13 Increasing particle therapy biological effectiveness by nuclear reaction-driven binary strategies

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13.1 INTRODUCTION

Charged particle inverted dose-depth profile represents the physical pillar of protontherapy. On the other hand, there is no obvious radiobiological advantage in the use of protons since their LET in the clinical energy range (a few keV/μm, at mid-Spread-Out Bragg Peak, SOBP) is too low to achieve a cell-killing effect significantly greater than in conventional radiotherapy. This currently prevents protontherapy from being useful against intrinsically radioresistant cancers. Radioresistance of cancer cells implies dose-escalation regimes to achieve ~~tumour~~ local control. .In theory, every ~~tumour~~ can be controlled if a sufficiently high dose can be delivered that is able to suppress the proliferative potential of all cancer cells. However, in clinical practice, the maximum radiation dose is unfortunately limited by the tolerance of the surrounding normal tissue. A well-known relationship links physical radiation quality (LET) and its biological effectiveness (RBE), based on the notion that cellular lethality increases with the degree of DNA damage clustering, i.e. complexity, which reflects the nano-scale model of radiation action. Therapeutic 12C ion beams show a LET at mid-SOBP of about 50 keV/μm, conferring these particles a greater RBE for ~~tumour~~ cell killing, which is the radiobiological justification for their use against radioresistant cancers. However, the non-negligible dose deposition beyond the SOBP due to nuclear fragmentation and economical issues encumber this form of hadrontherapy. Additionally, limited radiobiological data exist on long-term normal tissue radiotoxicity. It is already known from previous studies that many different factors are associated with radioresistance of cancer cells and multiple reviews have already described some of the possible mechanisms underlying radioresistance during conventional radiotherapy. Examples are cancer stem cells and hypoxia, as well as perturbations in survival pathways, DNA damage repair pathways, developmental pathways. Many molecular inhibitors have been tested in combination with conventional radiotherapy, while only very few have been tested in combination with protons or carbon ions. Since particle therapy is on the rise, this calls for further exploration of these combined therapies in a preclinical setting. Previously, particle radiation facilities provided limited access for biological experiments, which limited the time to perform such experiments. However, international consortia on particle therapy research are growing and now recognize the potential of radiobiological experimental work. Therefore, the European Particle Therapy Network is producing a considerable effort to form a network of research and therapy facilities in order to coordinate and standardize radiobiological experiments. For carbon ions specifically, limited data on combination therapies are available. This is mainly due to the high RBE of carbon ions by which the additional benefit of molecular inhibitors might be difficult to demonstrate. Furthermore, the use of carbon ions worldwide is limited, which could also explain why fewer studies have been published regarding combination treatment with carbon ions. In the next paragraph, combined molecular approaches targeting specific repair pathways will be briefly illustrated, together with an outlook of recently proposed systemic approaches where radiation may upregulate the fundamental anti-cancer response by the immune system. In the context of achieving greater RBE at cell ~~tumour~~ inactivation while maintaining reasonably low-dose levels in healthy tissues, the role of physics and, specifically of certain nuclear reactions, has recently re-gained center stage in the form of so-called binary strategies. Historically, the first approach to predict a ~~tumour~~-confined increase of radiobiologically effective doses by irradiation with a primary beam is the Boron Neutron Capture Therapy (BNCT) which exploits the 10B(n,α)7Li reaction. The BNCT is defined as a binary approach since an external neutron beam serves no therapeutic purpose by itself but is needed to trigger the secondary particles which bring about the radiobiologically effective action on the ~~tumour~~ A boron-10 (10B)-labelled carrier must deliver higher concentrations of 10B to target ~~tumour~~ cells compared to the concentrations uptaken by surrounding normal tissues. The administration of borated formulation is followed by irradiation with low-energy neutrons. When a neutron collides with 10B, high-LET particles, i.e., α-particles and recoiling 7Li particles, are released within one cell's diameter by the 10B(n, α)7Li neutron capture reaction, which occurs with a high cross section (3738 b) at thermal energy. These high-LET particles can destroy the 10B-containing cells without exerting hazardous

effects on the adjacent normal cells. Therefore, if sufficient quantities of boron compounds can be made to accumulate selectively in ~~tumour~~ cells with enough contrast to surrounding normal cells, the BNCT becomes an ideal radiotherapy modality. The selective properties of BNCT make it a radiotherapy option potentially useful also for disseminated or infiltrated malignancies. BNCT requires: a) low-energy neutron beams, whose availability is not trivial; b) selectivity in boron uptake by ~~tumour~~ cells only; c) a complex dosimetry of the mixed-field arising from neutron interaction with the tissue elements. Recently another binary approach has been proposed that exploits the 11B(p, α)8Be reaction, whose cross-section resonates at 675 keV, hence being termed Proton-Boron Capture Therapy (PBCT). In protontherapy such energies are those of protons as they slow down across the ~~tumour~~ region. The latter eliminates the requirement for selective boron uptake by cancer cell as alpha particles will be not generated, in principle, in healthy tissues at the beam entrance channel where incident proton energy is too high from that of the cross-section maximum; thus if proven viable, PBCT would elegantly bypass one of the most critical requirements of BNCT.

