Targeting Strategies against Radioresistant Tumors

Subjects: Cell Biology

Contributor: Fabio Busato , Badr El Khouzai , Maddalena Mognato

A radiosensitizer is a drug that makes cancer cells more sensitive to radiation therapy. These compounds apparently promote the scavenging of free radicals produced by radiation damage on the molecular level. Radiation therapy generally affects DNA; mainly, it leads to DNA DSBs. Therefore, many radiosensitizing agents have been formulated to target the clinically developed DNA DSB repair pathways. Other agents instead target different pathways, e.g., DNA-PKcs, ATM, and ATR signaling cascades. More than seven PARP inhibitors, for example, are currently being developed considering their role in DNA repair, especially for tumors with DNA repair defects, such as BRCA mutation, because of their synthetic lethality.

radiobiology radiation therapy radioresistance radiosensitizers

1. Hypoxic Cell Radiosensitizers

1.1. Hyperbaric Oxygen

Hyperbaric oxygen (HBO) therapy is the inhalation of 100% oxygen at elevated pressure >1.5 atmospheres absolute (ATA; 150 kPa), typically 2–3 ATA (200–300 kPa). The physiological effects of HBO include short-term effects such as vasoconstriction and enhanced oxygen delivery, edema reduction, and phagocyte activation [1].

Most tumors contain oxygen-deprived compartments. The sterilization of hypoxic tumor cells requires a three-times higher radiation dose than cells with normal oxygen tension. HBO therapy is an effective approach to cope with the phenomenon of hypoxia by increasing the oxygen load of the tumor and, therefore, enhancing the response to irradiation [2]. Some studies have shown an increase in the 5-year survival rate of patients with cancers of the uterine cervix and head and neck.

1.2. Carbogen

The idea of improving the oxygenation of tumors by breathing highly oxygenated air was recently revived by experiments in which participants breathe carbogen, a mixture of 95% oxygen and 5% carbon dioxide that does not produce the vasoconstriction associated with breathing 100% oxygen. Breathing carbon at atmospheric pressure is an attempt to overcome chronic (diffusion-limited) hypoxia by much simpler means than using hyperbaric chambers [3].

1.3. Nicotinamide

Nicotinamide is an amide of vitamin B3. Acute hypoxia within tumors arises from the intermittent closure of blood vessels, resulting in fluctuations in the tumor's microcirculation. Nicotinamide overcomes acute hypoxia by reducing these changes in the microcirculation. Furthermore, when nicotinamide is combined with carbogen, additional tumor sensitivity to radiation has been demonstrated, with overall enhancement ratios of between 1.8 and 2.1 in animal models using a clinically relevant dose schedule of 2 Gy day. It has, therefore, been proposed that the combination of carbogen and nicotinamide provides the optimal means of overcoming tumor hypoxia [4]. The cellular mechanism of action for a representative molecule from this class was found to be the G1 arrest, accompanied by the activation of p53/p21 DNA-damage signaling pathways, and most complexes induce high levels of apoptosis in low micromolar doses.

1.4. Metronidazole and Its Analogs

Knowledge of the oxygen effect led to the development of compounds that mimic the radiosensitizing property of oxygen. The radiosensitizing abilities of hypoxic cell sensitizers have been observed to correlate with electron affinity [5]. Metronidazole and its analogs, such as misonidazole, etanidazole, and nimorazole, have been found to be effective in sensitizing hypoxic tumor cells [6].

The "oxygen fixation" hypothesis was proposed to explain the mechanism of action for this class of sensitizers [7]. They fix the radiation damage by preventing the chemical restitution of free radicals. Misonidazole has been observed to deplete sulfhydryl groups in cells, inhibit glycolysis, and repair potentially lethal radiation-induced cellular damage [8].

Nimorazole is a member of the same structural class as metronidazole but is less toxic, allowing for higher doses. As an oxygen mimetic, nimorazole induces free radical formation and is able to sensitize hypoxic cells to the cytotoxic effects of IR, thus preventing DNA repair and enhancing damage to DNA strands. A phase III study of nimorazole versus placebo in subjects with squamous cell carcinoma of the supraglottic larynx and pharynx demonstrated a statistically significant difference in improvement in the loco-regional control at 5 years post-treatment [9].

1.5. Hypoxic Cell Cytotoxic Agents

Hypoxic cell cytotoxic agents include mitomycin-C and tirapazamine. Mitomycin-c is a bioreductive alkylating agent that has been studied in pancreatic, anal, and head and neck cancer. Tirapazamine is another bioreductive agent that is preferentially cytotoxic to hypoxic cells in vitro. It differs from oxygen-mimetic sensitizers, in that it requires metabolic activation and enhancement, as seen when this agent is given prior to or after RT [10]. Mitomycin-C, combined with radiation therapy, remains the standard-of-care therapy for anal carcinoma based on multiple clinical studies.

2. Radiosensitizing Chemotherapy Agents

2.1. Fluoropyrimidines

5-fluorouracil (5-FU) and fluorodeoxyuridine (FdUrd) are analogs of uracil and deoxyuridine, respectively. They are thymidylate synthase (TS) inhibitors: interrupting the action of this enzyme blocks the synthesis of pyrimidine thymidylate (dTMP), which is a nucleotide required for DNA replication Randomized trials have demonstrated local control and survival advantages using systemic 5-FU and radiation compared with radiation alone in patients with rectal cancer, esophageal cancer, and pancreatic cancer [11]. 5-FU and FdUrd, through their metabolites, lead to cell cycle redistribution, DNA fragmentation, and cell death [12].

