**Differential superiority of carbon ion irradiation and radiosensitizing nanoparticles to X-Rays: studies on biological effectiveness in tumor cell models**

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Among the current innovations to treat radioresistant tumors, improvement of radiotherapy relies on either the delivery of a higher dose of radiation to the target volume by focused beams (3D conformational or intensity-modulated radiotherapy, hadrontherapy with protons or carbon ions…) while limiting their delivery to critical normal structuresor the increase in the local effect of a given dose of radiation by radiosensitizing agents (metallic nanoparticles…). This presentation will summarize our current results with carbon ions and AGuIX® nanoparticles in different tumor models.

We demonstrated the superior relative biological effectiveness (RBE) of carbon ions at different levels:

- Gene and chromosomal damage induced by carbon irradiation are so complex that they cannot be transmitted in the progeny of irradiated tumor cells, thus limiting genomic instability and improving local control*.* Chromosome/chromatid loss appears as a specific signature of carbon ion exposure in sensitive and resistant head and neck squamous cell carcinoma (HNSCC) cells *(Hanot et al. Plos One, 2012)*. Furthermore, the response to carbon ions is independent of the telomeres’ size. The presence of long telomeres in tumor cells of patients with glioblastoma is a well-known factor of poor prognosis as they become more resistant to oxidative stress induced by conventional radiotherapy. Thus, our data first underlines that patients with long telomeres can advantageously benefit from carbon-therapy *(Ferrandon et al., Mol Neurobiol, 2013).*

- Cell death is triggered earlier and more significantly by carbon ions in HNSCC or glioblastoma cellular models. It involves either early apoptosis in radiosensitive cells or mitotic catastrophe followed by late apoptosis in radioresistant ones. Apoptosis is activated through a pathway independent of p53, but dependent on ceramide (a lipid signaling mediator) *(Alphonse et al, BMC Cancer, 2013; Ferrandon et al., Cancer Letters, 2015)*, giving carbon ions a significant advantage since 50% of tumors have a mutated p53 gene.

 - Carbon ions are more effective than photons in killing cancer stem cells (CSCs) in HNSCC *(Bertrand et al., Stem Cell Rev, 2014)*. Furthermore, molecular connections between the stem-cell state and epithelio-mesenchymal transition program have recently emerged, pointing out a double danger for cancer patients since CSCs have the ability to renew indefinitely and are resistant to apoptosis. Our investigations point out a significant decrease in the migration and invasion of both parental and CSC populations irradiated with carbon ions, thus highlighting the great interest of carbontherapy in the prevention of recurrences and metastases *(Moncharmont et al., Oncotarget 2016)*.

Hypoxia seems to have a key role in the self-renewal of CSCs (located in hypoxic niches), their stemness maintain, tumor angiogenesis, growth tumor and therapeutic resistance. The protein HIF-1α (Hypoxia-Inducible Factor 1α) is considered as the major transcriptional regulator of the cellular and developmental response to oxygen homeostasis. In hypoxic conditions, HIF-1α plays a central role in radioresistance (OER> 1.2) and the increased invasion and migration phenomenon activated by a photonic irradiation. Conversely, since carbon ions appear unable to stabilize HIF-1α in CSCs, there is neither resistance linked to the oxygen effect (OER = 1) nor activation of the migration and invasion pathways *(Wozny et al., Br J Cancer, 2017).*

AGuIX® (Activation and Guidance by Irradiation X) is a non-toxic gadolinium-based nanoparticle (GBNs) developed by the Lyon University. It accumulates in the tumor through the enhanced permeability and retention (EPR) effect and clears rapidly through the kidneys due to its small size (sub-5nm). We performed a proof of concept on HNSCC, metastatic melanoma and chondrosarcoma tumors, known for their low survival rates, demonstrating the radiosensitizing efficacy of the AGuIX® nanoparticles in cellular (2D and 3D cultures) and preclinical models.

GBNs enter HNSCC cells by passive diffusion and macropinocytosis *(Rima et al, 2013)*, localize in cytoplasm, as free particle or entrapped in lysosomes, in close vicinity to mitochondria. GBNs combined with irradiation can produce a large variety of secondary emissions, such as secondary Auger and Compton electrons, leading to the production of reactive oxygen species that trigger an intra‐mitochondrial stress (ROS production, transmembrane potential decrease, mtDNA deletion) and nuclear DNA damage leading to cell death.

The RBE in cancer cells is quite comparable to that observed in response to carbon ions, suggesting the existence of common mechanisms through the amplification of the local dose *(Miladi et al., Nanomedicine 2015).* The radioenhancing effect of AGuIX® was also observed when combined to carbon ion irradiation *(Wozny et al., in revision)*.

The efficacy of AGuIX® has also been demonstrated in orthotopic xenograft models of HNSCC and metastatic melanoma *(Miladi et al., 2015; Kotb et al., Theranostics 2016)*; the experiments are ongoing for chondrosarcoma. Regulatory toxicity studies were conducted in rats and monkeys and a first human clinical study is ongoing in patients with multiple brain metastases (clinicaltrial.gov).