13.2 NOVEL FRONTIERS IN PARTICLE THERAPY: THE RADIOBIOLOGICAL POINT OF VIEW

13.2.1 OVERCOMING CANCER RADIORESISTANCE

The ultimate goal of any form of curative radiotherapy resides in achieving local tumor control by suppressing the proliferative ability of all clonogenic cancer cells. If, on the one hand, the dosimetric precision inherent to charged particle therapy, in principle, allows to reduce the risk of adverse effects due to unnecessary dose absorbed by healthy tissues and/or organs at risk, on the other hand cancer radioresistance continues to represent a cause for treatment failure, leading to local recurrence, metastases and poor prognostic outlook [70]. Most of the available knowledge on the molecular mechanisms underlying cancer radioresistance derives from the predominant experience with conventional radiotherapy [53], which has highlighted how ~~tumour~~ response to ionizing radiation is deeply influenced by the heterogeneity that characterizes the ~~tumour~~ microenvironment [59]. Such heterogeneity and the changes the ~~tumour~~ microenvironment undergoes during cancer progression and the course of a radiotherapy regime, contribute to a variety of ~~tumour~~ subpopulations exhibiting differing radiosensitivities [14, 2] and define what is referred to as intrinsic and acquired radioresistance, respectively [7, 73]. Hypoxia is arguably one of the most intensively studied factors contributing to cancer radioresistance [15, 37, 38, 40, 6]. Although the low Oxygen Enhancement Ratio (OER) presented by high-LET radiation is the obvious radiobiological pillar of 12C-ion based radiotherapy against hypoxia-induced cancer radioresistance, charged particle radiobiology still lacks a thorough knowledge on the impact that the different physical nature of radiation may have on the molecular signaling pathways encompassing radioresistance and ~~tumour~~ microenvironment. The need for a re-definition of cellular radiosensitivity to charged particles is exemplified by peculiar inconsistencies if observed radioresponse is compared against the expected behavior based solely on the known LET-RBE relationship, with reported instances of a greater effectiveness exhibited by protons compared to photons in sensitizing resistant glioma cancer cells, which was ascribed to proton-specific increased ROS levels [78, 3]. These findings, coupled with the observation of a greater proportion of clustered DNA damage left unrepaired in cells exhibiting defects in the Fanconi Anemia/BRCA repair pathway following proton irradiation compared to photons at comparable LET values [51], have led to the suggestion of a clinically useful “New Biology” of protons by Held et al. (2016). Thus, in line with the increasing awareness of a variable proton RBE as a function of physical parameters, the genetic heterogeneity of ~~tumours~~ with regard to their repair capabilities may be exploited to single out proton-susceptible ~~tumours~~ [25, 34, 22]. However, despite indications of a significantly high efficiency of 12C ions at killing cancer stem in hypoxic niches [60], overcoming cancer radioresistance is still an urgent necessity in charged particle therapy, hence various approaches to enhance its biological effectiveness are being explored.

13.2.2 EXPLOITING BIOMOLECULAR APPROACHES

Targeting specific molecular pathways involved in cancer radioresistance has been proposed as a potential avenue to sensitizing cancer cells to charged particles in approaches that are designed to combine the peculiar radiobiological properties of the latter with novel molecular inhibitors directed to suppress a number of cancer cell pro-survival mechanisms and/or counteract radioresistant cell niches [44]. In this context, few radiobiological and clinically relevant data exist. These strategies have in common the idea that charged particles may modulate or act on most of these potential targets in a different manner compared to photons [71, 72] and include the DNA Damage Response (DDR) machinery [53] as well as proliferation- and cell death-associated gene transcription signalling pathways [61], cancer stem cells [74] and, of course, hypoxia. In counteracting the latter by charged particles, their concomitant use with hypoxia inducible factor (HIF)-1 inhibitors has been proposed. This is because, in contrast with the fundamental role that physiological levels of oxygen play in the yield of photon irradiation-induced DNA damage, HIF-1 expression is strongly upregulated by photons in hypoxic cells and its activation is linked with neo-angiogenesis, ~~tumour~~ growth and metastasization. Whereas the use of HIF-1 inhibitors in conventional radiotherapy has yielded inconclusive results, their role in particle therapy is still unexplored and may be of potential interest for clinical protons, where OER is supposed to be similar to that of photons. Additionally, the use of vascular endothelial growth factor (VEGF) inhibitors is warranted, especially in combination with 12C-ion therapy, because of the importance of hypoxia-modulated angiogenesis via the HIF-1/VEGF signalling pathway [106] and the demonstrated efficacy of high-LET radiation against hypoxia. Closely linked with the presence of hypoxic niches within the ~~tumour~~ is the presence of the intrinsically radioresistant cancer stem cells [42, 24], whose functional properties including self-renewal capacity, long-term repopulation potential, and tumor initiation and progression capacity render them a determinant of cancer resilience to radiotherapy. A number of survival signaling pathways have been identified that contribute to cancer stem cell radioresistance (i.e., Wnt, Notch, Hedgehog, anti-apoptotic Bcl-2, TGF-beta, and PI3K/Akt/mTOR), for which several pharmacological inhibitors have been developed over the years, thereby representing as many potential targets for combined treatments [44, 59]. Finally, given the pivotal role that repair has in conferring radioresistance, targeting of DDR signalling pathways, such as those ~~centred~~ on Poly(ADP-ribose) polymerase (PARP), a key responder and effector of radiation-induced DNA breakage, or on the genome integrity regulator ~~tumour~~ suppressor protein p53, are an attractive strategy to explore in hadrontherapy [44]. For example,the PARP inhibitor, AZD2281, was shown to enhance DNA damage yield and cell-cycle arrest in the ~~tumour~~ conformed SOBP for clinical proton beams by Hirai et al (2016), while more recently [50] have shown an increased 12C-ion induced cancer cell sensitization when administered in combination with another PARP inhibitor, talazoparib. Similarly attractive appears the concomitant use in particle therapy of antagonists of the mouse double minute 2 and X (MDM2/X), negative regulators of p53 [56], based on promising pre-clinical findings on targeting the MDM2/X-p53 pathway for the treatment of glioblastomas shown by several inhibitors in co-therapy scenarios with drugs and photon irradiation [12].

13.2.3 EXPLOITING SYSTEMIC RESPONSES

In order to grow and spread, cancer cells develop mutations that allow them to escape recognition and elimination by the host's immune system [33]. Immunotherapy (IT) has gained importance in cancer treatment due to its potential to recover the individual patient's immune recognition of cancer and develop an acquired immune response against malignant cells in the entire body. Available IT agents are therefore designed to either re-activate the immune system or release its brakes to allow recognition of cancer cells as non-self, and successfully reject them. However, even in malignancies where IT has proven efficacy (e.g. melanoma, non-small cell lung cancer, and certain genitourinary malignancies), response rates remain low, highlighting the need for more effective agents or com-