Capecitabine is an oral prodrug of 5-FU. It is converted to its cytotoxic form in three enzymatic steps, the last of which is mediated by thymidine phosphorylase. One of the potential advantages of this mechanism for increasing tumor cytotoxicity is that thymidine phosphorylase is overexpressed in tumor tissues. Interestingly, radiation has been shown to stimulate the expression of thymidine phosphorylase, which provides a further rationale [13].

2.2. Gemcitabine

Gemcitabine is a nucleoside analog that mediates its antitumor effects by promoting apoptosis in malignant cells undergoing DNA synthesis [14]. It has demonstrated effectiveness as a single agent against solid tumors, including pancreatic cancer, non-small-cell lung cancer, head and neck cancer, and breast cancer. The mechanism by which gemcitabine radiosensitizes tumor cells is not yet clear. Preliminary studies indicate that the observed radiosensitization is not associated with either an increase in the radiation-induced DNA double-strand breaks or with a slowing of DNA double-strand break repair. This suggests that radiosensitization by gemcitabine is unlike that produced by fluoropyrimidines and thymidine analogs. The relationships between gemcitabine radiosensitization and DNA incorporation, alterations in DNA synthesis, and alteration in cell cycle kinetics remain to be investigated. In addition, it would be logical to investigate the role of apoptosis in gemcitabine-mediated radiosensitization, since this mechanism of cell death has been shown to be the pathway by which the drug exerts its cytotoxic action, at least in lymphoid cell lines [15].

2.3. Taxanes

This group of anticancer agents works by disrupting microtubule function, thus inhibiting cell division [16]. Paclitaxel is the prototype of taxanes. Docetaxel is another agent in this group. Paclitaxel may be the most efficacious single chemotherapy agent for head and neck cancer, with a 40% response rate for patients with recurrent disease. As it is possible to achieve durable control with radiotherapy on locally advanced head and neck cancers in only a minority of cases; chemotherapy drugs, such as paclitaxel, are used with radiotherapy in an attempt to improve tumor control. Paclitaxel stabilizes microtubules and leads to the accumulation of cells in the G2/mitosis phase of the cell cycle, which is a necessary condition for its antitumor effect, and it is the phase with the greatest relative radiosensitivity. Paclitaxel has been shown to be a radiosensitizer in vitro for some, but not all, cell lines studied [17].

2.4. Platinum-Based Drugs

This group of compounds, distinguished from most others by its metallic element base, has come to be recognized as one of the most potent chemotherapies available to date. Cisplatin (cis-diamminedichloroplatinum II), which is a prototype drug, has been acknowledged to be a potent radiosensitizer for many years and has had a significant role in clinical practice to date. Preclinical work performed using murine models by Rosenberg et al. in the late 1960s showed that cisplatin is an effective antitumor chemotherapy. Subsequent efforts have shown that its primary mechanism of inhibition for tumor growth appears to involve the inhibition of DNA synthesis. Another secondary mechanism includes the inhibition of transcription elongation by DNA interstrand cross-links [18]. Work on nonmammalian systems first demonstrated the radiosensitizing abilities of platinum-based compounds. This was confirmed in several mammalian systems as well [19] (Szumiel 1976).

This makes inherent sense because these platinum compounds have a high electron affinity and react preferentially with hydrated electrons. The exact mechanism of the increased cell death seen with combinations of IR and platinum drugs is not known for certain; however, the evidence would seem to point to the inhibition of PLDR49 and to the radiosensitization of hypoxic tumor cells [20]. Cisplatin-free-radical-mediated sensitization may involve the ability to scavenge free electrons formed by the interaction between radiation and DNA. The reduction in platinum moiety may serve to stabilize DNA damage that would otherwise be repairable.

Carboplatin, a second-generation platinum compound with a different toxicity profile, has also been studied as a radiosensitizer [21]. Its potential efficacy as a radiosensitizer has allowed for its incorporation into regimens used in several randomized trials. Interest exists in combining radiation with other platinum analogs, including oxaliplatin, as well as orally administered compounds such as satraplatin.

2.5. Temozolomide

Temozolomide, a relatively new drug, is a second-generation alkylating agent, which is orally administered, is readily bioavailable, and demonstrates broad-spectrum activity in a variety of difficult-to-treat malignancies. It is unique in its ability to cross the blood–brain barrier (about 30% to 40% of plasma concentration found in CSF). Radiosensitization appears to occur via the inhibition of DNA repair, leading to an increase in mitotic catastrophe. It has proven efficacy as a first-line therapy for glioblastoma multiforme (GBM) patients in conjunction with RT, based on a randomized phase III clinical study demonstrating its survival benefit [22]. Temozolomide spontaneously converts into the reactive methylating agent MTIC and transfers methyl groups to DNA, the most important one being at the O6 position of guanine, an important site for DNA alkylation [23]. The MGMT gene encodes a DNA repair protein that removes the alkyl group from the O6 position of guanine, and high MGMT activity levels abrogate the effectiveness of alkylating agents. In vitro, temozolomide enhances the radiation response most effectively in MGMT-negative glioblastomas, likely due to decreased double-strand DNA repair capacity and increased DNA double-strand break damage, which occurs when a combination of temozolomide and radiation therapy is administered.

