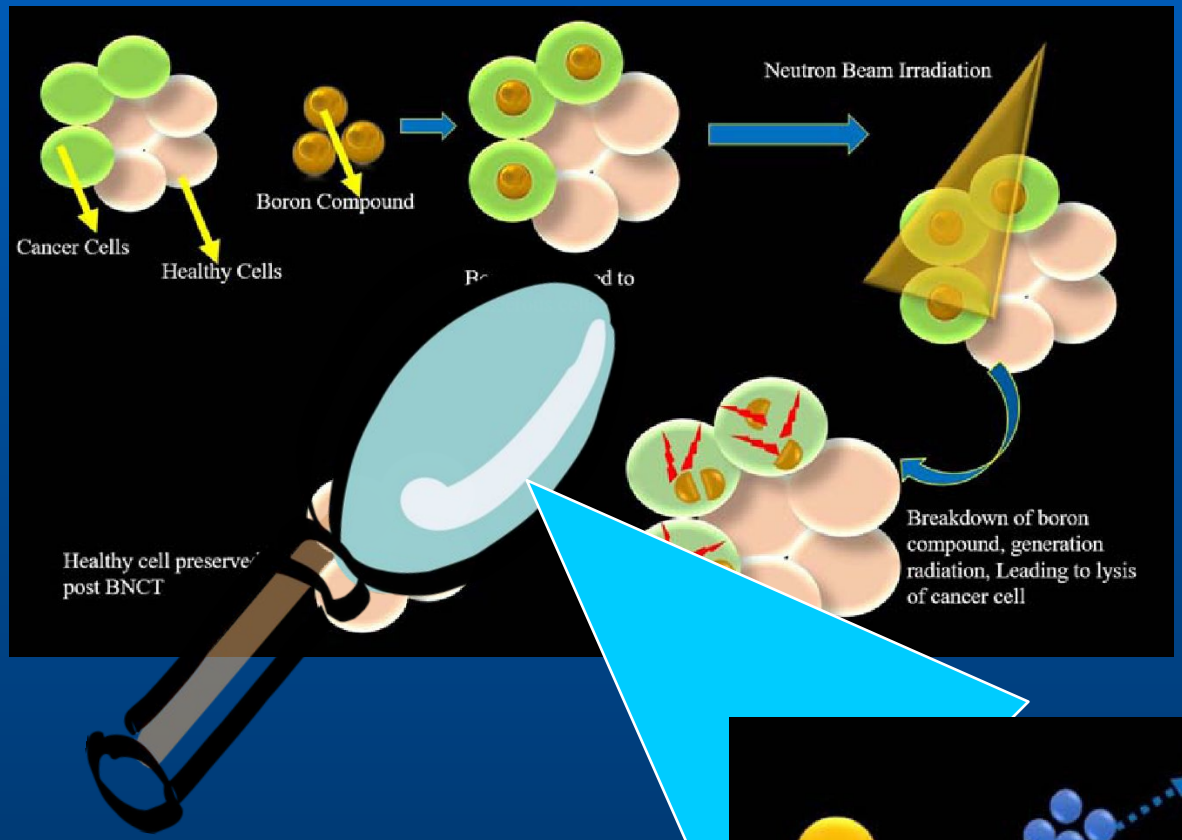
Department of Biological Chemical and Pharmaceutical Sciences and Technologies (STEBICEF, Section of Cellular Biology)
University of Palermo - Italy

Genetics and genomics of BNCT among basics and perspectives

Dr. Fabio Caradonna, PhD

CUIA Days 2017 - Universidad Favaloro
Facultad de Ingeniería y Ciencias Exactas y Naturales - Buenos Aires

The Boron neutron capture therapy (BNCT)



The Boron neutron capture therapy (BNCT) bases its therapeutic action on the selective ability to induce DNA damage of tumor cells with the aim to cause their death by apoptosis or necrosis.