bined modalities [64, 10, 32]. There exists a revival of interest in the modulatory effects of ionizing radiation on the immune system as mounting evidence has consistently shown the ability of locally administered radiotherapy to induce a system-wide immune response [20, 21, 27] in addition to exerting its cytotoxic action on the ~~tumour~~ site, suggesting that radiation can work together with the immune system to eliminate cancer [27, 28, 32, 58]. As a result, historically established concepts have been revisited, such as those describing a mere immunosuppressive action by radiation whereas it is now accepted that radiation causes multiple immunostimulatory effects [21]. Furthermore, in-vitro radiobiological phenomena collectively known as Non-Targeted Effects (NTEs), specifically the bystander effect, have been re-assessed, in light of new reports of regression of tumors outside of radiation field, thereby bridging the conceptual gap with the abscopal responses sporadically reported in vivo [66, 28]. Unfortunately, due to various immune escape mechanisms put in place by the tumor, radiotherapy alone rarely results in a systemic response of metastatic disease sites, that is the abscopal effect. The rarity of radiotherapy-induced abscopal effects, with 46 reported cases in the literature from 1969 to 2014 [1], reflects the fact that, once metastases are detected, a sustained cancer-related immunosuppression has already been established [75]. In order to elicit the abscopal effects of RT in preclinical models mimicking metastatic disease, some of this concurrent immune suppression must be relieved. This concept was initially tested in a series of experiments that first linked the abscopal effects to an immune-mediated mechanism. In a clinical trial, where 41 patients with metastatic cancer were treated with a combination of local radiotherapy and administration IT factors, abscopal response occurred in a remarkable 26.8% of the patients [31]. The radiobiological rationale in support of a synergistic use of radiotherapy and immunotherapy relies on the fact that radiation induces "immunogenic cell death" (ICD), a process that involves the release of various cytokines and signals that modify the microenvironment of tumors and stimulate influx of immune cells to recognize tumor-specific antigens presented by dying cells as a result of therapeutic doses of radiation. This, in turn, cross-primes T-cells in draining lymph nodes, causing their activation and eventual increase in tumor infiltration by cells facilitated in recognizing the tumor cells. By such processes, radiotherapy has been shown to significantly modify the tumor microenvironment and to possess the potential to convert an irradiated tumor into an in-situ vaccine to provide systemic, long-lasting protection against cancer. [26, 27]. Thus, the view that radiation may indeed revert a host's suppressed immune status, not only by locally enhancing radiation-induced lethality in directly irradiated cancer cells through the (re)-activation of the innate and adaptive immune system, but also leading to out-of-field immune-mediated anti-metastatic responses, has been consolidating [21]. The idea that combining radiotherapy with immunotherapy may allow better local and systemic ~~tumour~~ controls, has naturally led to the proposal of exploring if, and to what extent, such immune system radiomodulation is influenced by radiation quality [23]. The possibility that charged particle exposure may elicit stronger immune responses than after photon irradiation is based on the aforementioned physical advantages and radiobiological peculiarities exhibited by protons and 12C ions [71]. Several pre-clinical and clinical trials are ongoing to experimentally verify whether charged particle therapy may potentiate the benefits brought by immunotherapy to overcome radioresistance and ~~tumour~~ invasiveness. For example, Gameiro et al. (2016) [30] have shown that proton irradiation is able to induce hallmarks of immunogenic modulation by upregulating the expression of calreticulin and other ~~tumour~~ cell-surface markers involved in immune recognition in stem-like breast cancer cells. Several studies are also examining the immunoadjuvant effects of protontherapy against non-small cell lung cancer on the grounds of differential biological responses induced by protons, i.e. more immunogenicity and less immunosuppression if compared to photons [49]. Recently, in-vivo work by Takahashi et al. (2019) [52] for the first time showed that carbon ion irradiation combined with dual immune checkpoint blockade therapy enhanced local anti-tumor efficacy and induced an abscopal effect by inhibiting distant metastases in a preclinical murine model of osteosarcoma. Equally promising are the results by Mirjolet et al. (2021), demonstrating for the first time in a clinical protontherapy setting and using an ectopic mouse model with

a transplanted colon carcinoma cell line, that a single proton dose of 16.4 Gy activated several immune response pathways, inducing intra-tumour infiltration of CD8+ T cells, CD4+ T cells and type 1 tumor associated-macrophage (TAM1). In conclusion, validation of a clinical advantage deriving from coupling of hadrontherapy and immunotherapy would arguably represent a strong argument in favour of a further expansion of anticancer therapies based on charged particles as they remain, overall, just a fraction of all radiotherapy modalities.

13.3 THE RADIOBIOLOGICAL RATIONALE OF PHYSICS-BASED STRATEGIES FOR CHARGED PARTICLE-MEDIATED RADIOSENSITIZATION

Strategies based on physical interaction between ionizing radiation and metallic nanoparticles or that exploit nuclear fusion reactions have gained momentum in recent years and shown promising results to achieve clinically effective radiosensitization. In the following sections, an overview of such methods to reverse cellular radioresistance is presented.

13.3.1 NANOPARTICLE-MEDIATED RADIOSENSITIZATION

The use of metallic complexes has been actively investigated as a means of enhancing tumour cytotoxicity induced by high-energy photons based on amplification of primary processes as comprehensively reviewed in Kobayashi et al. (2010) [43]. To avoid size-dependent poor diffusion of such active products into the cancer tissues, research was soon directed to developing metallic nanoparticles (MNPs) due to their ability to passively accumulate in higher concentrations in tumour tissue than in the surrounding normal tissues when injected into the bloodstream. The selective delivery of NPs occurs because of the enhanced permeability and retention effect (EPR), whereby systems that are small enough (diameter ¡200 nm) tend to permeate through the tumour blood vessel walls[55]. Tumours recruit their own blood supply by angiogenesis, and their vasculature has leaky capillary walls that allow for NPs to easily pass through the wall. The most commonly considered formulation has been traditionally gold nanoparticles (GNPs), i.e. particles of gold with a diameter of 100 nm or less. In addition to good biocompatibility because of their inert chemical nature, GNPs were mostly expected to be ideal photosensitisers for those reactions characterized by a strong dependence on Z, such as photoelectric and pair production interactions of X-rays [47]. Among the several emissions that occur, Auger electrons can play an important radiosensitizing role when produced by photon interaction with high-Z GNPs since they have much shorter range than fluorescent photons, with much higher ionization density, thereby deploying a highly localized clustered DNA damage (Ku et al., 2019). Generally, it is the high absorption of photons by NPs to augment secondary electrons, increasing the levels of DNA damage by physically enhancing the dose. However, high-Z metal NPs (MNPs) have also been shown to act through distinct biological mechanisms including higher levels of oxidative stress, increasing levels of reactive oxygen species (ROS) and inducing highly reactive hydroxyl radicals which go on to cause further DNA damage [16]. To improve tumour targeting, then NPs may be functionalized with tumour specific agents such as antibodies or other peptides [29]. Other MNPs have been investigated over the years as radiosensitizers, and sophisticated NPs, composed of other heavy elements such as hafnium [54] and gadolinium [68], are already being considered for clinical usage and possible theranostic agents. Equally sophisticated is the theranostic use of Superparamagnetic Iron Oxide Nanoparticles (SPIONs) in light of the development of MR-Linacs, whose radiosensitising properties have recently assessed in combination with kVp X-rays over a panel of cancer cell lines in vitro, as well as for the first time, in vivo with a H460 lung xenograft model [67]. Although initially devised and long investigated for photons/electrons, the strategy based on the use of nano-radiosensitizers has been proposed and is being increasingly tested also for charged particles, mainly protons and, to a lesser extent, 12C ions. This approach was not initially regarded as capable of leading to significantly measurable radiosensitizing effects for proton and ion radiotherapy because of the decrease in collision stopping power of charged particles