2.6. Histone Deacetylase Inhibitors (HDACi)

The clinical effectiveness of histone deacetylase inhibitors (HDACis) as radiosensitizers has been demonstrated in vitro in cancer cells [24][25]. Even though HDACis have been validated in clinical trials and approved for cancer patients by the FDA for the treatment of cutaneous/peripheral T-cell lymphoma and multiple myeloma, many other HDACis are under investigation in clinical trials [26][27]. The evidence of HDACis being radiosensitizers in clinical practice is limited, and more experimentation is required to determine their potential [28].

2.7. DNA Repair and Cell Cycle Inhibitors

Several drugs affecting DNA repair mechanisms and the progression of the cell cycle have demonstrated preclinical activity, and for this reason, they have promising clinical potential. Poly (ADP-ribose) polymerase (PARP) is a class of proteins with an essential role in DNA repair, detecting SSBs, recruiting DNA repair proteins, and ultimately stabilizing DNA [29]. The activity of PARP proteins is enhanced in many tumors; therefore, PARP inhibitors, which work by binding the SSB site and blocking the recruitment of repair proteins, are considered a promising strategy in combination with RT to enhance the efficacy of oncological cures [30]. The researchers can consider the combination PARPi + RT feasible and usually safe, with hematological toxicities being the most commonly reported adverse events [31]. However, the clinical efficacy of this combination, as well as of other therapies such as immunotherapy, remains to be determined [32].

The PI3-kinase-like family of protein kinases includes DNA-PKcs (DNA-dependent protein kinase catalytic subunit), ATM (ataxia–telengiectasia mutated), and ATR (ataxia–telangiectasia and Rad3-related). This family recruits DNA repair proteins and activates cell cycle checkpoints in response to DSBs [33]. Silencing DNA-PKcs leads to increased radiosensitivity and DSBs [34][35]. Preclinical experiments have demonstrated that DNA-PKcs inhibitors increase the sensitivity of in vitro gastric cancer cells and that they can be effective and tolerable when associated with local RT [36][37].

The serine/threonine kinase ATM is activated by DNA DSBs to orchestrate the cellular response to IR. ATM inhibitors were studied in a phase I trial, which closed early due to a non-optimal pharmacokinetics profile [38]. Even though the development of that drug has been halted, the ATM pathway still represents an attractive therapeutic target, and second-generation ATM inhibitors are being investigated (ClinicalTrials.gov NCT04882917). Not only have these drugs been tested in monotherapy but also in combination. Indeed, the dual inhibition of DNA-PKcs and ATR represents a promising approach, concomitant with radiation [39]. Both ATR and its major downstream effector, checkpoint kinase 1 (CHK1) inhibitor, have been studied, and the results of phase I and II clinical trials have shown a low safety and efficacy profile, despite the promising preclinical studies [40].

Next-generation drugs with reduced toxicity and the possibility of selecting patients who benefit the most are future goals for this class of drugs.

3. Nanoparticles (NPs)

NPs usually have a simple structure composed of a core, a shell, and a surface. In the case of radiosensitizing NPs, the core is usually made of high-Z materials, such as silver, lanthanides, and (most extensively) gold, to exploit the increased photon absorption. The shell, which is chemically or physically bound to the core, acts as a base on which surface molecules (which sometimes include active agents) are anchored or bound with or without spacers. However, the high-Z elements can also be chelated by ligands present on the surface or inside the nanoparticle. The surface molecules usually consist of site-, tissue-, cell-, and/or receptor-specific molecules (targeting units) [41].

4. Immunomodulators

Immunotherapy has recently emerged as one of the major advances in prolonging overall survival in several cancers. It utilizes the patient's immune system to induce tumor cell killing and can be either active or passive in nature. Active immunotherapy directly targets tumor cells and includes antibody therapy and chimeric antigen receptor T cell therapy. In contrast, passive immunotherapy enhances the ability of the immune system to eradicate tumor cells and includes immune checkpoint inhibitors (anti PD/PDL-1 and anti-CTLA-4) and cytokines [42]. The biological ways through which radiotherapy can stimulate the immune system and, thus, work synergically are: (i) by killing tumor cells and thus promoting the release of tumor antigens and the activation of cytotoxic T cells; (ii) by stimulating antigen-presenting cells [43]; (iii) by increasing MHC-1 expression [44]; and (iv) by releasing damage-associated molecular patterns (DAMPs) that can activate the immune system against tumor cells [45].

Several clinical trials have demonstrated the efficacy of radiotherapy combined with immune checkpoint inhibitors in real life: the PACIFIC trial and the Keynote 001 in NSCLC, Keynote-522 in triple-negative breast cancer, and many other practice-changing trials. Efforts are ongoing to better understand the detailed interaction between radiation therapy and the immune environment (sequencing, doses, timing) to increase their efficacy among cancer cures. One of these efforts includes making the abscopal effect more frequent in daily practice, as only 46 clinical cases using RT alone have been reported from 1969 to 2014 [46]. This is described as the ability of localized radiation to induce an antitumor response throughout the body in sites that were not subjected to targeted radiation [47]. Even though the abscopal effect was first described in 1953 [48], it has recently obtained great attention as a way to increase radiation therapy efficacy in combination with immune checkpoint inhibitors, as these drugs are revolutionizing cancer treatments and patient prognoses [49].

