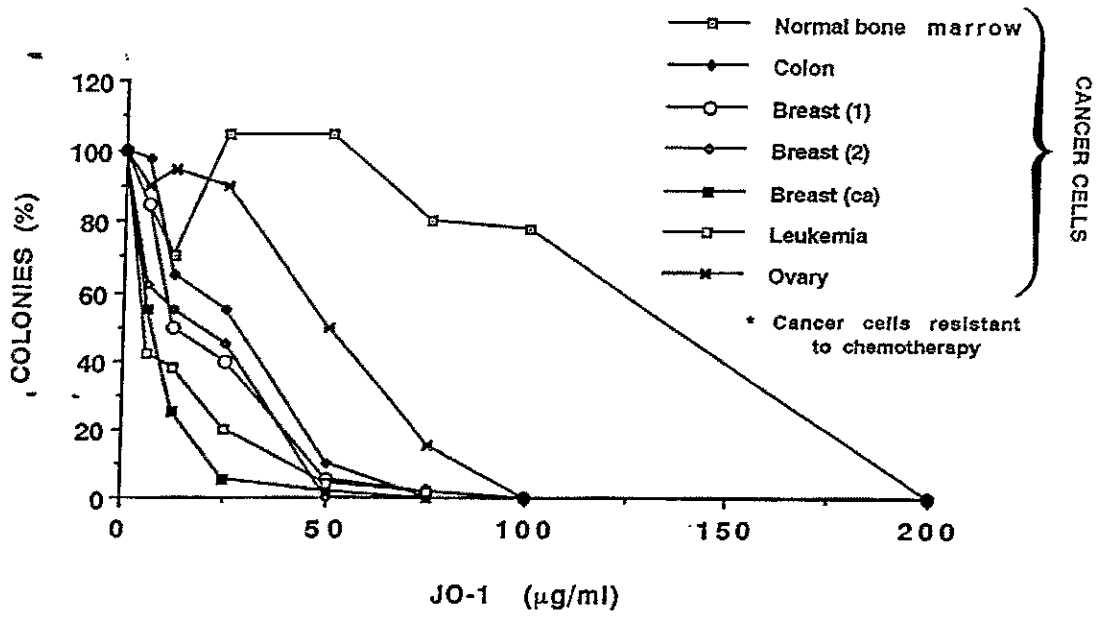

Fig. 9 : The presence of JO-1. Doses which destroy cancer cells are well below those which affect normal cells.