as a weak logarithmic function of Z in contrast with the high photoelectric absorption with strong Z-dependence exhibited by kV X-rays. However, as recently reviewed by Lacombe et al. (2017) [48] and by Peukert et al. (2018) [62], experimental evidence has been accumulated that seems to dispel this notion, although only limited in vitro and in vivo data are available and much remains to be understood about the underlying physical and radiobiological mechanisms responsible for the observed radiosensitizing effects such as those of proton irradiation in the presence of GNPs. Indeed, charged particles are able to give rise to a nonlinear avalanche of electron emissions from high-Z NPs through impact ionisation and ensuing Auger cascades. Importantly, surface plasmon excitation can result in a large production of secondary electrons that can continue to excite and ionise surrounding biomolecules and neighbouring nanoparticles, providing a solid rationale for proton-therapy radiosensitization [48] In addition, Coulomb nanoradiator (CNR) effects that produce burst emission of fluorescent X-rays and low-energy electron (LEEs) via Auger cascade and a generalised increase in chemical damage by reactive oxygen species (ROS) are thought as major players in the dose enhancement effects that are observed for high-Z NPs and high-energy proton beams as shown by Geant4-based simulations by Peukert et al., (2019) [63]. To confirm such theoretical predictions, a number of high-Z MPNs were tested by Rashid et al. (2019) [65] and all showed a radiosensitizing action compatible with an increased intracellular ROS production in a human colon carcinoma cell line exposed within the SOBP of a 150-MeV proton beam. More recently, increased induction of cytogenetic damage but lack of an LET dependence on the incident particle LET were shown in a proof-of-principle study aimed at investigating the radiosensitizing properties of large (50 nm diameter) GNPs on CHO-K1 cells exposed at varying depths along a 50-mm SOBP modulated from a 200-MeV clinical proton beam (Cunningham et al., 2021) [19]. Finally, Zhang et al. (2021) [79] were able to show for the first time evidence of in-vivo radiosensitization by GNPs of 12C ion irradiation in tumor-bearing mice, which also these authors attribute to an increased production of ROS and, more specifically, to the activation of the mitochondrial apoptotic pathway in line with cytoplasmic GNP localization of the incorporated GNPs. Though intriguing, these results warrant further radiobiological studies to unveil the full potential of nano-material radiosensitization of charged particle therapy.

13.3.2 BINARY APPROACHES BASED ON NUCLEAR PHYSICS REACTIONS

13.3.2.1 Boron-Neutron Capture Therapy (BNCT)

BNCT, as explained above, exploits the neutron capture reaction in 10B, carried into ~~tumour~~ cells by suitable drugs before low-energy neutron irradiation. The neutron capture reaction occurs with a cross section of 3837 b at thermal energy and has the following two branches:

$$n + 10B \rightarrow 11B \rightarrow 7Li + \alpha + 2.79MeV\,(6.3\%) \tag{13.1}$$

$$n + 10B \rightarrow 11B \rightarrow (7Li)^{*} + \alpha + 2.31MeV\,(93.7\%) \rightarrow 7Li + \gamma + 0.478MeV \tag{13.2}$$

Suitable boron drugs have been developed for clinical use and many strategies are being followed to design new carriers able to guarantee a high boron concentration between ~~tumour~~ and normal tissues. Today, the only drug used for patients is Boronophenylalanine (BPA), ensuring ~~tumour~~ to healthy tissue concentration ratios of about 3.5:1 [8]. Albeit not high, this ratio is still useful for a safe and effective BNCT treatment.

BNCT was first applied to treat a patient affected by malignant glioma in 1951, using the Brookhaven Graphite Research Reactor [8]. Since then, it has been applied in institutions equipped with research nuclear reactors, where suitable neutron beams were designed according to the ~~tumours~~ to be treated: epithermal for deep-seated and thermal or hyperthermal for shallow cancer (i.e. nodular melanoma). A review of the clinical trials performed since the recent years is described in [120]. The pathologies that have been treated with a higher number of patients are Glioblastoma Multiforme (GBM), recurrent and newly diagnosed head and neck ~~tumours~~ and skin melanoma. In

recent years, an important technological innovation has opened the way to more clinical applications, since suitable neutron beams can now be obtained with proton accelerators coupled to Be or Li targets. In Japan, clinical ~~centres~~ with neutron beams obtained by cyclotron and Be target are already applying BNCT to patients [121] ; all over the world new facilities based on accelerators are being projected and constructed.

BNCT is based on the emission of two high-LET particles following the neutron capture in 10B, having a much higher cross section compared to the interaction with other elements in biological tissues. Globally, neutron irradiation of biological tissues in the presence of 10B generates a mixed radiation field. Apart from 10B(n, alpha)7Li reaction, thermal neutrons are also captured in 14N, producing a proton. Alpha, lithium ions and protons have moderate to high ionization density values (164, 151 and 44 keV/mm, respectively), and short ranges in tissue (9, 5 and 11 μm, respectively). Moreover, epithermal neutrons thermalize in tissue mainly through elastic scattering in hydrogen, producing a recoil proton that deposits dose. Finally, there is a low-LET component of the dose, due to gamma generated by neutrons capture in hydrogen and by the structural photons, which is always present in a neutron beam. The only selective component of the absorbed dose is the one due to neutron capture in 10B, because there is a differential accumulation in ~~tumour~~. The other components are a background which must be kept as low as possible. Due to this unavoidable dose absorbed by healthy tissues, BNCT treatment planning prescribes the dose to reach the maximum tolerated dose in the most radiosensitive tissue involved in the irradiation, and calculated ~~tumour~~ dose according to the known boron concentration.

From the radiobiological point of view, one of the most exploited models is the ~~tumour~~ cell culture irradiation in presence and in absence of boron. Survival of ~~tumour~~ cells as a function of the absorbed dose is a measure of the biological effectiveness of BNCT compared to a reference radiation, typically photons. As explained later with more details, the first model employed to express biologically-weighted BNCT dose, was multiplying each dose component by the RBE/CBE fixed factors, obtained by cell-survival curves [122]. The RBE measured for different cell lines show that BNCT is around 5 times more effective than photons in reducing ~~tumour~~ cell survival. Figure 13.1 shows a typical experiment irradiating rat osteosarcoma cells in a thermal neutron field in presence and in absence of BPA and using a 60Co source as the photon reference radiation [123]. These curves, opportunely fitted, allow the calculation of RBE for the neutron dose component of 2.2$\pm$0.5 and a CBE for the boron dose component of 5.3$\pm$1.5. It is important to note that BNCT poses an important requirement: in order to calculate properly the effectiveness, dose delivered to cell cultures must be calculated in detail, taking into account that equilibrium of charged particles may not hold, thus the Monte Carlo transport of each secondary charged component must be performed.