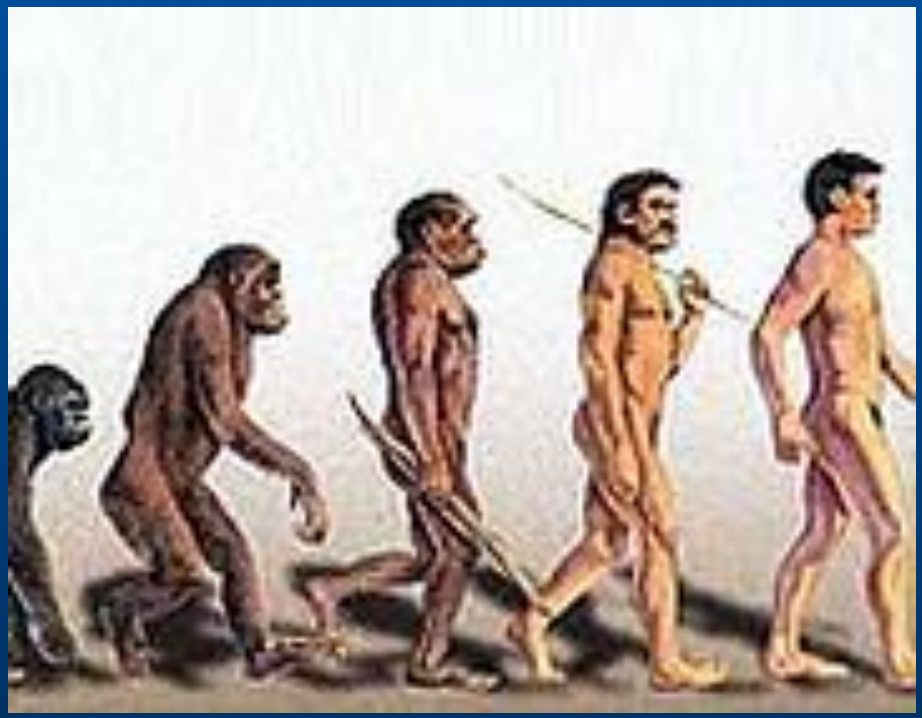
Genetic variability

Originated from spontaneous DNA mutations…..

…from generation to generation distributes phenotipic diversity in a population

Genetic variability

- Is an evolutionary *long-time* mechanism for the species improvement;



Genetic variability

- As *short-time* consequence, it's responsible for individual susceptibility:

- different subjects have different reacting behaviours towards the same environment;
- in the same population is very rare to find two subjects which could react with the same behaviour towards a same environmental insult.

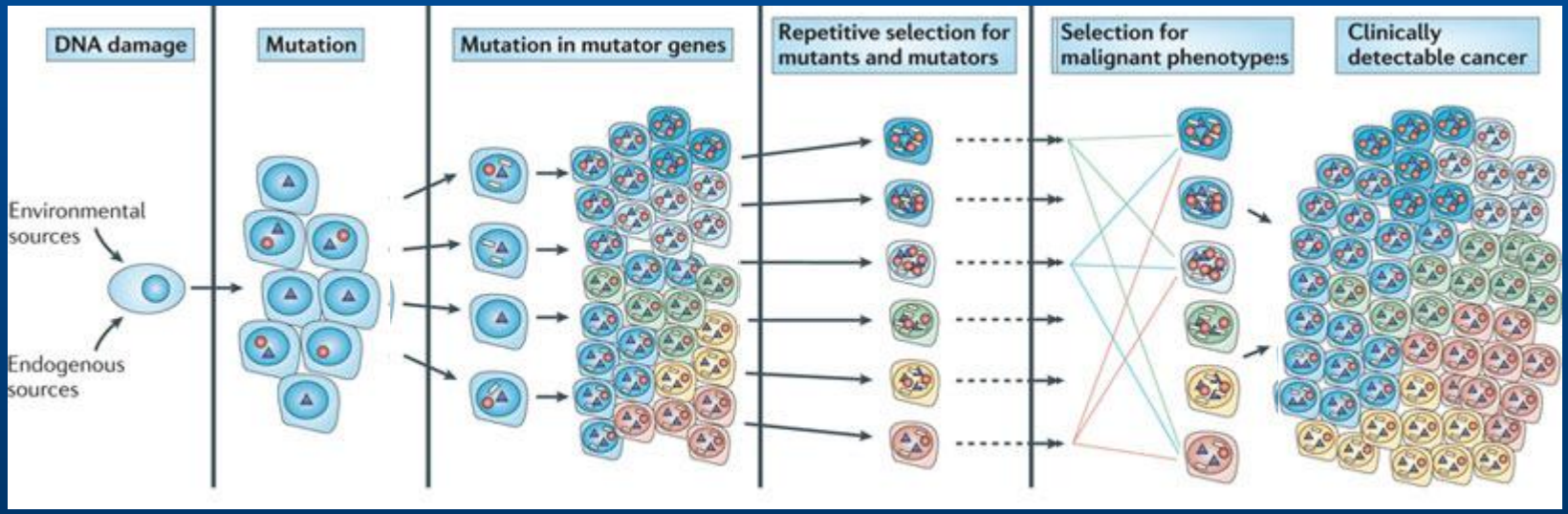
Genetic variability

It's spread in a population by genetic polymorphisms:

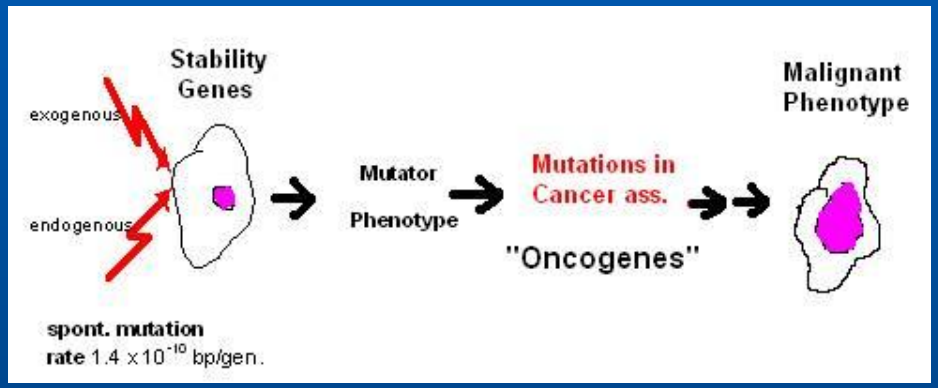
- Mutations which are present in a population with a frequency greater than 1%.
- They can be originated either by point mutations of DNA sequence (RFLP) or by a variable number of short sequence repeats (VNTR).
- Depending on DNA site they occur, are able to modify gene expression or gene product.
- Finally they modify a determined phenotypic characteristic of a subject.
- **For example, the different tolerance to insults and reparative ability of DNA damage induced by physical and chemical agents**

A similar more speedy micro-evolution occours in a tumor

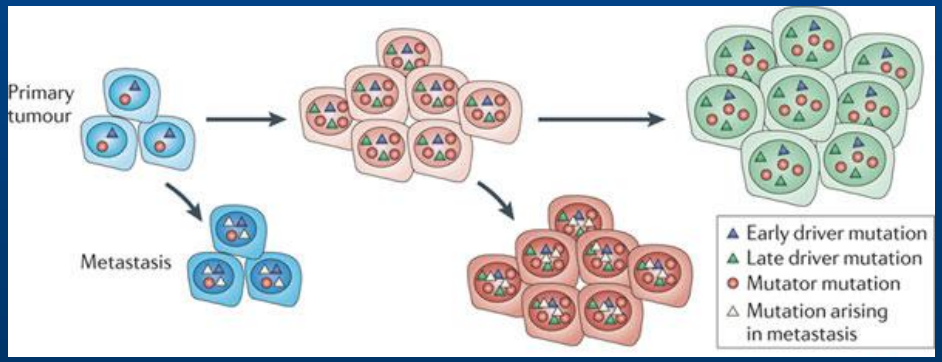
A tumor cell, due to its intrinsic genomic instability, acquires the mutator phenotype and may, during the cell duplications, generate, in the same individual, neoplastic cells with different mutations which, by clonal selection and expansion, may change some characteristics of the tumor, for example, the aggressiveness and the over-capability to repair DNA breaks to withstand therapies DNA damage-based, such as BNCT.



In particular….



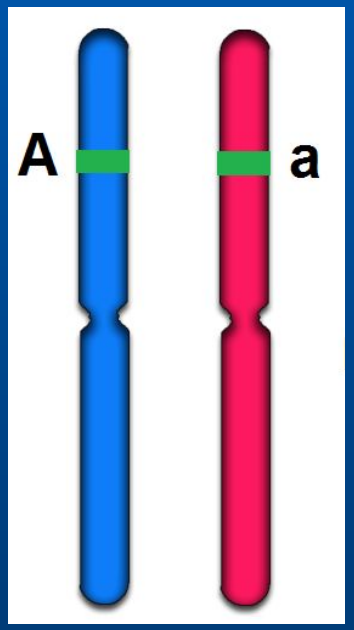
An external insult, physical or chemical, could break the genomic instability of a **normal cell** of a subject by mutating the genes of cell cycle control. Thus, the cell acquires a so called "mutator phenotype" and become prone to generate others mutations up to transform itself in a tumor cell.



For the same mechanisms and even more so, a **tumor cell** could become metastatic with a genetic configuration different in respect of the first cancer cell.

But Genetics ….

……remembers us that every human cell, in chromosomes, for each gene has two allelic copies i.e. two genes with the same function but with some small difference in their nucleotide sequence and in phenotipic behavior.