5. Radiation Therapy beyond Photons: Protons and Carbon Ions

Historically, radiation therapy has been associated with photons. Today, heavy ion accelerators, mostly carbon ions and proton ones, are being studied for their properties and, specifically, for their effects on radioresistant tumors. When compared to photons, carbon ions and protons show an inverted depth dose profile. Their energy deposition follows the Bragg curve, where low levels of energy are delivered to the normal tissue in the entrance channel and the maximum energy levels are delivered in the spread-out Bragg peak inside the tumor tissue, where the particles

stop. Due to the steep energy drop after the Bragg peak, the normal tissue and the organs at risk beyond the tumor volume can be spared from radiation exposure [50]. Carbon ions also exhibit higher linear energy transfer (LET) than photons and protons [51]. This leads to a higher RBE, where damage caused by carbon ions is clustered in the DNA, overwhelming the cellular repair systems [52].

Thanks to its promising properties, heavy ion radiotherapy is being studied in several clinical trials, both for primary tumors (prostate cancer, bone cancer, sarcomas, and head and neck cancers) and recurrent tumors [53][54]. A review of head and neck cancers showed that, for malignant mucosal melanoma, the 5-year OS is higher with carbon ions than with photons (44% versus 25%), and for sino-nasal and paranasal cancers, the 5-year local control rate is higher with protons than with photons (88% versus 66%) [55].

In addition to its physical characteristics, high-LET radiation has been shown to induce complex DNA damage by inactivating hypoxic cells via direct ionization without the radiolysis of water [56]. Moreover, they perform increased immunogenicity in radiation-induced cell death compared to photon radiation through a variety of mechanisms, thus leading to a hypothesized advantage in the setting of combined immunotherapy [57] (Helm 2018). In mouse studies, carbon-ion irradiation correlates with stronger immune activation when paired with dendritic cell injection. Combining carbon-ion therapy with immunotherapy demonstrates increased antitumor immunity and reduces the number of metastases compared with RT or immunotherapy alone, or in combination with photons [58][59].

5.1. SBRT

Conventional normo-fractionated radiotherapy (2 Gy/fraction) is ineffective for some tumors such as renal cell carcinoma (RCC), and they have been called “radioresistant” for this reason in the past few years. The recent use of SBRT (stereotactic body radiation therapy), thanks to the use of higher doses per fraction, has overcome some of these radioresistance scenarios. In the clinic, SBRT has been used to treat RCC, showing high local control and low toxicity rates. These data can be explained by the low α/β-ratio exhibited by RCC: low alpha–beta tumors are classically radioresistant to standard fractionation regimens and benefit from dose escalation using hypofractionation, which consists of delivering higher doses per fraction [60]. Using higher radiation doses, alternative cell death mechanisms, such as ceramide-induced apoptosis, have become more relevant in RCC cells [61]. Molecularly, a secretory form of acid sphingomyelinase is translocated to the extracellular leaflet of the cell membrane and transforms sphingomyelin into the pro-apoptotic protein ceramide via enzymatic hydrolysis [62]. The fact that acid sphingomyelinase, especially its secretory form, is predominantly expressed in endothelial cells explains the high sensitivity of endothelium to ceramide-induced apoptosis in RCC, a highly vascularized tumor. In vivo studies comparing sphingomyelinase-knockout mice with wildtype mice demonstrated that sphingomyelinase-knockout mice exhibited an increased threshold to irradiation-induced endothelial apoptosis and were resistant to single-dose RT with 20 Gy. The importance of sphingomyelinase activity regarding tumor response after SBRT was further underlined in the study by Sathishkumar et al.: 75% of the patients with partial or complete tumor response after SBRT exhibited significantly increased serum ceramide and serum sphingomyelinase levels, whereas none of the non-responders had increased levels of these proteins [63]. A systematic review showed that ablative SBRT can be effectively used to treat RCC with high local control rates (84–100%) [64].

5.2. FLASH Radiotherapy

Favaudon et al. [65] discovered that pulsed and ultrahigh-dose-rate irradiation (≥40 Gy/s, FLASH) causes less damage to the healthy lung than conventional radiotherapy (≤0.03 Gy/s, CONV) in mouse models while preserving efficacy against tumor cells. They called this technology FLASH radiotherapy, and it has two major advantages: a low toxicity rate in irradiated healthy tissues, thus providing a chance to increase the dose to tumor targets, and a short delivery time; e.g., the first patient affected by cutaneous T-cell lymphoma was irradiated in 90 ms [66]. FLASH RT represents a new and promising field to fight cancer. Higher doses in the tumor target will hopefully increase efficacy for radioresistant tumors. Once better know its standard dosimetry, 3D treatment planning, volumetric image guidance, and motion management, more clinical trials will start to enroll patients [67].

6. Diet

Short-term fasting and calorie restriction have been associated with the mechanisms of radioresistance. Klement et al. [68] summarized them according to the 5Rs: (a) DNA Repair: short-term fasting likely selectively improves DSB repair in normal cells but not cancer cells (mTOR inhibition), thus favoring normal tissue repair and cancer cell death; (b) Repopulation (cell proliferation occurring during the course of fractionated RT in both tumors and normal tissue): calories restriction in rodents reduces IGF-1/insulin–PI3K–Akt–mTor signaling, which has been shown to be correlated with significant tumor growth delay [69]; (c) Redistribution: fasting seems to promote cell cycle progression, M phase accumulation, and energy expenditure, and in this way, it renders such cells synthetically vulnerable to the combination of nutrient restriction with RT or chemotherapy [70]; (d) Reoxygenation: calorie restriction downregulates VEGF [71], thus decreasing areas of hypoxia in tumors [72].

Though there is a large amount of preclinical data, further clinical data are necessary to establish the effects of calorie restriction and intermittent fasting on irradiated cancer cells. In accordance, recent ASCO guidelines state that, currently, there is insufficient evidence to recommend for or against dietary interventions such as ketogenic or low-carbohydrate diets, low-fat diets, functional foods, or fasting to improve outcomes related to QoL, treatment toxicity, or cancer control.

