

## THE NCT TURIN PROJECT FOR PRIMARY AND SECONDARY HEPATIC CANCER

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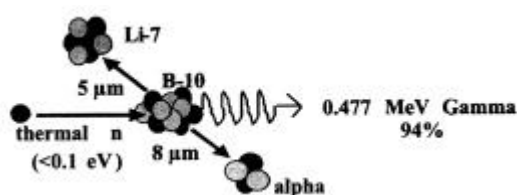
The development of new kind of therapy in the treatment of cancer in which a surgical approach is not possible, or which does not respond to chemio or radio traditionally therapy, actually is object of international interest; in particular international studies are applied to the neutron capture therapy, known as NCT.

The neutron capture therapy is based on the possibility to give, using an adequate source of termic neutronic beams of sufficient intensity able to produce by opportune nuclear reactions characterised by high energetic release, the selective cellular death.

Historically, NCT had been initially applied to the oncological pathologies since 1930, and immediately pointed out the two mayor difficulties 1) find an adequate source of neutrons, with no pollution of other type of radiation 2) introduce an elevate concentrations of neutron's absorbent substances, like Boron, Gadolinium, Gold, or other substances which can release, in consequence of a nuclear reaction induced by neutrons, particles with high linear energy transfer (LET).

At present the  $^{10}\text{B}$  Boron atoms is the more studied in the different international studies (BNCT) in different compounds as the 4-dihydroxiboryl-phenylalanina (BPA) and the polyhedral mercaptoboron compounds such as sodium mercaptoundecahydrododecarbate(BSH).

In fact  $^{10}\text{B}$  boron nuclei have a large cross- section capture for thermal neutrons and , when irradiated with a flux of neutrons, the formed boron particles give al alpha particle and a  $^{7}\text{Li}$  ion bearing 2,4 MeV.



If the boron atoms are within a cell, the fission products dissipate their kinetic energy before travelling one cell diameter so that the destructive effect is highly localised to boron-loaded tissue.

The fundamental requirements for an efficient therapy are 1) an elevated boron concentrations within the cancer cells (20-35 mg of  $^{10}\text{B}$  for g of tissue) 2) that the ratio between the boron concentration into the tumor cells with respect to that in surrounding tissues and in the blood is high enough.3) that the maximum of the neutronic beams arrives in the tumbrel zone.

In order to reach the maximum concentrations of neutron's absorbent substances within the cancer cells, now the research is about compounds that should be able selectively accumulate the boron near the genetic material, then around or within the nucleus.

The loco-regional therapy, with a intro arterial introduction of a catheter, seems to be a good way to obtain an elevated boron concentrations within the cancer cells, respect to the intravenous systemic way: different studies have demonstrated that with the intra carotic infusion of boron compounds (loco- regional therapy) in the treatment of brain cancer, is possible to obtained elevated cellular concentrations within the cancer cells of BSH or PBH, higher respect to the intravenous systemic infusion.

First significant experiences have brought forward in Japan (Nagakawa, Hatanaka) as from the end of the seventy as possible therapy of brain cancer as astrocitoma, glioblastoma.

Now the NCT is getting up of a great interest in the treatment of different cancer as melanomi, linfomi, hepatic cancer (hepatocarcinoma or hepatic metastasis).; Japanese studies have recently demonstrated the efficacy in the treatment of hepatic metastasis from colon-rectal cancer, and the efficacy in the treatment in the primary hepatocarcinoma (Suzuki M et Al., Jpn J Cancer Res 2000 Oct;91(10):1058-64; Takahashi T et Al, Jpn J Exp Med 1987 Apr;57(2):83-91).

Although currently the research about the NCT is focused on the Boron-10 compounds, also the isotope Gd-157 can be used for this purpose. The natural abundance of isotope Gd-157 is about 15.7%, similar to that one of Boron-10 (19.9%). The capture of a neutron from Gd-157 gives the formation of Gd-158 and the emission of radiation of a Auger electron. As demonstrated in vitro, better radio-chemical damage is found when the Gd-157 is bound to DNA. The Nuclide Gd-157 has two advantages respect to the B-10: i) Gd-157 has a section of capture for neutrons greater (255,000 barn) than B-10 (3,840 barn) and ii) the Gd(III) compounds are normally employed as contrast agents in MRI. This property is extremely important because the high resolution of MR images allows the visualization of the region to subject to neutronic irradiation.

