Next to surgery, radiotherapy and chemotherapy are the two most commonly applied treatments against cancer. These therapies are often combined, but the therapeutic outcome is still limited. Nanocarriers may be the solution to many of the issues encountered when combining these therapies. In this review, the role of nanocarriers in combined radiotherapy and chemical cancer treatment is discussed, divided into four distinct strategy classes. For each strategy, examples of the literature are given to come eventually to conclusion on the possible translation to the clinic.

1. Introduction

Malignant tumors that have not metastasized are commonly treated using surgery, followed by external beam radiotherapy, possibly combined with chemotherapy. In case of metastases, chemotherapy and radionuclide therapy are the primary options for treatment. External radiotherapy and radionuclide therapy both use ionizing radiation to damage the DNA of cancerous cells, although the type of radiation differs. In external radiotherapy, high energy X-rays or gamma rays are applied, as well as protons and heavy ions,[1] while in radionuclide therapy beta minus particles (electrons) are the common type of radiation employed.[2] Chemotherapy uses cytotoxic drugs to induce cell death, targeting fast-dividing cells as present in cancerous cells, although the type of radiation differs. In external radiotherapy, high energy X-rays or gamma rays are applied, as well as protons and heavy ions.[1] while in radionuclide therapy beta minus particles (electrons) are the common type of radiation employed.[2] Chemotherapy uses cytotoxic drugs to induce cell death, targeting fast-dividing cells as present in cancerous tissue.[3] The effectiveness of both treatments is driven by the maximal achievable tumor control versus toxicity to normal tissue and will differ per tumor type and therapy plan. The main purpose of combining treatments is to improve this dose-response curve, that is, better therapeutic outcome is achieved for the same level of toxicity. It is often wrongly assumed that concurrent therapies have mostly additive therapeutic effects. However, combining radiotherapy with systemic medication (also including inhibitors of cell-repair processes and the like) may have a variety of different consequences leading to substantially enhanced therapeutic outcome. The most important additional effects are: improved drug delivery due to changes in tumor structure, reoxygenation and therefore more effective radiotherapy, more rapid cell proliferation as result of tumor volume decrease and consequently better sensitivity to chemotherapeutics, and tumor reduction requiring smaller radiation field sizes and allowing for higher dose deposition.[4] For all of these reasons, combinations of radiotherapy and drug therapies have been pursued in the clinic for several decades. Positive results were reported for a variety of cancer types.[5–7] For instance in the treatment of stage IIB squamous cell carcinoma of the uterine cervix, chemo–radiotherapy had significantly higher five year disease free rate as well as better overall survival.[8] There are also cancer types such as non-small cell lung cancer (NSCLC), where chemo–radiotherapy using cisplatin has become the standard.[9]

Still, the outcome of these combined therapies is often limited due to toxicity of the applied chemotherapeutics. Attempts to reduce these effects have been to switching to new drugs such as gemcitabine or trying new drug combinations.[10,11] Although in some cases, in particular for NSCLC, better toxicity profiles could be achieved, for many cancer types lasting improvements are still lacking. For instance, fluorouracil and cisplatin combined with radiotherapy in the treatment of cancer of the esophagus led to a longer life expectancy but with increased side effects, that is, lower quality of life for the patient.[12] It is clear that to really benefit from a combined treatment, toxic side effects need to be eliminated or at least significantly reduced. Besides toxicity, such concurrent treatments often suffer from low efficacy due to the low solubility of the drugs in aqueous solution or due to suboptimal timing of both therapies leading to a diminished combined effect.

Nanocarriers may offer a solution to many of these issues. Nanotechnology has developed tremendously over the last decades enabling the preparation of a broad range of nanocarriers having different sizes, morphologies and functionalities helping to solve problems of solubility as well as reducing toxic effects.[13,14] Large variety of nanocarriers has been reported, of which liposomes, polymeric, and inorganic nanocarriers are the most studied. Since nanocarriers are larger than small molecular drugs they often rely on a different mechanism to target tumor cells, that is, the so-called Enhanced Permeation and Retention
(EPR) effect (also referred to as passive targeting).[15] The EPR effect is based on the defective endothelial layers of blood vessels supplying the tumor with oxygen and nutrients and the lack of lymphatic drainage of cancer tissue which results in the retention of entities above certain size. Besides passive targeting, the functionalization of nanocarriers with targeting agents such as peptides, anti-bodies, or small molecules helps to increase uptake and retention in cancer cells or target specific cell organelles, offering in this way better control of the treatment.