13.3.2.2 Proton-Boron Capture Therapy (PBCT)

PBCT, as aforementioned, is a novel binary approach, first theoretically proposed by Yoon et al. (2014) [118] with the aim of potentiating the efficiency of protontherapy, as to render it amenable to treat radioresistant cancers . Specifically, in order to increase the clinical RBE of protons, commonly assumed to be 1.1, the PBCT strategy is based on the exploitation of the nuclear fusion reaction between low-energy protons and 11B (p-B, in brief):

$$p + 11B \rightarrow 3\alpha + 8.68MeV \tag{13.3}$$

ensuing the simultaneous release of three high-LET alpha particles [11]. The average energy of the emerging alpha particles is between 3 and 4 MeV [39] , which translates to maximal ranges of approximately 18 - 27 μm, corresponding to the mean diameter of a cell nucleus. The high-LET of such particles provides thus the basic radiobiological rationale for the clinical use of PBCT since it would in principle allow for deployment of highly localized clustered damage in the hit 11B-containing cancer cells. The maximum for the reaction cross section is believed to occur for incident proton energies of around 700 keV (see Figure 13.2, hence the probability of PBCT increases with

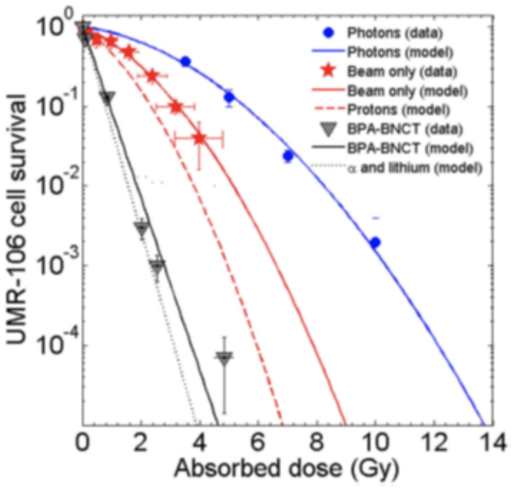

Figure 13.1: Rat osteosarcoma UMR-106 cell survival curves as a function of the absorbed dose

the protons slowing down within the SOBP, which corresponds to the region where the tumor is located.

Conversely, at the beam entrance, corresponding in vivo to the healthy tissues, the mean proton energy is far too high for the reaction to be triggered, hence physics would selectively drive and confine the radiobiological enhancement of protontherapy to the ~~tumour~~ volume. This would therefore eliminate one of the most stringent and limiting criteria for clinical viability of BNCT, that is the necessity that the boron carrier be uptaken almost exclusively by the cancer cells.

Besides theoretical considerations on the actual feasibility of such an approach, it was only with the work by Cirrone et al. (2018) [17] that the first experimental proof by radiobiological measurements was provided by showing that irradiation in the presence of an 11B carrier resulted in a significant increase of the effectiveness of a clinical 62-MeV proton beam at inducing cell killing in a prostate cancer cell line (Figure 13.3). The dose-modifying factor at 10 % survival (DMF10) at the middle position of the SOBP was 1.46 ± 0.12 for cells irradiated in the presence of BSH (sodium mercaptododecaborate, Na2B12H11SH) at a concentration of 80 ppm of 11B.

These results were attributed to the expected increase in cell lethality due to the high-LET radiation generated by the p-B reaction as theoretically predicted [118, 39] as well as inferred experimentally [77]. That the putative radiosensitizing action of the p-B reaction is brought about by high-LET alpha particle and, more importantly, that the PBCT could represent a viable approach in the high-energy clinical protontherapy scenario are supported by recent work studying the yield and level of complexity of radiation-induced chromosome aberrations (CAs) in normal mammary epithelial MCF-10A cells irradiated at the clinical proton beam (131.5-164.8 MeV) of the Centro Nazionale di Adroterapia Oncologica (CNAO), Pavia, Italy [13]. In this study, while DU 145 cells cells irradiated in the presence of the 11B carrier BSH underwent increased clonogenic cell death along the SOBP but not at the entrance, MCF-10A cells displayed substantially increased fractions of complex CAs (Figure 13.4), which are a well-documented biomarker of high-LET exposure [5].

To gain further radiobiological evidence on the involvement of the alpha particles in the observed PBCT-based radiosensitization, the degree of CA complexity was examined , here presented as frequencies of chromosomes and breaks per complex exchange (Figure 13.5) and resulted greater in boron-treated cells compared to the samples irradiated by protons alone.

Following the research already performed for BNCT, the 11B carriers being evaluated in PBCT studies are BSH (described above) and BPA (boronophenylalanine, C9H12BNO4), which are cur-

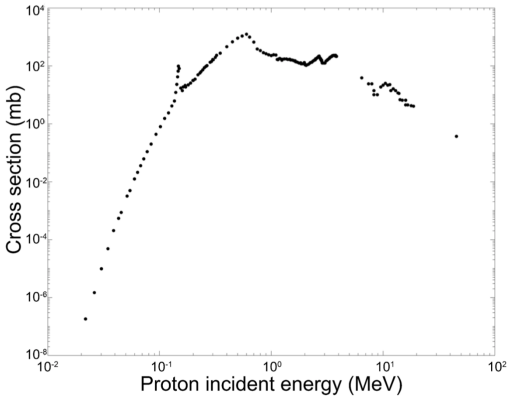

Figure 13.2: Total p-B experimental reaction cross section for the most probable $\alpha 1$ channel decay from EXFOR database

rently being used in the BNCT treatment, with others being under consideration [7] [35]. The PBCT binary approach may be a promising way how to overcome radioresistance of some types of tumors (e.g. hypoxic, such as pancreatic) as a possible alternative to the use of heavier particles (i.e. 12C ions) and the related issues, such as high cost, fragmentation, and/or radiobiological uncertainties connected with late toxicity, while maintaining the inherent benefits of charged particle therapy in the form of healthy tissue sparing. In spite of the accumulating experimental evidence from a radiobiological point of view [17] [13] the exact mechanisms remain to be properly elucidated. The discrepancy between the yield of alpha particles and the observed biological effects remains a source of controversy [88] [2] [41]. Possible explanations currently being investigated include the contribution of concomitant bystander effects. A modality that conceptually stems from PBCT is another binary approach utilizing the reaction between protons and 19F atoms:

$$p + 19F \rightarrow \alpha + 16O \tag{13.4}$$

where the created alpha particle as well as the recoiled 16O atom would lead to increase in cancer cell killing The cross section maximum for the p-F reaction is reached for proton energies of around 2-3 MeV, hence comparable to those of the PBCT, thus offering the same advantage of being effective exclusively in the tumor region, which would be strengthened by the high affinity for tumor cells of the 19F carriers, such as 19FDG (19Fluorodeoxyglucose) whose isotopic analogue, 18FDG, is commonly used during positron emission tomography [9]. Indeed, a possible advantage over PBCT offered by the use of the p-F reaction is its theranostic potential. warranting experimental investigation on this binary approach.