The concentration of the absorbent neutron substance inside the neoplasm so that only tumoral cells were destroyed by the neutron beams, can follow two different ways, taking advantage from the innovations of cellular and molecular biology and form the clinical experience of the loco-regional treatment

**Molecular approach.** the most interesting route is based on the exploitment of receptors' recognition capabilities followed by endocytosis process. It is well established that a number of receptors are overexpressed in tumour cells in respect to the normal tissue (for instance receptors for growth factors, like somatostatin, cholecystokinins, folate receptor=). Alternatively one may consider the exploitment of transport systems for cellular nutrients, analogously to what used for Borophenylalanin which enters the cells through LNAATS (Large Neutral AminoAcid Transport System). Such a system is 3-4 times more active in neoplastic cells that normal. More in general, in this process several transport systems will be considered to assess whether their change in activity may be exploited for a differential accumulation of the neutron absorbing substances inside tumoural cells.

The neutron absorbing substances synthetized in this project will consist of two parts, according to the following scheme:

X----Y

Where X is the moiety responsible for the targeting to the molecules of interest, i.e. a receptor/ a transporting system which carries out the internalization of the substance. We'll select receptors endowed with a high recycling rate in order to deliver a large amount of neutron absorbing substance (either B-10 or Gd-157 containing one) into the cell in relative short times.

Y is the moiety containing the atoms responsible for the absorption of neutrons. In the case it contains Gd-157, this moiety has to be designed in a way to provide proper characteristics for MRI visualisation. Y may contain a high number of Boron atoms and/or a high number of Gd(III) complexes (ranging from systems containing 3-10 units up to dendrimeric structures containing more than 100 atoms of interest).

X and Y are bound through a spacer whose role is important in limiting the effect of Y and the capabilities of X to bind the receptor /transporter of interest and the successive internalization process.

Internalization procedures based on the use of micelles or liposomes (suitably functionalized for the cellular targeting) will also be considered in order to pursue the goal of the entrapment of a high number of neutron absorbing molecules. In the case of cells endowed with macrophagic activity, the internalization will be pursued through the use of solid particles (nanometer size) which eventually are degraded by the intracellular enzymes with consequent release of single units. Particularly important for the attainment of high efficient therapies is the design of systems able to address the nuclear region (to bind DNA), when internalized in the cells. In this regard, we will exploit the wide knowledge obtained in the field of investigations dealing with the binding of several chemotherapeutics to DNA as well as the studies on the adducts formed between cationic polymers and the negatively charged nuclear acid macromolecule. The DNA recognition ability will be provided through the introduction of suitable chemical substituent at the Y moiety or on the spacer itself. The extent of internalization of each X---Y system (in given cell typology) will be investigated "in vitro" on cellular cultures. These cell lines will be provided by the cellular biology teams of Prof. Silengo and Palestro and will be selected at the light of the therapeutic perspectives indicated by dr. Zanon. For Gd-containing systems, the determination of the amount of internalized substance will be assessed by means of relaxometric techniques. This methodology is based on the measure of water proton relaxation times ( $T_1$  and  $T_2$ ) which suffer a dramatic decrease in the presence of Gd(III) ions (as a consequence of its high paramagnetism due to 7 unpaired electrons and its long electronic relaxation time). This property is the basis of the contrast enhancement observed in MR images in the presence of Gd(III) complexes. The MR images will be acquired either on cell pellets (few millions of cells are sufficient) or on animal models bearing the neoplasm of interest. At the Bioindustry Park at Colleretto Giacosa (TO) is operating an Imaging Centre equipped with two tomographs operating at 7.0 and 0.2 Tesla respectively. The 7.0 Imager is specifically dedicated to investigations, under high resolution conditions, of mice and small-size rats.

Loco-regional approach: Acquired experience at S. Giovanni Battista Hospital in Turin (Servizio di Patologie Neoplastiche Complesse in Chirurgia Oncologica) about loco-regional treatment in Port-A-Cath implantation (it is possible put a catheter in every artery of whole body) let us to test neutron absorbent substances administered per arterial infusion to improve their concentration in neoplastic cells and to reach a better concentration than with systemic infusion.

This technique gives us better outcomes on hepatic target, because primitive and metastatic liver neoplasms have a predominantly arterial vascularization.

We can administer high concentration of neutron absorbent substances directly on neoplastic tissue implanting a permanent catheter into hepatic artery or in one of its branches.

The cooperation between molecular biology and loco-regional treatment clinic experience is needed to guarantee high specificity of the NCT technique to cause only tumoral cells death.