For example: regarding an hypothetical gene A, every human cell has allele "Big A" and allele "small a"; together they make the three possible genotype of a subject: **AA, Aa, aa**

Every genotype corresponds to a different capability to positively respond to BNCT and to develop secondary tumors

Strong DNA repairer

His cancer cells needs an high-dose BNCT to go in apoptosis or necrosis

His normal cells will be protect by mutations BNCT-induced. No secondary tumor will occur

Bland DNA repairer

His cancer cells needs a normal-dose BNCT to go in apoptosis or necrosis

His normal cells will be in risk to have mutations BNCT-induced and to go in neoplastic trasformation

Intermediate DNA repairer

His cancer cells needs an intermediate-dose BNCT to go in apoptosis or necrosis

His normal cells will be in low risk to have mutations BNCT-induced and rarely go in neoplastic trasformation

It follows that we must also take account of genetic / genomic parameters of every patient in order to give greater success to BNCT

It would be desirable to act on more than one front:

1- Improve boric carriers to give less systemic toxicity

(Imperio et al., 2017)

Organic LETTERS

Letter

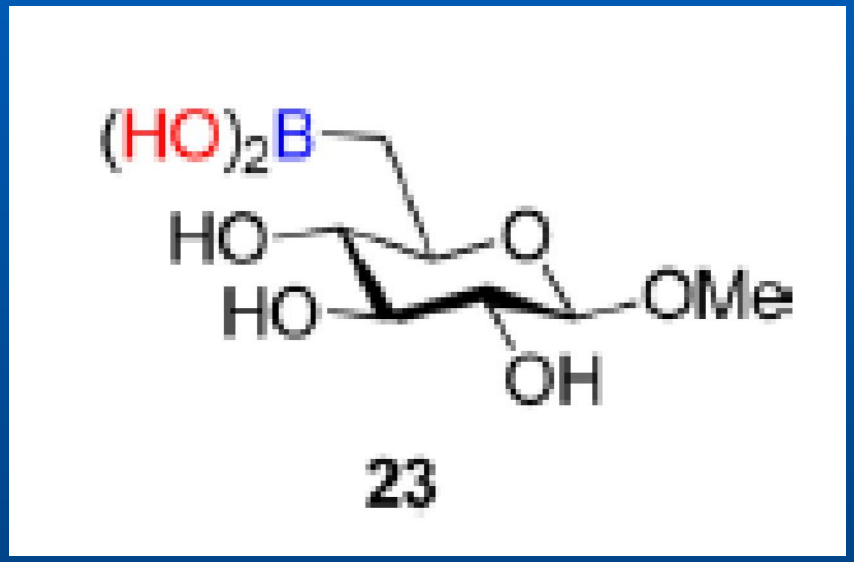
pubs.acs.org/OrgLett

DOI: 10.1021/acs.orglett.7b00382
Org. Lett. 2017, 19, 1678–1681

Synthesis of Sugar–Boronic Acid Derivatives: A Class of Potential Agents for Boron Neutron Capture Therapy

Daniela Imperio, Erika Del Grosso, Silvia Fallarini, Grazia Lombardi, and Luigi Panza*

Department of Pharmaceutical Sciences, Università del Piemonte Orientale, Largo Donegani 2, 28100 Novara, Italy



(Imperio et al., 2017)

Best sugar used in boric carriers to give less systemic toxicity *(data in vitro)*

2 – Obtain greater specificity for a mirate delivery of Boron compounds

(Wang et al., 2017)