In this review, we focus on the combination of radiotherapy and delivery of various drugs mediated by nanocarriers, based on four different strategies: 1) delivery of multifunctional drugs; 2) enhanced targeting or tumor uptake in concurrent radiotherapy and drug delivery; 3) enhancing the effect of ionizing radiation; 4) controlled release using ionizing radiation as a trigger. It is important to note that the third strategy only focuses on therapies involving cytotoxic approaches similar to chemotherapy in combination with enhanced dose deposition due to the creation of secondary radiation. This review does not include pure radiation dose enhancement effects as typically reported for gold nanocarriers.[16]

In each section we will show examples of concurrent treatments summarizing the characteristics of the used nanosystems and the obtained results. The review will end with a short perspective on clinical translation and possible obstacles for an optimal therapeutic outcome.

2. Delivery of Multifunctional Drugs

In concurrent therapy studies, nanocarriers are often used to deliver chemotherapeutics that could have additional radiosensitization effects or DNA repair inhibitors helping to improve the outcome of radiotherapy. In some cases the mechanism of action of chemotherapeutics is obvious such as in the case of doxorubicin (DOX). DOX is known to limit DNA repair capabilities of cells which, when combined with ionizing radiation, leading to unrepaired DNA breaks and hence eventual cell death. Still, in some cases radiation effects might be more subtle. For instance the mechanism of cisplatin radiosensitization is suggested to be related to the electron-transfer reactions of this molecule with electrons originating from the radiolysis of water leading to higher cytotoxicity rather than impending DNA repair mechanisms.[17] The exact mode of action of such treatment combinations is important to determine the optimal timing of drug delivery relative to the radiotherapy sessions.

The majority of reported combined treatment studies using nanocarriers use either liposome and lipid particles, polymer- or silica- nanocarriers. Here, each class will be discussed.

2.1. Liposomes and Lipid Nanocarriers

Soft nanocarriers are often capable of degrading when in the body, which prevents inflammation effects from developing in the long term. For this reason, they are the preferred vehicles in combined therapies. The most studied soft carriers are liposomes (i.e., vesicles composed of phospholipids), probably because of the simplicity of formulation as well as the fact that they have been approved for use in the clinic.[18] Liposomes loaded with various standard chemotherapeutic drugs were investigated in combination with radiotherapy.[19–22] The time and administration rate of the liposomes before radiotherapy appeared to influence the therapeutic outcome as demonstrated by Zhang et al.[23] and Harrington et al.[20] This effect was proposed to be related to the mechanism of drug radiosensitization and was therefore expected to differ per chemotherapeutic. Similar results were also reported for lipid particles (i.e., particles having a solid lipid core) carrying docetaxel, where the most optimal time of irradiation appeared to differ considerably when the free drug was administered versus the encapsulated one.[24] Besides simple enclosing of drugs, liposomes were also applied to oxygenate the tumor environment. A simple example is the encapsulation of perfluorooctylbromide functioning as an oxygen carrier in liposomes loaded with paclitaxel, which was found to improve treatment efficacy in vivo.[21] Liu et al. on the other hand used the hypoxic conditions in tumors as a trigger for drug release.[25] For this purpose, the authors conjugated nitroimidazoles via a hydrolysable ester group to phospholipids which could self-assemble into liposomes. In hypoxic conditions, the bond was cleaved breaking the liposomal structure and releasing any encapsulated drugs. In addition, the nitroimidazoles acted as radiosensitizers enhancing radiotherapy, so that when combined with chemotherapy these nanocarriers demonstrated to be more effective in the treatment
of glioma tumors than the separate therapies. More elaborate examples include liposomes encapsulating gallic acid-ferrous (GA–Fe(II)) complexes catalyzing the conversion of H₂O₂ to hydroxyl radicals (·OH), and l-buthionine sulfoximine (BSO), suppressing the synthesis of glutathione (GSH).\[60\] According to animal studies this BSO-liposomal system was proven to be very efficient in tumor growth suppression when applied in combined chemo- and radiotherapy. Alternatively, Zhang et al. reported the use of liposomes composed of phospholipids conjugated to cisplatin, which served as a vehicle for catalase (CAT), an antioxidant enzyme.\[67\] Figure 1 shows a schematic drawing of the Pt containing liposomes with and without the enzyme CAT. The function of this enzyme was to decompose H₂O₂ produced by tumor cells to oxygen, increasing the radiotherapeutic efficacy in hypoxic tumors. In vivo studies indeed confirmed the synergistic effect of cisplatin, CAT and radiotherapy leading to much more enhanced tumor growth suppression when compared to the single treatments.