References

1. Al-Waili, N.S.; Butler, G.J.; Beale, J.; Hamilton, R.W.; Lee, B.Y.; Lucas, P. Hyperbaric Oxygen and Malignancies: A Potential Role in Radiotherapy, Chemotherapy, Tumor Surgery and Phototherapy. Med. Sci. Monit. 2005, 11, RA279–RA289.
2. Churchill-Davidson, I.; Foster, C.A.; Wiernik, G.; Collins, C.D.; Pizey, N.C.; Skeggs, D.B.; Purser, P.R. The Place of Oxygen in Radiotherapy. Br. J. Radiol. 1966, 39, 321–331.
3. Hall, E.; Cox, J. Physical and Biological Basis of Radiation Therapy. In Radiation Oncology: Rationale, Technique, Results; Cox, J.D., Ang, K.K., Eds.; Mosby: St. Louis, MO, USA, 2003; pp.

3–62.

4. Hoskin, P.J.; Saunders, M.I.; Phillips, H.; Cladd, H.; Powell, M.E.; Goodchild, K.; Stratford, M.R.; Rojas, A. Carbogen and Nicotinamide in the Treatment of Bladder Cancer with Radical Radiotherapy. Br. J. Cancer 1997, 76, 260–263.
5. Adams, G.E.; Dewey, D.L. Hydrated electrons and radiobiological sensitisation. Biochem. Biophys. Res. Commun. 1963, 12, 473–477.
6. Asquith, J.C.; Foster, J.L.; Willson, R.L.; Ings, R.; McFadzean, J.A. Metronidazole (“Flagyl”). A Radiosensitizer of Hypoxic Cells. Br. J. Radiol. 1974, 47, 474–481.
7. Alper, T. The Modification of Damage Caused by Primary Ionization of Biological Targets. Radiat. Res. 1956, 5, 573–586.
8. Guichard, M.; Malaise, E.P. Radiosensitizing Effects of Misonidazole and SR 2508 on a Human Melanoma Transplanted in Nude Mice: Influence on Repair of Potentially Lethal Damage. Int. J. Radiat. Oncol. Biol. Phys. 1982, 8, 465–468.
9. Overgaard, J.; Hansen, H.S.; Overgaard, M.; Bastholt, L.; Berthelsen, A.; Specht, L.; Lindeløv, B.; Jørgensen, K. A Randomized Double-Blind Phase III Study of Nimorazole as a Hypoxic Radiosensitizer of Primary Radiotherapy in Supraglottic Larynx and Pharynx Carcinoma. Results of the Danish Head and Neck Cancer Study (DAHANCA) Protocol 5-85. Radiother. Oncol. 1998, 46, 135–146.
10. Kovacs, M.S.; Hocking, D.J.; Evans, J.W.; Siim, B.G.; Wouters, B.G.; Brown, J.M. Cisplatin Anti-Tumour Potentiation by Tirapazamine Results from a Hypoxia-Dependent Cellular Sensitization to Cisplatin. Br. J. Cancer 1999, 80, 1245–1251.
11. Pu, A.T.; Robertson, J.M.; Lawrence, T.S. Current Status of Radiation Sensitization by Fluoropyrimidines. Oncology (Williston Park) 1995, 9, 707–714; discussion 714, 717–718, 721.
12. Pinedo, H.M.; Peters, G.F. Fluorouracil: Biochemistry and Pharmacology. J. Clin. Oncol. 1988, 6, 1653–1664.
13. Hasegawa, K.; Okamoto, H.; Kawamura, K.; Kato, R.; Kobayashi, Y.; Sekiya, T.; Udagawa, Y. The Effect of Chemotherapy or Radiotherapy on Thymidine Phosphorylase and Dihydropyrimidine Dehydrogenase Expression in Cancer of the Uterine Cervix. Eur. J. Obstet. Gynecol. Reprod. Biol. 2012, 163, 67–70.
14. de Sousa Cavalcante, L.; Monteiro, G. Gemcitabine: Metabolism and Molecular Mechanisms of Action, Sensitivity and Chemoresistance in Pancreatic Cancer. Eur. J. Pharmacol. 2014, 741, 8–16.
15. Huang, P.; Plunkett, W. Fludarabine- and Gemcitabine-Induced Apoptosis: Incorporation of Analogs into DNA Is a Critical Event. Cancer Chemother. Pharmacol. 1995, 36, 181–188.