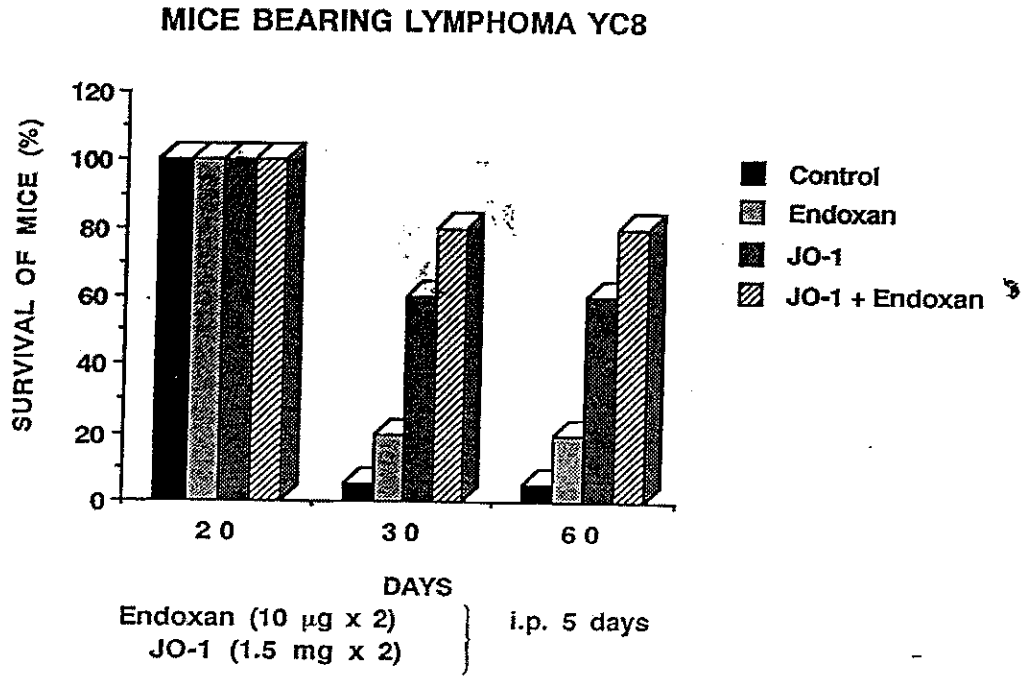

Fig. 10 : Synergistic effect of cyclophosphamide (Endoxan) with Naringin (both given in low doses) in Balb C mice infected with YC8 lymphoma cells (5×10^3 cells/mouse).

REFERENCES

1. Fox, R.M., Mendelsohn, J., Barbosa, E. et al., RNA in Nascent DNA from Cultured Human Lymphocytes. Nature New Biol., 245,234-236 (1973).

2. Beljanski, M., Beljanski, M.S., Plawecki, M. et al., ARN-fragments amorceurs nécessaires à la réplication in vitro des ADN. C.R. Acad. Sci. (Paris) (D) 280, 363-366 (1975).

3. Stockdale, F.E., Topper, Y.J. The Role of DNA Synthesis and Mitosis in Hormone-Dependent Differentiation. Proc. Natl. Acad.Sci. (USA), 56, 1283-1289 (1966).

4. Bischoff, R., Holtzer, H.J., The Effect of Mitotic Inhibitors on Myogenesis in vitro. J. Cell Biol., 36, 111-127 (1968).

5. Lotem, J., Sachs, L., Regulation of Normal Differentiation in Mouse and Human Myeloid Leukemia Cells by Phorbol Ester and the Mechanism of Tumor Promotion. Proc. Natl. Acad. Sci. (USA) 76,5158-5162 (1979).

6. Farber, E., Biochemistry of Carcinogenesis. Cancer Res., 28,2338-2349 (1968).

7. Beljanski, M., The Regulation of DNA Replication and Transcription. The role of Trigger Molecules in Normal and Malignant Gene Expression. Ed. A. Wolsky. Exper. Biol. and Medicine. Karger, Basel. Vol. 8, 1-189 (1983).

8. Darnell, J.E., Implications of RNA, RNA Splicing in Evolution of Eukaryotic Cells Science, 202, 1257-1261 (1978).

9. Borek, C., Cellular transformation by Radiation : Induction, Promotion and Inhibition. J. Supramol. Struct. Cell Biochem., 16,311-336 (1981).

10. Urano, M., Koike, S., Ohara, K., Secondary Malignant Neoplasms Following Radiotherapy of a Mouse Mammary Carcinoma. Cancer (Phila.) 43, 151-156 (1979).

11. Braun, D., Harris, J.E., Modulation of the Immune Response by Chemotherapy .Pharmac. Ther, 14, 89-122 (1981).

12. Filder, I.J., Gernstern, D.M., Hart, I.R., The Biology of Cancer Invasion and Metastasis. Ed. Klein, Weinhouse: Advances on cancer research. Academic Press, New York, 149-250 (1978).

13. Sugino, A., Hirose, S.,Okazani, R., RNA-linked Nascent DNA Fragments in Escherichia Coli. Proc. Nat. Acad. Sci. (USA), 69, 1863-1867 (1972).

14. Beljanski, M., Plawecki, M., Particular RNA Fragments as Promoters of Leukocyte and Platelet Formation in Rabbits. Expl. Cell Biol., 47, 218-225 (1979).

15. Donadio, D., Lorho, R., Causse, J.E., Nawrocki, T.,Beljanski, M., RNA fragments (RLB) and Tolerance of CytostaticTreatments in Hematology : a Preliminary Study about two non-Hodgkin Malignant Lymphoma Cases. Dtsch. Zschr. Onkol., 22,33-35 (1991).