13.4 MONTE CARLO SIMULATIONS STUDIES FOR THE EVALUATION OF THE RADIOBIOLOGICAL EFFECT ENHANCEMENT IN NUCLEAR REACTION-DRIVEN BINARY STRATEGIES

13.4.1 MONTE CARLO SIMULATIONS FOR THE EVALUATION OF RADIOBIOLOGICAL EFFECT IN BNCT

The theoretical advantage of BNCT compared to fast neutrons or, in general, to external beam therapies is the possibility to localize the absorbed dose to the tumor by selective incorporation of 10B. Suitable neutron beams are produced at research nuclear reactors and, recently, by high-current proton accelerators coupled with beryllium or lithium targets [124] Neutron beams of suitable energy

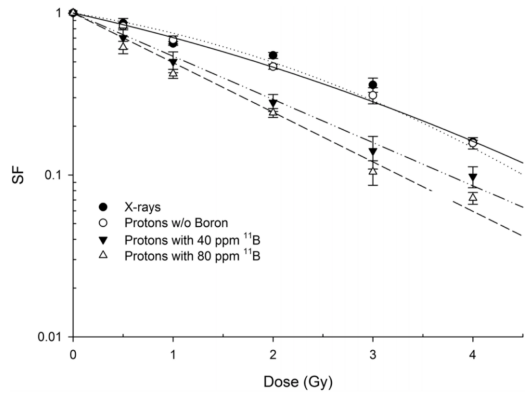

Figure 13.3: Boron-mediated increase in proton irradiation-induced cell death. Clonogenic dose response curves of prostate cancer cells DU145 irradiated with therapeutic protons in the presence or the absence of BSH at mid-SOBP. Data are weighted mean values plus standard error from four independent experiments in the case of proton irradiation in the absence of BSH (open circles) and in the presence of the compound at the highest concentration used (80 ppm, open triangles). X ray-irradiation survival data are also shown for comparison (from Cirrone et al., 2018)

to treat deep-seated ~~tumours~~ are obtained with proper beam shaping assemblies (BSA) [124]. In tissue the neutrons are thermalized, causing the neutron capture reaction 10B(n,α)7Li, producing 7Li (average LET: 190 keV/μm) and alpha particles (160 keV/μm), both of them are of short range and highly damaging to cells. The energy deposition of such particles is limited within a short range (¡10 μm). The remaining contribution to the absorbed dose is given by 580 keV protons (38 keV/μm) ejected following the 14N(n,p)14C reaction, as well as by gamma rays.

Several models have been developed to calculate the absorbed dose of the 10B(n,α)7Li reaction to the nuclei of cells exposed to boron localized to different cellular locations. Although analytical models have been used in the past **[?, ?]**, the Monte Carlo (MC) technique allows a more realistic simulation of cell and tissue geometry [94, 119]. At present, one of the main reference MC codes for BNCT is MCNP [95, 85], which was also used for treatment planning. More recently also PHITS [111, 115] has been used for this purpose [107]. Other general purpose codes, such as Geant4 [28, 82] and Fluka [92, 29] are also used. A comparison between these two codes for BNCT evaluations is reported in [89]. One of the complications in the evaluation of the biological effect following the neutron capture reaction arises from the fact that the tissues targeted with boron are exposed to a mixed radiation field composed by particles with different radiobiological effectiveness [105]. The first strategy to calculate a photon-equivalent dose was proposed by J. Coderre, and it consisted in multiplying each component of the BNCT dose by fixed Relative Biological Effectiveness factors, obtained from radiobiological in vitro and in-vivo studies. The boron component was weighted by a Compound Biological Factor, which considered that different borated formulations may cause different biological effects at the same dose [122]. More recently, it has been demonstrated that this approach, albeit still used in clinical dose reporting, gives artificially high doses in the ~~tumour~~. For this reason, new, more refined models have been proposed. One of these is the Photon Iso-Effective dose model **[?]**. Photon iso-effective dose is defined as the reference dose that produces the same level of cell survival as a given combination of the absorbed dose components of a mixed field BNCT radiation. Dedicated radiobiological experiments provide the dose-response relationship, choosing proper in-vitro or in-vivo models, irradiated with neutrons, neutrons in presence of boron, and reference radiation (typically photons from 60Co or from conventional radiotherapy facility). A mathematical expression quantifying the effect of interest under

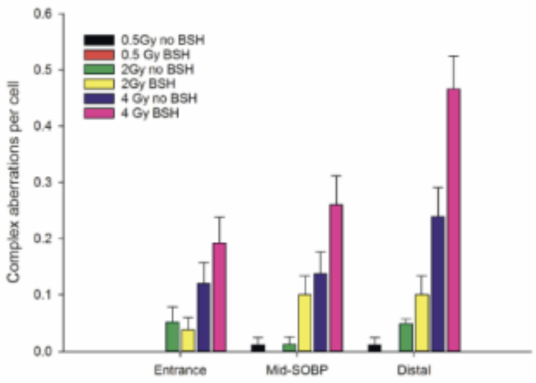

Figure 13.4: Evidence for the action of high-LET radiation generated by clinical protons via the p-B reaction. Frequency of complex CAs as revealed by mFISH as a function of dose and position along the CNAO proton beam SOBP for samples irradiated in the presence or the absence of BSH (from Bláha et al., 2021).

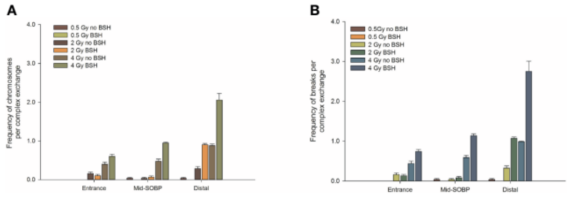

Figure 13.5: Classification of complex exchanges revealed by mFISH analysis in terms of frequencies of number of chromosomes, left panel (A), and number of breaks (right panel (B)) involved; for MCF-10A cells irradiated at entrance, mid, and distal positions of the CNAO clinical proton beam.

both photon and BNCT treatment conditions is necessary, considering also important biological phenomena such as synergism between different radiations and sublethal damage repair. The general expression for the photon isoeffective dose, when both synergism and repair mechanism are considered, and using survival-dose curves obtained with in-vitro experiments is:

$$\alpha_R D_R + G_R(\theta')\beta_R D_R^2 = \sum_{i=1}^{4} \alpha_i D_i + \sum_{i=1}^{4}\sum_{j=1}^{4} G_{ij}(\theta)\sqrt{\beta_i \beta_j} D_i D_j \tag{13.5}$$