16. Abal, M.; Andreu, J.M.; Barasoain, I. Taxanes: Microtubule and Centrosome Targets, and Cell Cycle Dependent Mechanisms of Action. Curr. Cancer Drug Targets 2003, 3, 193–203.
17. Rosenthal, D.I.; Carbone, D.P. Taxol plus Radiation for Head and Neck Cancer. J. Infus. Chemother. 1995, 5, 46–54.
18. Rosenberg, B.; VanCamp, L.; Trosko, J.E.; Mansour, V.H. Platinum Compounds: A New Class of Potent Antitumour Agents. Nature 1969, 222, 385–386.
19. Szumiel, I.; Nias, A.H. The Effect of Combined Treatment with a Platinum Complex and Ionizing Radiation on Chinese Hamster Ovary Cells in Vitro. Br. J. Cancer 1976, 33, 450–458.
20. Stratford, I.J.; Williamson, C.; Adams, G.E. Combination Studies with Misonidazole and a Cis-Platinum Complex: Cytotoxicity and Radiosensitization in Vitro. Br. J. Cancer 1980, 41, 517–522.
21. O'Hara, J.A.; Douple, E.B.; Richmond, R.C. Enhancement of Radiation-Induced Cell Kill by Platinum Complexes (Carboplatin and Iproplatin) in V79 Cells. Int. J. Radiat. Oncol. Biol. Phys. 1986, 12, 1419–1422.
22. Stupp, R.; Mason, W.P.; van den Bent, M.J.; Weller, M.; Fisher, B.; Taphoorn, M.J.B.; Belanger, K.; Brandes, A.A.; Marosi, C.; Bogdahn, U.; et al. Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma. N. Engl. J. Med. 2005, 352, 987–996.
23. Hegi, M.E.; Diserens, A.-C.; Gorlia, T.; Hamou, M.-F.; de Tribolet, N.; Weller, M.; Kros, J.M.; Hainfellner, J.A.; Mason, W.; Mariani, L.; et al. MGMT Gene Silencing and Benefit from Temozolomide in Glioblastoma. N. Engl. J. Med. 2005, 352, 997–1003.
24. Camphausen, K.; Tofilon, P.J. Inhibition of Histone Deacetylation: A Strategy for Tumor Radiosensitization. J. Clin. Oncol. 2007, 25, 4051–4056.
25. Johnson, A.M.; Bennett, P.V.; Sanidad, K.Z.; Hoang, A.; Jardine, J.H.; Keszenman, D.J.; Wilson, P.F. Evaluation of Histone Deacetylase Inhibitors as Radiosensitizers for Proton and Light Ion Radiotherapy. Front. Oncol. 2021, 11, 735940.
26. Cappellacci, L.; Perinelli, D.R.; Maggi, F.; Grifantini, M.; Petrelli, R. Recent Progress in Histone Deacetylase Inhibitors as Anticancer Agents. Curr. Med. Chem. 2020, 27, 2449–2493.
27. Bondarev, A.D.; Attwood, M.M.; Jonsson, J.; Chubarev, V.N.; Tarasov, V.V.; Schiöth, H.B. Recent Developments of HDAC Inhibitors: Emerging Indications and Novel Molecules. Br. J. Clin. Pharmacol. 2021, 87, 4577–4597.
28. Antrobus, J.; Parsons, J.L. Histone Deacetylases and Their Potential as Targets to Enhance Tumour Radiosensitisation. Radiation 2022, 2, 149–167.
29. Patel, A.G.; Sarkaria, J.N.; Kaufmann, S.H. Nonhomologous End Joining Drives Poly(ADP-Ribose) Polymerase (PARP) Inhibitor Lethality in Homologous Recombination-Deficient Cells. Proc. Natl. Acad. Sci. USA 2011, 108, 3406–3411.

30. Lesueur, P.; Chevalier, F.; Austry, J.-B.; Waissi, W.; Burckel, H.; Noël, G.; Habrand, J.-L.; Saintigny, Y.; Joly, F. Poly-(ADP-Ribose)-Polymerase Inhibitors as Radiosensitizers: A Systematic Review of Pre-Clinical and Clinical Human Studies. Oncotarget 2017, 8, 69105–69124.
31. Barcellini, A.; Loap, P.; Murata, K.; Villa, R.; Kirova, Y.; Okonogi, N.; Orlandi, E. PARP Inhibitors in Combination with Radiotherapy: To Do or Not to Do? Cancers 2021, 13, 5380.
32. Césaire, M.; Thariat, J.; Candéias, S.M.; Stefan, D.; Saintigny, Y.; Chevalier, F. Combining PARP Inhibition, Radiation, and Immunotherapy: A Possible Strategy to Improve the Treatment of Cancer? Int. J. Mol. Sci. 2018, 19, 3793.
33. Blackford, A.N.; Jackson, S.P. ATM, ATR, and DNA-PK: The Trinity at the Heart of the DNA Damage Response. Mol. Cell 2017, 66, 801–817.
34. Peng, Y.; Zhang, Q.; Nagasawa, H.; Okayasu, R.; Liber, H.L.; Bedford, J.S. Silencing Expression of the Catalytic Subunit of DNA-Dependent Protein Kinase by Small Interfering RNA Sensitizes Human Cells for Radiation-Induced Chromosome Damage, Cell Killing, and Mutation. Cancer Res. 2002, 62, 6400–6404.
35. Toulany, M.; Kehlbach, R.; Florczak, U.; Sak, A.; Wang, S.; Chen, J.; Lobrich, M.; Rodemann, H.P. Targeting of AKT1 Enhances Radiation Toxicity of Human Tumor Cells by Inhibiting DNA-PKcs-Dependent DNA Double-Strand Break Repair. Mol. Cancer Ther. 2008, 7, 1772–1781.
36. Geng, W.; Tian, D.; Wang, Q.; Shan, S.; Zhou, J.; Xu, W.; Shan, H. DNA-PKcs Inhibitor Increases the Sensitivity of Gastric Cancer Cells to Radiotherapy. Oncol. Rep. 2019, 42, 561–570.
37. Willoughby, C.E.; Jiang, Y.; Thomas, H.D.; Willmore, E.; Kyle, S.; Wittner, A.; Phillips, N.; Zhao, Y.; Tudhope, S.J.; Prendergast, L.; et al. Selective DNA-PKcs Inhibition Extends the Therapeutic Index of Localized Radiotherapy and Chemotherapy. J. Clin. Investig. 2020, 130, 258–271.
38. Waqar, S.N.; Robinson, C.; Olszanski, A.J.; Spira, A.; Hackmaster, M.; Lucas, L.; Sponton, L.; Jin, H.; Hering, U.; Cronier, D.; et al. Phase I Trial of ATM Inhibitor M3541 in Combination with Palliative Radiotherapy in Patients with Solid Tumors. Investig. New Drugs 2022, 40, 596–605.
39. Hafsi, H.; Dillon, M.T.; Barker, H.E.; Kyula, J.N.; Schick, U.; Paget, J.T.; Smith, H.G.; Pedersen, M.; McLaughlin, M.; Harrington, K.J. Combined ATR and DNA-PK Inhibition Radiosensitizes Tumor Cells Independently of Their P53 Status. Front. Oncol. 2018, 8, 245.
40. Qiu, Z.; Oleinick, N.L.; Zhang, J. ATR/CHK1 Inhibitors and Cancer Therapy. Radiother. Oncol. 2018, 126, 450–464.
41. Fukumori, Y.; Ichikawa, H. Nanoparticles for Cancer Therapy and Diagnosis. Adv. Powder Technol. 2006, 17, 173.
42. Ukleja, J.; Kusaka, E.; Miyamoto, D.T. Immunotherapy Combined With Radiation Therapy for Genitourinary Malignancies. Front. Oncol. 2021, 11, 663852.