Ceramics International 43 (2017) 6371–6376

Contents lists available at ScienceDirect

Ceramics International

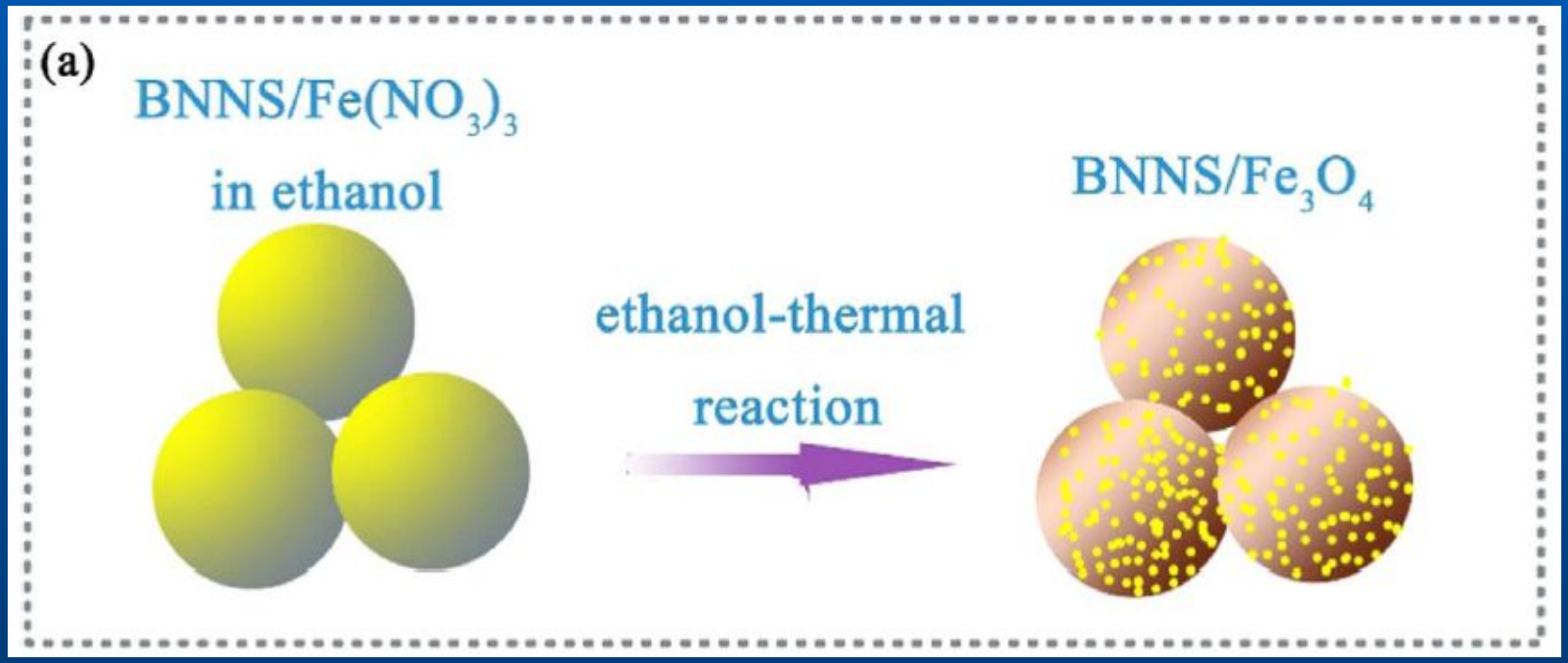
journal homepage: www.elsevier.com/locate/ceramint

Fe_3O_4 nanoparticle-coated boron nitride nanospheres: Synthesis, magnetic property and biocompatibility study

Weijia Wang[a,b], Jing Lin[a,b,*], Chengfen Xing[c,**], Ran Chai[c], Saleem Abbas[a,b], Tao Song[a,b], Chengchun Tang[a,b], Yang Huang[a,b,*]

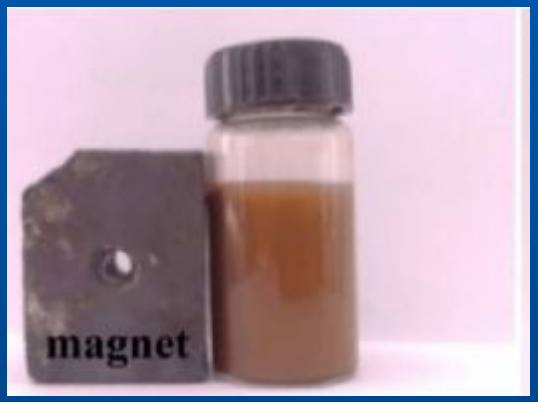
[a] School of Materials Science and Engineering, Hebei University of Technology, Tianjin 300130, PR China
[b] Hebei Key Laboratory of Boron Nitride Micro and Nano Materials, Hebei University of Technology, Tianjin 300130, PR China
[c] Institute of Biophysics, Hebei University of Technology, Tianjin 300401, PR China

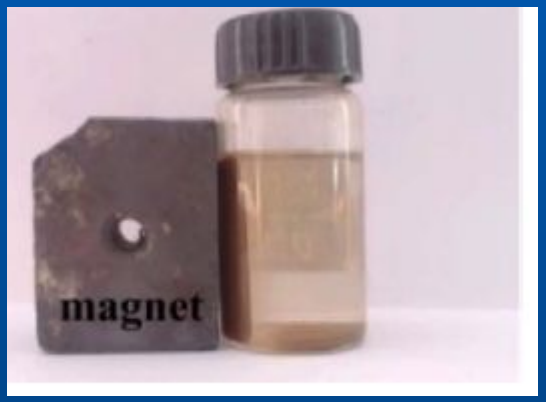
Fe_3O_4 nanoparticle-coated boron nitride nanospheres….