In this case, the survival curves are fitted with the generalized Linear Quadratic Model, where α and β are the parameters of the fit for the reference radiation and for the 4 dose components in BNCT and Gij(θ) is the generalized Lea-Catcheside factor which modifies the quadratic term of LQ model by taking into account the probability of repair (θ is the irradiation time) [127]. In [126] the same concept has been described using radiobiological data in-vivo, i.e. using as the effect to be compared the ~~Tumour~~ Control Probability. The Photon Iso-effective dose model has been proved as a more realistic tool to interpret the clinical outcome of patients treatment in different clinical trials in the light of the dosimetry re-calculated with this method. Another strategy that has been proved useful to account the different radiobiological effect of the dose components in the same framework is to base the biological computations on microdosimetric evaluations [100], such as in the Microdosimetric Kinetic Model [96, 7, 98]. Using the MKM the cell survival of various charged particles can be

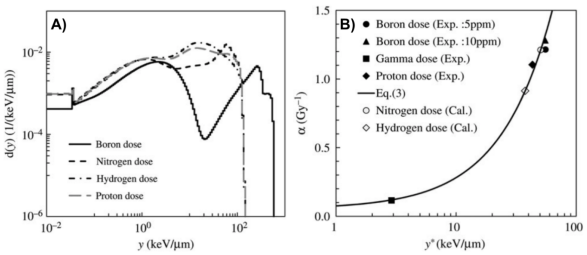

Figure 13.6: A) Probability densities of lineal energy, d(y), for the "boron" (10B(n, α)7Li reaction), "nitrogen" (14N(n, p)14C reaction), "hydrogen" (1H(n, n)1H) and "proton" (1H(n, γ)2H reaction) doses calculated using the microdosimetric function in the PHITS code. B) Measured α value (V79 cells) for each of the four major BNCT dose components as a function of calculated y*. The solid line and open markers denote the relationship between α and y* expected from Equation (mkm-bnct). Figures taken from (Horiguchi et al. 2015).

estimated from the probability of specific energies deposited in subvolumes (domains) of the cell nucleus. Following the LQ formalism (McMahon 2019) the dependence of linear parameter to the microdosimetric spectra can be expressed as

$$\alpha = \alpha_0 + \beta z*_{1D} \tag{13.6}$$

where α_0 is a constant parameter that represents the initial slope of the surviving fraction curve in the limit of LET = 0, β is the quadratic term, assumed to be independent on radiation type and LET, and z*1D is the saturation-corrected dose-mean specific energy of the domain delivered in a single event [14]. While α_0 and β depend on the specific cell line and biological endpoint and are usually phenomenologically determined from survival curves of a low-LET reference radiation, the physical dependence on the radiation is completely encapsulated in $z*_{1D}$, which can be evaluated from

$$z*_{1D} = \frac{\int_0^\infty z_{sat} z f_1(z) dz}{\int_0^\infty z f_1(z) dz} \tag{13.7}$$

where f1(z) is the probability density of energy z deposited by a single deposition event in the domain, and z_{sat} represents the saturation-corrected specific energy defined as follows

$$z_{sat} = \frac{z_0^2}{z}(1 - exp(-z^{"}/z_0^2)) \tag{13.8}$$

where z_0, the saturation coefficient, indicates the energy above which the saturation correction due to the overkilling effect became important. This coefficient is usually determined phenomenologically. A strategy to fix the value of this parameter is given in [14].

MC codes, such as PHITS used in [100], are exploited to determine the microscopic probability f1(z) tracking main particle components (α particles, 7Li, protons and gammas) and secondary electrons that are generated from the neutron capture. An example of these microdosimetric evaluations is reported in Figure [?].

Another microdosimetric approach has been described by Sato et al in [115]. The model is based on the Stochastic Microdosimetric Model [128] and calculates the photon iso-effective BNCT dose considering the intra- and intercellular heterogeneity in 10B distribution. The dose distributions in domains are calculated by the microdosimetric function implemented in PHITS, taking into account the 10B distribution inside cells and the dose rate effect. The SMK model approximates the dose by their mean value, which is important in BNCT because of the higher heterogeneity of the absorbed dose in each cell nucleus due to the stochastic nature of the intercellular 10B distribution.

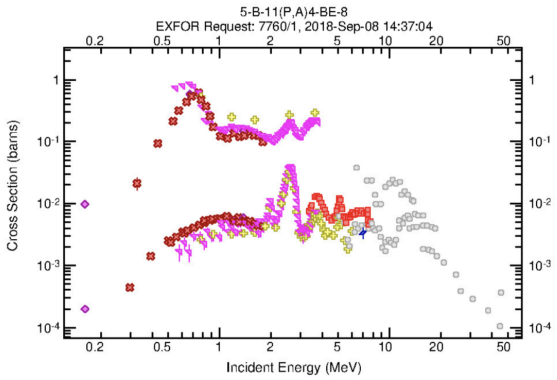

Figure 13.7: Experimental data for the proton-boron fusion reaction (p+11B α3). Data Taken from EXFOR database [113]. Different colours correspond to different experimental campaigns. Two curves can be seen: the one with higher cross section relates to the channel known as $alpha_0$: after the carbon decay the 8Be is created in its fundamental state, while the latter relates to the $alpha_1$ channel, where the beryllium is created in one of its excited states.

13.4.2 MONTE CARLO SIMULATIONS FOR THE EVALUATION OF THE RADIOBIOLOGICAL EFFECT ENHANCEMENT IN PBFT AND NCEPT

Other topics in which MC simulation can be important in the context of radiobiology of binary radiotherapy, is the comprehension of possible mechanisms leading to the enhancement of radiobiological effectiveness the additions of specific radioisotopes (like 11B or 19F) to exploit nuclear reactions triggered by protons on these nuclei [17, 13]. In analogy to the BNCT, these reactions can generate short- range high-LET alpha particles inside the tumors, thereby allowing a highly localized DNA-damaging action. In the case of Proton-Boron Fusion Therapy (PBFT) with 11B, an open question that MC studies have been done to determine the potential role of the p+11B $\rightarrow$ 3 αreactions in the biological enhancement of proton therapy effectiveness. The alpha particles produced in the reaction have an average of 4 MeV kinetic energy and are generated at the point of interaction of the proton and the 11B. The breakup of the boron is not prompt in most cases but usually an unstable 12C is created thanks to a resonance at 675 keV [11]. This nucleus mostly decays α, emitting a first alpha particle, and then the 8Be decays α as well, splitting in another pair of alpha particles. The experimental total cross section for p-11B reaction can be seen in Figure 13.7. The upper curve in the figure clearly shows a resonance pattern: cross section is peaked at proton energies about 675 keV where it reaches a maximum of 0.8 b. This resonance has been highlighted to be exploited for therapeutic use in combination with proton therapy: if boron is located preferentially inside the ~~tumour~~ region, the proton effect would be enhanced where proton energy is low, i.e. at the end of their range inside the ~~tumour~~, improving the therapeutic index [118].