NCT represent a technological innovation in tumour treatment. Its rationale is to discriminate tumoral cells from healthy ones. Selective treatment has been always wanted because standard protocol (surgery, chemotherapy, radiotherapy) has consistent side effects that do not allow therapy in every patients.

Moreover, to perform a highly selective NCT, we can image the NCT pharmaceuticals uptake in neoplastic lesions during their administration. We will use gadolinium-containing molecules as MRI probes, as well as radiopharmaceuticals. In the last few years radioligands (receptor-seeking radiopharmaceuticals) were developed for imaging and treatment. The specificity of the binding of these radiopharmaceuticals to target cells is relevant both in biological research and in clinical applications (imaging and treatment) and explain their wide use in diagnosis and treatment.

The receptor expression by a single cell type (sometimes only in some phases of its development) allows a specific identification of the target cells: normal tissues (as in central nervous system during receptor distribution analysis with radioligands), pathological structures (neuroendocrine tumors) and normal tissues in particular phases of their development (endothelium during angiogenesis) can be identified.

Radioligands show generally a high target-to-background ratio due to the high chemical affinity to receptors with strong uptake and low aspecific distribution. This high target-to-background ratio allows radioligands use also in imaging and therapy of small amounts of pathological tissues, when receptor or antigens are available for binding on the tissue itself.

Octreotide, a somatostatin analogue, was the first radioligand used in clinical identification of pathological tissue. It is now used to detect many benign or malignant, active or inactive neoplasms, generally belonging to the "neuroendocrine tumors" group.

Octreotide can be labelled with various radionuclide by a chelating group: the widest used are indium-111 and technetium-99m. The choice of the radionuclide is made according to imaging or therapeutic needs: the goal is to "give" the radionuclide a specific tropism to cells exposing somatostatin receptors.

Now, not only many others radioligands are under development, but the researchers actively identify new receptors for cell labeling. On the other side, there are many studies to transfer technologies from radioligands to gadolinium-containing contrast media to be used in MRI. The radionuclide imaging with radioligands, very sensitive, can be considered a step in the development of contrast media for MRI.

The synthesis of a gadolinium-labelled ligand could allow a synergy between the high specificity of the ligand-receptor binding and the high spatial resolution of MRI.

If used in NCT application, a gadolinium-labelled ligand could allow optimal irradiation: high dose to target tissue with negligible irradiation of surrounding areas. Target-to-background dose ratio will be far higher than with radionuclide therapy because neutron irradiation could be delayed until optimal gadolinium uptake, according to standard data or assessed in that patient with MRI. Moreover, with NCT there will be no risk to remote tissues, often target of radiopharmaceuticals or of its metabolites during excretion.

**Neutron Irradiation Facility.** For the experiments and the clinical application of NCT, it is of utmost importance the availability of a suitable neutron source.

On this item it has to be reminded in advance that the "European Interregional Committee for the Development of Innovative Energy-Environment Systems (EUROSEA)", a no profit organization created by some (about 20) minor companies with advanced technological background and by research institutions as Departments of the Polytechnic of Milano, Torino and the Universities of Torino, Pisa and Genova, and by other scientific institutions, started in 1998-1999 the *BoroterapiaPiemonte* project, in order to develop feasibility studies and, later on, the installation of a therapeutic unit for BNCT in Piemonte. This research project is, to some extent, complementary to the one proposed here, It is focused mainly on the development of new types of neutron sources for irradiation for the treatment of endocranial tumors, while most biological, chemical and therapeutic aspects are not deeply investigated.

*BoroterapiaPiemonte* has been planned on two phases: the first, started in the middle 2001, scheduled for two years, is concerned with the feasibility project and on the determination of the scientific, technologic and therapeutic aspects of a BNCT plant, with particular attention to the different neutron sources nowadays available; the second, planned for three years, focuses on the realization of the plant at a qualified regional hospital unit.

In the first phase of BoroterapiaPiemonte, it was proposed to analyze and compare, from the physical and engineering points of view, the different neutron sources acceptable in a hospital environment. that can be bought or developed with the present day technology. In particular, the high current accelerator-based systems, coupled to target-converter suitable to produce a high neutron yield, will be compared with new alternative devices based on low current accelerators, used as primary source generators and coupled with neutron amplifier and a spectrum converter system, actually under development at Energetic Department of the Politecnico in Turin. Neutrons escaping from the amplifier play the role of secondary, amplified source, for therapeutic use.