(Wang et al., 2017)

…. able to be manipulated under a magnetic field





(Wang et al., 2017)

- This nanocomposites, uniformly dispersed in an ethanol solution, present a dark brown color.
- When a magnet was placed to the side wall of the solution for several minutes, all the Fe_3O_4@BNNS moves to the sidewall of the bottle.

3 - Avoid that even a few genomic insult can trigger oncogenic mutations in normal cells, especially in subjects with a risk-genotype(s).

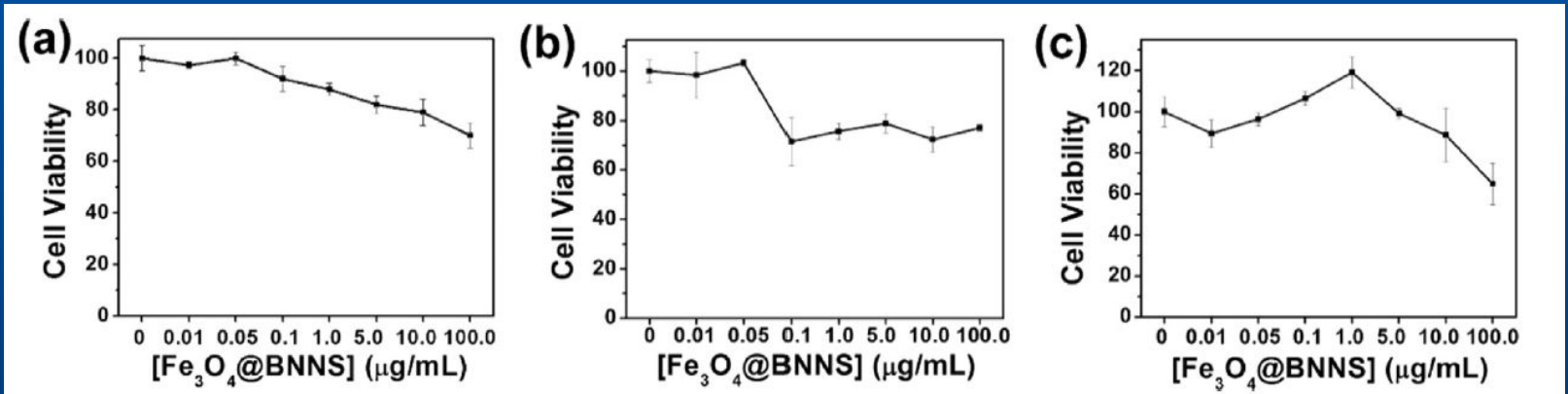


Fig. 7. Cell viability data of the nanocomposities by MTT assay (a) on MCF-7 cell line for 24 h; (b) on MCF-10 cell line for 24 h; (c) on Hela cell line for 24 h.

(Wang et al., 2017)

4 - Associate genetic studies to determine the preventive patient genotype for some key-genes for an evaluation of the risk / benefit of BNCT cycles

By genotyping every patient for the so called ”genome guardians genes” as *TP53*, *BRCA*, *P16*, ...

- A patient with a constitutionally heterozygous genotype for some of these key-genes responds to a BNCT curative effects **but** has certainly some risk to have greater genomic instability, BNCT-induced, in normal cells and to generate other secondary tumors.
- By remembering of microevolution capability of a tumor, it would also be necessary, when logistically possible, to have serial data from tumor biopsies taken with cyclical time, to detect the genetic / genomic evolution of the tumor.

DNA Ligase IV: a novel interesting key-gene, BNCT-related

Radiat Environ Biophys (2016) 55:89–94
DOI 10.1007/s00411-015-0625-2

ORIGINAL ARTICLE

DNA damage induced by boron neutron capture therapy is partially repaired by DNA ligase IV

Natsuko Kondo[1] · Yoshinori Sakurai[2] · Yuki Hirota[3] · Hiroki Tanaka[2] · Tsubasa Watanabe[1] · Yosuke Nakagawa[1] · Masaru Narabayashi[1] · Yuko Kinashi[1] · Shin-ichi Miyatake[3] · Masatoshi Hasegawa[4] · Minoru Suzuki[1] · Shin-ichiro Masunaga[1] · Takeo Ohnishi[4] · Koji Ono[1]