The Monte Carlo simulations studies have been carried out using mainly MCNPX [109, 99] and Geant4 [28, 82]. These studies suggest that this contribution is basically negligible in ordinary clinical irradiation conditions [88, 87] and that the observed enhancement in the radiobiological effectiveness of the PBFT is not related to the alpha particles produced in the abovementioned nuclear reactions. Some studies [114] have proposed an alternative nuclear process for the production of secondary low-energy particles by capturing thermal neutrons produced inside the treatment volume during irradiation in presence of boron-10 and gadolinium-157-based drugs, the Neutron Capture Enhanced Particle Therapy (NCEPT). During particle therapy, such as proton and carbon ion therapy, a fraction of the primary particles undergo non-elastic collisions with nuclei in the target. This

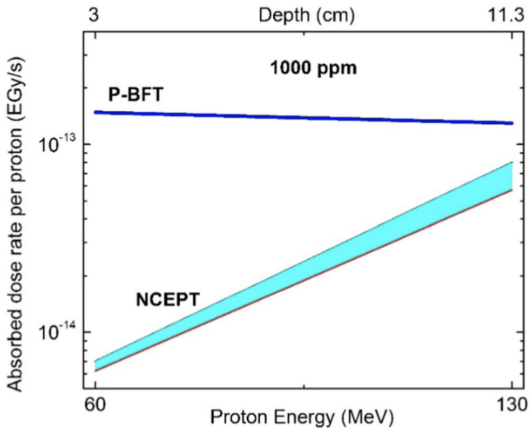

Figure 13.8: A comparative evaluation for NCEPT and PBFT in terms excess dose rate transfer to the tumor site when 1000 ppm of 10B and 11B has been applied respectively. The PBFT has been evaluated through an approximated analytical calculation while, in NCEPT, MCNPX and GEANT4 have been used (defining the lower and upper limits).

results in the production of a range of nuclear fragments at the target site (see also Section Mixed field approaches for evaluating biological impact of projectile and target fragmentation), including a mixture of fast and thermal neutrons. In presence of 10B or 157Gd, the thermal neutron component can exploited through the nuclear reactions:

$$10B + n_{th} \rightarrow [11B]* \rightarrow \alpha + 7Li + \gamma(2.31MeV) \tag{13.9}$$

$$157gd + n_{th} \rightarrow [158Gd]* \rightarrow 158Gd + \gamma + 7.94MeV \tag{13.10}$$

In the case of 10B, the the capture mechanism results in the production of several high LET products and , while, in the case of 157Gd, it results in high energy and the production of low-energy Auger electrons, the latter exploitable for therapeutic effects. A comparative analysis between PBFT and NCEPT has been carried out using both MCNPX and GEANT4 based MC simulations in [117], showing a lower effectiveness of the NCEPT approach, although the excess dose rate from both the methods are about more than several orders of magnitude lower than the dose rate from proton beam therapy even when using 1000 ppm of boron (see figure 13.8).

We remark however that at present, the results of these studies are somewhat contradictory, depending on the used Monte Carlo implementations and assumptions. Furthermore, the majority of them are simply based on the valuation of the yield of alpha particles and other high-LET particles produced from the nuclear reaction, and the corresponding physical dose amplification. A study with a proper biological insight based on a quantitative radiobiological modeling to evaluate the expected increase in the tumor killing effectiveness is still missing. An exception is the study carried out in [88] that also suggest a negligible radiobiological role of the alpha particles in the case of the (p+11B $\rightarrow 3\alpha$) reaction. However, even in this case, the radiobiological evaluations are based on simplified models that account the mixed-field irradiation through the evaluation of a dose-averaged LET that does not properly account for the mixed contribution of proton and low-energy nuclear products (Se also Sections Monte Carlo to link RBE with radiation quality quantities and Mixed field approaches for evaluating biological impact of projectile and target fragmentation).

13.4.3 ISSUES IN THE EVALUATION OF THE BIOLOGICAL EFFECT OF SLOW SECONDARY PARTICLES

At present, the radiobiological models clinically used to predict the RBE in high-LET external beam charged particle therapy are the Local Effect Model (LEM) [116, 91, 93] and the MKM (see previous section). These models often rely on MC codes for the macroscopic tracking of the particle beams, including the secondary particles generated from nuclear fragmentation, coupled with a simplified analytical models for the description of the track structure at a nanometric level (amorphous track models) [90, 104, 9]. One of the basic assumptions of this approach is the so-called track-segment condition, in which the particles are assumed to have enough energy to completely cross the cell nucleus, assumed to be the radiosensitive part of the cell, without a significant change in kinetic energy. In general, the track-segment condition is not verified in a binary radiotherapy process, due to the presence of very low-energy particles in the mixed field. This is particularly relevant in the case of alpha particles generated in both neutron and proton capture nuclear reactions. The most part of the energy of these particles is released in the same cell where they are produced, and often only in a subvolume of the cell. An approximate correction to account the partial cell nucleus crossing of slow particles can be applied through a corrective weighting in the mixed field formalism for the LQ parameters:

$$\alpha = \sum_i \alpha_i D_i \Delta L_i / \sum_i D_i \Delta L_i \tag{13.11}$$

$$\sqrt{\beta} = \sum_i \sqrt{\beta_i} D_i \Delta L_i / \sum_i D_i \Delta L_i \tag{13.12}$$

Where the index i is the track index, Di is the dose contribution of the track, (α_i,β_i) are the LQ parameters of the track and ΔLi is the length of the track in the cell nucleus. The high local energy density found in the track results in complex DNA damage. In principle, a more precise evaluation of these effects should rely on the complete spatial knowledge of the track structure. MC codes capable of simulating the track structure down to a nano-scale level can also be used for these evaluations. Examples of these codes are Geant4-DNA [102, 112] or Trax [108, 84]. Due to their relevance for the evaluation of radiobiological effects in the case of complex mixed radiation fields, attempts to include nanometric evaluations in treatment planning simulation and optimization have also been made. An example of these studies is reported in [86], where, exploiting the Geant4-DNA code, a treatment plan has been optimized using a cost function based on nanodosimetric quantities scored in cell sub-volumes, such as the ionization cluster size, the probability distribution P(ν,Q) of the number of ionizations per particle of quality radiation Q, and other derived quantities.

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