This EUROSEA idea was appreciated and supported by Compagnia di San Paolo that, since 2001, has financed the first year of research.

During the first stage of the work EUROSEA attention has been attracted by a new generation of neutron primary sources, based on the nuclear fusion reactions (D-D) and (D-T), that are likely to become of great interest for the scientific community. In particular we refer to the new compact generators, now in advanced phase of project at Lawrence Berkeley National Laboratory (LBNL) in California.

EUROSEA has started a scientific and technical relationship with this Laboratory and, in particular with Prof. W. Barletta, Director of "Accelerator Fusion Research Division"- AFRD- (Berkeley) and with Prof. Ka\_Ngo Leung, Responsible of "Plasma and Ion Source Technology Group" (Berkeley), who has projected and realized the prototype of these sources. In several meetings between EUROSEA and AFRD the overall characteristics of a first class of fusion generators suited for BNCT have been started. D-D reaction was chosen instead of D-T one, that could allow, under the same conditions, a larger neutron yield, but it would be hardly acceptable in a hospital, because of Tritium radiation protection problems.

On 6<sup>th</sup> September 2001 EUROSEA and AFRD had signed a Memorandum of Understanding, here attached, for a collaboration between the two partners on the following subjects: firstly on NCT and in general on fusion-based neutron sources and on their possible uses. Then AFRD proposed to EUROSEA to develop a D-D compact generator of  $10^{11}$  n/s neutron and a moderating system suited for BNCT.

Low emission fusion-based neutron sources for industrial applications, research, geophysical investigations and safety are available on the market since many years.

But high emission, neutron generators based on light atoms fusion were developed, according to international opinion, for the first time in the world, at LBNL and they are already suitable for BNCT experiments and some applications.

Consequently EUROSEA considers that these new fusion-based systems developed by LBNL are to be given due consideration. It must be taken into account that some of these devices could also produce time oscillating neutron sources, with different frequencies. EUROSEA considered also this kind of sources among the ones to be considered in the development of BoroterapiaPiemonte project.

As a consequence EUROSEA has modified and amplified the first phase of its project related to the physical and engineering areas as follows:

Cooperation for the development of the system proposed by LBNL, according to Memorandum of Understanding of 6<sup>th</sup> September 2001;

continuation of the project about the development of neutron amplifiers and spectral converters proposed by the Politecnico of Turin, coupling them with both the fusion neutron generators proposed by LBNL, and the other low fluxes primary sources already being considered in the first project, for all of the NCT applications that require higher neutron yields.

Meanwhile the Oncology Department of Azienda San Giovanni Battista of Turin became interested on NCT application in the oncological surgery field, with particular interest on liver tumors and it has constituted an interdisciplinary work group, that collects a large amount of competence, ranging from experimental medicine to the oncology surgery, nuclear medicine, molecular and cellular biology and chemistry.

The project herewith proposed by this interdisciplinary group is to some extent widening and deepening the one planned by BoroterapiaPiemonte. This project considers as neutron capture agents both boron compounds and gadolinium compounds. Liver tumors are considered with priority and the use for BNCT in a short while, and for the first time in Europe, of the fusion-based sources developed at LBNL, both for the experiments and for the clinical preliminary applications in a hospital is considered.

Following the project of the Oncology Department interdisciplinary group, EUROSEA has declared itself available to transfer the LBNL proposal to Oncology Department, under consensus of LBNL and with the approval of Compagnia di San Paolo that promotes the cooperation among the different Institutions operating in the oncological field

After the quality assurance and the commissioning procedures of the generator developed and installed in Turin by LBNL, EUROSEA will provide Oncology Department with the technical-scientific aid requested for radiation protection, for the neutron generator operation, for the optimization of neutron field conformation for BNCT. Furthermore EUROSEA will be in a position to coordinate Experts, Institutions and Companies of its supporting team in the field of nuclear measurements, dosimetry, mathematical modelling of pharmacodynamic problems, robotics and treatment planning, devoted to NCT.

EUROSEA reserves for itself the possibility of using ( for a short time period and without prejudice for the experiments and the clinical applications planned) the LBNL fusion-based neutron generator, bought by the Oncology Department, in order to investigate the coupling of such generators with the neutron amplifiers under development at Energetic Department of Politecnico in Turin, if the sudden need of a higher neutron flux facility should arise, either in Molinette therapeutic unit or elsewhere in Italy.