Received: 10 February 2014 / Accepted: 3 November 2015 / Published online: 16 November 2015

These studies open hopeful scenarios in terms of efficacy of BNCT for cancer cells

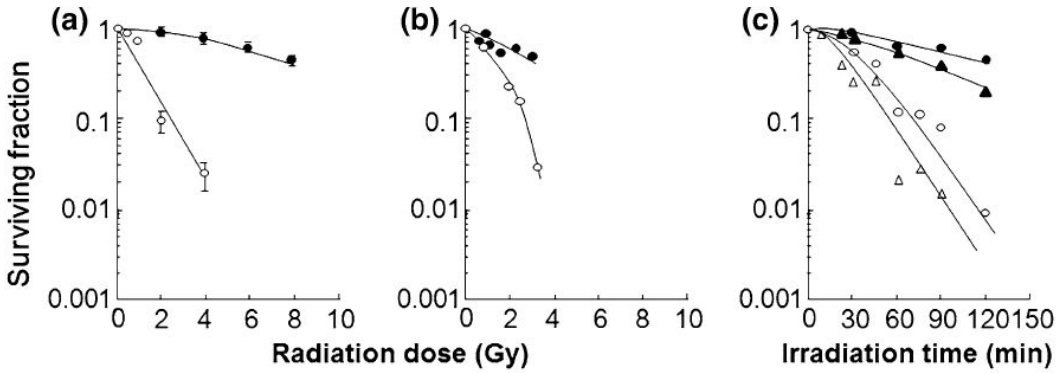


Fig. 1 Cell survival curves for *Lig4−/−p53−/−* cells (Lig4 deficient; *open circles*) and *Lig4+/+p53−/−* (*closed circles*). **a** γ-ray irradiation: plot of radiation dose (Gy) against surviving cell fraction. **b** Neutron beam (thermal, epithermal and fast neutron) irradiation: plot of radiation dose (Gy) against surviving cell fraction. **c** Neutron beam (thermal, epithermal and fast neutron) and γ-ray irradiation: plot of irradiation time (min) against surviving cell fraction in the presence of 10 ppm BPA (*closed triangles*, *Lig4+/+p53−/−*; *open triangles*, *Lig4−/−p53−/−* cells) or in the absence of BPA (*closed circles*, *Lig4+/+p53−/−*; *open circles*, *Lig4−/−p53−/−* cells)

(Kondo et al., 2016)

Recent in vitro studies show that deficient cells for **DNA ligase IV** gene, with a -/- *LIG4* genotype, are much more sensitive to the effects of BNCT of other proficient cells with +/+ or +/- *LIG4* genotypes.

Presence of mutated *LIG4* in oncologically healthy persons



J Clin Immunol (2016) 36:341–353
DOI 10.1007/s10875-016-0266-5

ORIGINAL ARTICLE

Ligase-4 Deficiency Causes Distinctive Immune Abnormalities in Asymptomatic Individuals

Kerstin Felgentreff[1,2] · Sachin N. Baxi[1] · Yu Nee Lee[1] · Kerry Dobbs[1] · Lauren A. Henderson[1] · Krisztian Csomos[3] · Erdyni N. Tsitsikov[4] · Mary Armanios[5] · Jolan E. Walter[1,3] · Luigi D. Notarangelo[1,6]

Received: 12 January 2016 / Accepted: 7 March 2016 / Published online: 11 April 2016