43. Obeid, M.; Tesniere, A.; Ghiringhelli, F.; Fimia, G.M.; Apetoh, L.; Perfettini, J.-L.; Castedo, M.; Mignot, G.; Panaretakis, T.; Casares, N.; et al. Calreticulin Exposure Dictates the Immunogenicity of Cancer Cell Death. Nat. Med. 2007, 13, 54–61.
44. Reits, E.A.; Hodge, J.W.; Herberts, C.A.; Groothuis, T.A.; Chakraborty, M.; Wansley, E.K.; Camphausen, K.; Luiten, R.M.; de Ru, A.H.; Neijssen, J.; et al. Radiation Modulates the Peptide Repertoire, Enhances MHC Class I Expression, and Induces Successful Antitumor Immunotherapy. J. Exp. Med. 2006, 203, 1259–1271.
45. Ashrafizadeh, M.; Farhood, B.; Eleojo Musa, A.; Taeb, S.; Najafi, M. Damage-Associated Molecular Patterns in Tumor Radiotherapy. Int. Immunopharmacol. 2020, 86, 106761.
46. Abuodeh, Y.; Venkat, P.; Kim, S. Systematic Review of Case Reports on the Abscopal Effect. Curr. Probl. Cancer 2016, 40, 25–37.
47. Craig, D.J.; Nanavaty, N.S.; Devanaboyina, M.; Stanbery, L.; Hamouda, D.; Edelman, G.; Dworkin, L.; Nemunaitis, J.J. The Abscopal Effect of Radiation Therapy. Future Oncol. 2021, 17, 1683–1694.
48. Mole, R.H. Whole Body Irradiation; Radiobiology or Medicine? Br. J. Radiol. 1953, 26, 234–241.
49. Dagoglu, N.; Karaman, S.; Caglar, H.B.; Oral, E.N. Abscopal Effect of Radiotherapy in the Immunotherapy Era: Systematic Review of Reported Cases. Cureus 2019, 11, e4103.
50. Blanchard, P.; Gunn, G.B.; Lin, A.; Foote, R.L.; Lee, N.Y.; Frank, S.J. Proton Therapy for Head and Neck Cancers. Semin. Radiat. Oncol. 2018, 28, 53–63.
51. Malouff, T.D.; Mahajan, A.; Krishnan, S.; Beltran, C.; Seneviratne, D.S.; Trifiletti, D.M. Carbon Ion Therapy: A Modern Review of an Emerging Technology. Front. Oncol. 2020, 10, 82.
52. Mohamad, O.; Sishc, B.J.; Saha, J.; Pompos, A.; Rahimi, A.; Story, M.D.; Davis, A.J.; Kim, D.W.N. Carbon Ion Radiotherapy: A Review of Clinical Experiences and Preclinical Research, with an Emphasis on DNA Damage/Repair. Cancers 2017, 9, 66.
53. Yang, J.; Gao, J.; Wu, X.; Hu, J.; Hu, W.; Kong, L.; Lu, J.J. Salvage Carbon Ion Radiation Therapy for Locally Recurrent or Radiation-Induced Second Primary Sarcoma of the Head and Neck. J. Cancer 2018, 9, 2215–2223.
54. Hayashi, K.; Koto, M.; Ikawa, H.; Hagiwara, Y.; Tsuji, H.; Ogawa, K.; Kamada, T. Feasibility of Re-Irradiation Using Carbon Ions for Recurrent Head and Neck Malignancies after Carbon-Ion Radiotherapy. Radiother. Oncol. 2019, 136, 148–153.
55. Ramaekers, B.L.T.; Pijls-Johannesma, M.; Joore, M.A.; van den Ende, P.; Langendijk, J.A.; Lambin, P.; Kessels, A.G.H.; Grutters, J.P.C. Systematic Review and Meta-Analysis of Radiotherapy in Various Head and Neck Cancers: Comparing Photons, Carbon-Ions and Protons. Cancer Treat. Rev. 2011, 37, 185–201.