16. Beljanski, M., Radioprotection of Irradiated Mice. Mechanisms and synergistic Action of WR-2721 and BLR. Dtsch. Zschr. Onkol., 23, 155-159 (1991).

17. Plawecki, M., Beljanski, M., Comparative Study of Escherichia Coli Endotoxin, Hydrocortisone and Beljanski Leukocyte Restorers Activity in Cyclophosphamide-treated Rabbits. Proc. Soc. Expl.Biol. Med., 168, 408-413 (1981).

18. Beljanski, M., Plawecki, M., Bourgarel, P., Beljanski, M.S., Leukocyte Recovery with Short Chain RNA-fragments in Cyclophosphamide-treated Rabbits. Cancer Treatment Reports, 67,611-619 (1983).

19. Beljanski, M., Overview : BLRs as Inducers of in vivo Leukocyte and Platelet Genesis. Dtsch. Zschr. Onkol., 24, 41-45 (1992).

20. Vasilescu, D., Rix-Monteil, M.A., Interaction of Sulfur-containing Radioprotectors with DNA; a Spectrophotometric Study. Physiol. Chem-phys., 12, 51-55 (1980).

21. Hotchkiss, R.D., Methods for Characterization of Nucleic Acid (I. Characterization of Nucleic Acids by Spectrophotometry. Methods in Enzymology. Ed. Colowick S.P. and Kaplan N.O., Academic Press, New York, 708-715 (1957).

22. Beljanski, M., Bourgarel, P., Beljanski, M.S., Correlation between in vitro DNA Synthesis, DNA Strand Separation and in vivo Multiplication of Cancer Cells. Expl. Cell Biol., 49, 220-231(1981).

23. Mong, S., Daskal, Y., Prestayko, A.W., Crooke, S.T., DNA Supercoiling Shortening and Induction of Single-Strand Regions by Cis-Diaminedichloroplatinium (II). Cancer Res., 41, 4020-4026 (1981).

24. Neubort, S., Liegeskind, D., Mendez, F., Hsu, K.C., Bases, R., Morphological Transformation, DNA Strand Separation and Antinucleoside Immunoreactivity Following Exposure of Cells to Intercalating Drugs. Molec. Pharmacol., 31, 739-743 (1982).

25. Center, M.S., Induction of Single-strand Regions in Nuclear DNA by Adriamycin. Biochem. Biophys. Res. Commun., 89, 1231-1238 (1979).

26. Pillans, P.I., Ponzi, S.F., Parker, M.I., Cyclophosphamide-induced DNA Strand Breaks in Mouse Embryocephalic Tissue in vivo. Carcinogenesis 10, 83-85 (1989).

27. Rebouleau, C.P., Shapiro, H.S., Chemical Inducers of Differentiation Cause Conformational Changes in the Chromatin and Deoxyribonucleic Acid Murine Erythroleukemia Cells . Biochemistry ,22, 4512-4517 (1983).

28. Le Goff, L., Roussaux, J., Aaron Da Cunha, M.Y., Beljanski,M., Growth Inhibition of Crown gall Tissues in Relation to the Structure and Activity of DNA. Phys. Plant, 64, 177-184 (1985).

29. Le Goff, L., Beljanski, M., The in vitro Effects of Opines and other Compounds on DNAs originating from Bacteria and from Healthy and Tumorous Plant Tissues . Expl. Cell Biol., 53, 335-350 (1985).

30. Beljanski, M. Oncotest, a DNA Assay System for the Screening of Carcinogenic Substances . IRCS Medical Science , 7, 476 (1979).

31. Beljanski, M., Le Goff, L., Beljanski, M.S., In vitro Screening of Carcinogens using DNA of the His- Mutant of Salmonella typhimurium. Expl. Cell Biol., 50, 271-280 (1982).