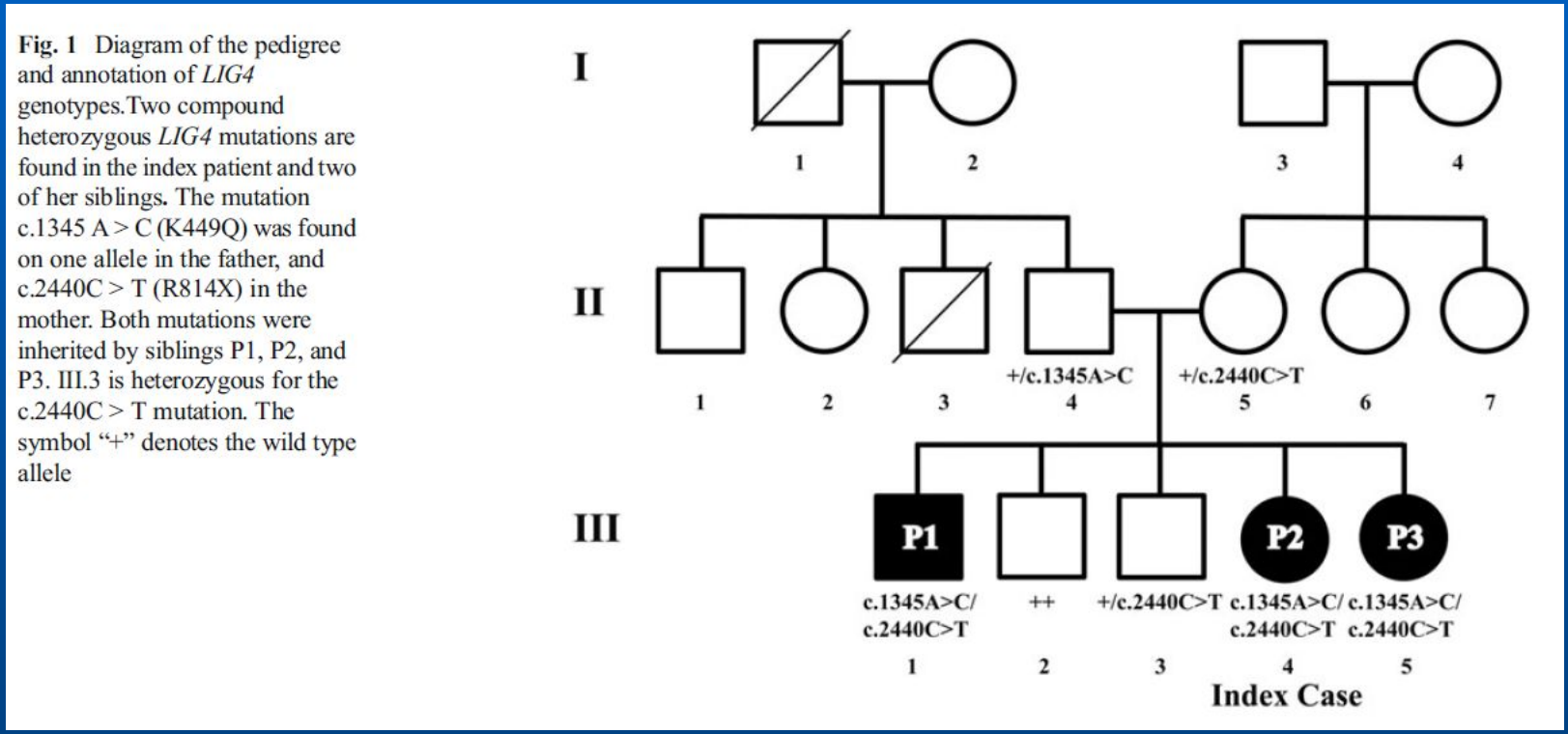


Fig. 1 Diagram of the pedigree and annotation of *LIG4* genotypes.Two compound heterozygous *LIG4* mutations are found in the index patient and two of her siblings. The mutation c.1345 A > C (K449Q) was found on one allele in the father, and c.2440C > T (R814X) in the mother. Both mutations were inherited by siblings P1, P2, and P3. III.3 is heterozygous for the c.2440C > T mutation. The symbol "+" denotes the wild type allele

(Felgentreff et al., 2016)

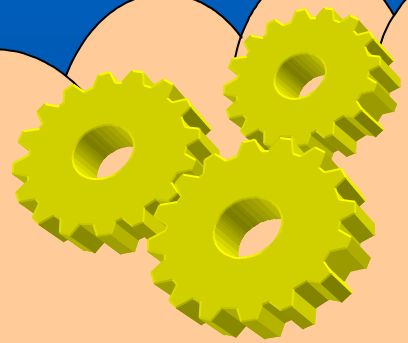
Allarmingly, this study:

- says us that may exist apparently healthy subjects, carriers of mutated *LIG4* but totally asymptomatic for cancer or related syndromes,
- raises the possibility that cycles of BNCT in these subjects can expose them to develop secondary tumors in tissue districts unrelated to the primary tumor.

In conclusion:

Today it is necessary to apply the principles of Pharmacogenetics and Pharmacogenomics to the BNCT. In particular:

- Associate genetic studies to determine the patient genotypes for the so called ”genome guardians”;
- Associate a constitutional genotyping for *LIG4* gene.



Thanks to the massive DNA sequencing techniques, with the help of genetics and genomics, tomorrow it will be possible personalize a BNCT treatment to maximize its curative effect.

Thanks to lab and collaborators

Ilenia Cruciata

Chiara La Rosa

Flores Naselli

Claudio Luparello