56. Schlaff, C.D.; Krauze, A.; Belard, A.; O'Connell, J.J.; Camphausen, K.A. Bringing the Heavy: Carbon Ion Therapy in the Radiobiological and Clinical Context. Radiat. Oncol. 2014, 9, 88.
57. Helm, A.; Ebner, D.K.; Tinganelli, W.; Simoniello, P.; Bisio, A.; Marchesano, V.; Durante, M.; Yamada, S.; Shimokawa, T. Combining Heavy-Ion Therapy with Immunotherapy: An Update on Recent Developments. Int. J. Part Ther. 2018, 5, 84–93.
58. Matsunaga, A.; Ueda, Y.; Yamada, S.; Harada, Y.; Shimada, H.; Hasegawa, M.; Tsujii, H.; Ochiai, T.; Yonemitsu, Y. Carbon-Ion Beam Treatment Induces Systemic Antitumor Immunity against Murine Squamous Cell Carcinoma. Cancer 2010, 116, 3740–3748.
59. Ando, K.; Fujita, H.; Hosoi, A.; Ma, L.; Wakatsuki, M.; Seino, K.-I.; Kakimi, K.; Imai, T.; Shimokawa, T.; Nakano, T. Intravenous Dendritic Cell Administration Enhances Suppression of Lung Metastasis Induced by Carbon-Ion Irradiation. J. Radiat. Res. 2017, 58, 446–455.
60. Nguyen, L.; Dobiasch, S.; Schneider, G.; Schmid, R.M.; Azimzadeh, O.; Kanev, K.; Buschmann, D.; Pfaffl, M.W.; Bartzsch, S.; Schmid, T.E.; et al. Impact of DNA Repair and Reactive Oxygen Species Levels on Radioresistance in Pancreatic Cancer. Radiother. Oncol. 2021, 159, 265–276.
61. De Meerleer, G.; Khoo, V.; Escudier, B.; Joniau, S.; Bossi, A.; Ost, P.; Briganti, A.; Fonteyne, V.; Van Vulpen, M.; Lumen, N.; et al. Radiotherapy for Renal-Cell Carcinoma. Lancet Oncol. 2014, 15, e170–e177.
62. Garcia-Barros, M.; Paris, F.; Cordon-Cardo, C.; Lyden, D.; Rafii, S.; Haimovitz-Friedman, A.; Fuks, Z.; Kolesnick, R. Tumor Response to Radiotherapy Regulated by Endothelial Cell Apoptosis. Science 2003, 300, 1155–1159.
63. Sathishkumar, S.; Boyanovsky, B.; Karakashian, A.A.; Rozenova, K.; Giltiay, N.V.; Kudrimoti, M.; Mohiuddin, M.; Ahmed, M.M.; Nikolova-Karakashian, M. Elevated Sphingomyelinase Activity and Ceramide Concentration in Serum of Patients Undergoing High Dose Spatially Fractionated Radiation Treatment: Implications for Endothelial Apoptosis. Cancer Biol. Ther. 2005, 4, 979–986.
64. Siva, S.; Pham, D.; Gill, S.; Corcoran, N.M.; Foroudi, F. A Systematic Review of Stereotactic Radiotherapy Ablation for Primary Renal Cell Carcinoma. BJU Int. 2012, 110, E737–E743.
65. Favaudon, V.; Caplier, L.; Monceau, V.; Pouzoulet, F.; Sayarath, M.; Fouillade, C.; Poupon, M.-F.; Brito, I.; Hupé, P.; Bourhis, J.; et al. Ultrahigh Dose-Rate FLASH Irradiation Increases the Differential Response between Normal and Tumor Tissue in Mice. Sci. Transl. Med. 2014, 6, 245ra93.
66. Lin, B.; Gao, F.; Yang, Y.; Wu, D.; Zhang, Y.; Feng, G.; Dai, T.; Du, X. FLASH Radiotherapy: History and Future. Front. Oncol. 2021, 11, 644400.
67. Taylor, P.A.; Moran, J.M.; Jaffray, D.A.; Buchsbaum, J.C. A Roadmap to Clinical Trials for FLASH. Med. Phys. 2022, 49, 4099–4108.

68. Klement, R.J.; Champ, C.E. Calories, Carbohydrates, and Cancer Therapy with Radiation: Exploiting the Five R's through Dietary Manipulation. Cancer Metast. Rev. 2014, 33, 217–229.
69. Saleh, A.D.; Simone, B.A.; Palazzo, J.; Savage, J.E.; Sano, Y.; Dan, T.; Jin, L.; Champ, C.E.; Zhao, S.; Lim, M.; et al. Caloric Restriction Augments Radiation Efficacy in Breast Cancer. Cell Cycle 2013, 12, 1955–1963.
70. Lee, C.; Raffaghello, L.; Brandhorst, S.; Safdie, F.M.; Bianchi, G.; Martin-Montalvo, A.; Pistoia, V.; Wei, M.; Hwang, S.; Merlino, A.; et al. Fasting Cycles Retard Growth of Tumors and Sensitize a Range of Cancer Cell Types to Chemotherapy. Sci. Transl. Med. 2012, 4, 124ra27.
71. Mukherjee, P.; Abate, L.E.; Seyfried, T.N. Antiangiogenic and Proapoptotic Effects of Dietary Restriction on Experimental Mouse and Human Brain Tumors. Clin. Cancer Res. 2004, 10, 5622–5629.
72. Goel, S.; Duda, D.G.; Xu, L.; Munn, L.L.; Boucher, Y.; Fukumura, D.; Jain, R.K. Normalization of the Vasculature for Treatment of Cancer and Other Diseases. Physiol. Rev. 2011, 91, 1071–1121.