32. Beljanski, M., Oncotest : Dépistage des potentiels cancérogènes et spécifiquement anticancéreux. Conceptions et perspectives nouvelles en cancérologie. Environnement et médecine nouvelle, N°2, 18-23 (1982).

33. Hackenberg, R., Hofmann, J., Hölzel, F., and Schulz, K.D., Stimulatory Effects of Androgen and Antiandrogen on the in vitroProliferation of Human Mammary Carcinoma Cells. J. Cancer. Res. Clin. Onkol., 114, 593-601 (1988).

34. Gottardis, M.M., Wagner, R.J., Borden, E.C., Jordan, V.C.,Differential Ability of Antiestrogens to Stimulate Breast Cancer Cell (MCF-7) Growth in vivo and in vitro. Cancer Res., 49,4765-4769 (1989).

35. Ts'o, P.O.P., Helmkamp, G.K., Sander, C., Interaction of Nucleosides and Related Compounds with Nucleic Acids as Indicated by the Change of Helix-coil Transition Temperature. Proc. Natl. Acad. Sci. (USA), 48, 686-698 (1962).

36. Ts'o, P.O.P., Helmkamp, G.K., Sander, C., Secondary Structure of Nucleic Acids in Organic Solvents. II. Optical Properties of Nucleotides and Nucleic Acids. Biophys. Biochim. Acta, 55, 584-600(1962).

37. Lee, C.H., Mizusawa, H., Kakefuda, T., Unwinding of double-stranded DNA helix by Dehydration. Proc. Natl. Acad. Sci. (USA), 78, 2838-2842 (1981).

38. Neville, D.M., Jr Davies, D.R.. The Interaction of Acridinedyes with DNA: an X-ray Diffraction and Optical Investigation. J. Mol. Biol., 17, 57-74 (1966).

39. Beljanski, M., Beljanski, M.S., Selective Inhibition of in vitro Synthesis of Cancer DNA by Alkaloids of beta-carboline Class. Expl. Cell Biol., 50, 79-87 (1982).

40. Le Goff, L., Beljanski, M., Agonist and/or antagonist Effects of Plant Hormones and an Anticancer Alkaloid on Plant Structure and Activity. IRCS Med. Sci., 10, 689-690 (1982).

41. Beljanski, M., Le Goff, L., Faivre-Amiot, A., Preventive and Curative Anticancer Drug. Application to Crown-gall Tumors. Acta Horticulturae, 125, 239-248 (1982).

42. Vervoitte, V., Intérêt des cultures in vitro de tissus végétaux pour dépister de nouveaux composés anticancéreux. Application à l'alstonine. Bio-Sciences, III, N°14, 14-16,(1984).

43. Beljanski, M., Beljanski, M.S., Three Alkaloids as Selective Destroyers of the Proliferative Capacity of Cancer Cells. IRCS Med. Sci., 12, 587-588 (1984).

44. Beljanski, M., Beljanski, M.S., Three Alkaloids as Selective Destroyers of Cancer Cells in Mice - Synergy with Classic Anticancer Drugs. Onkology, 43, 198-203 (1986).

45. Beljanski, M., Nawrocki, T., Le Goff, L., Possible Role of Markers synthesized during Cancer Evolution: I. Markers in Mammalian Tissues. IRCS Med. Sci., 14, 809-810 (1986).

46. Le Goff, L., Beljanski, M., Possible Role of Markers synthesized during Cancer Evolution: II. Markers in Crown gall Tissues. IRCS Med. Sci., 14, 811-812 (1986).

47. Grisworld D.P., Schabel, F.M., Wilcox, W.S., et al., Successand Failure in the Treatment of solid Tumors: I. Effects of Cyclophosphamide (NSC-26271) on Primary and Metastatic Plasma cytoma in the Hamster. Canc. Chemoth., 52, 345-387 (1968).

48. Israël, L., Les Résistances, causes d'échec des chimiothérapies anticancéreuses. Path. Biol., 37, 125-127 (1989).

49. Beljanski, M., Cancer Therapy: a New Approach. Deutsche Zeitschrift für Onkologie, (5) 22, 145-152 (1990).