2.2. Polymeric Nanocarriers

Polymer-derived nanocarriers make up the other major class of soft particles used in combination therapies. The main functions of these nanocarriers are: 1) to deliver a chemotherapeutic drug or radiosensitizer in sufficient quantities, 2) to limit drug toxicity to healthy tissue or 3) to exploit properties of the tumor microenvironment, all aiming at increasing the efficacy of radiotherapy.\[28–30\] Simple encapsulation of chemotherapeutic drugs (e.g., paclitaxel) in clinically approved micelles composed of poly(ethylene glycol)-block-poly(ε-caprolactone) (PEG-PDLLA) is an example of the first strategy. Such studies are essential for clinical translation and in this particular case showed that micelle-mediated delivery leads to much better tumor growth suppression than using the pure drug (paclitaxel), when combined with radiation.\[31\] Tactics to decrease adverse effects to healthy tissue are even more important in combination with radiation and rely on the direct conjugation of drugs (e.g., cisplatin, camptothecin) to polymers. The drug-polymer conjugates are able to self-assemble in nanocarriers and deliver the medicine primarily at the tumor\[32\] using for instance the reducing environment in cancer cells.\[13\] Examples of the third strategy involve the use of chemical compounds as linkers (e.g., metronidazole, gelatinizes-cleavable peptide) between the polymers or drugs to trigger release by the tumor environment such as hypoxia\[34\] or the presence of metalloproteases,\[15\] enzymes involved in cancer development.

While most of the literature focuses on the use of external beam therapy, Wang et al. designed lipid-polymer nanocarriers that enclosed DOX and were radiolabelled with ⁹⁰Y, a therapeutic radionuclide used in systemic therapy.\[66\] The addition of a therapeutic radionuclide enables attacking metastasized tumors, which nicely complements chemotherapy. This particular study was limited only to cell survival evaluation but demonstrated the improved efficacy of the combination of drug and radionuclide especially when targeting vectors were used. Huang et al. followed a similar approach but in this case a thermosensitive polymeric hydrogel encapsulating DOX and ¹³¹I was prepared, which in theory can enable the treatment of metastasized tumors.\[37\] In vivo these hydrogel nanocarriers (NPs) indicated much better ability to reduce tumor volume, however, only peritumoral injections were studied, so the full potential of these nanocarriers for attacking metastases was not evaluated.

DNA repair inhibitors are well known to improve radiotherapy and are often combined with this treatment.\[38,39\] Such inhibitors can also be encapsulated in nanocarriers, which can help in reducing toxicity\[40\] to healthy tissue and/or increasing uptake, particularly when dealing with hydrophobic compounds.\[41\] An example of such a system is the work of Wu et al. who employed nanocarriers with the purpose to bring a PARP (Poly(ADP-ribose) polymerase) to the tumor. Where PARP inhibitors are generally not harmful to the cell, when combined with radiation they act as a radiosensitizer, stopping the ability of cells to repair DNA breaks.\[45\] Olaparib is a common PARP inhibitor, which has a very limited solubility leading to only low amounts reaching the tumor. The authors demonstrated that by encapsulating this radiosensitizer in PEO–PCL nanocarriers much better tumor reduction could be achieved than when administering the inhibitor in its native form. Another nice example of the use of inhibitors is the study by Chen et al. who explored the possibility to deliver the ATR inhibitor VE822 to the brain using polymeric nanocarriers composed of poly(ω-pentadecalactone-co-p-dioxanone) [poly(PDL-co-DO)].\[42\] An important aspect of this delivery system was to enable drug release over a prolonged period of time since radiotherapy is normally given in fractions extending over tens of days. The prepared nanocarriers were small enough to cross the brain parenchyma and showed two phases of release, a fast initial phase followed by slow delivery lasting for more than 28 days. In vivo studies in rats having brain tumors revealed a much better survival of the animals which were treated with the loaded NP and radiotherapy versus NP-free drug delivery. This effect was attributed to the extended half-life of the drug when encapsulated in the particles and sustained supply over time.

Examples of bio-polymer carriers were explored by Peng et al. who utilized chitosan and cis-dichlorodiaminoplatinum thermoresponsive hydrogels for the combined treatment of...
nasopharyngeal carcinoma.\[^{[43]}\] In vivo evaluation revealed that the hydrogels had sustained release of dichlorodiaminoplatinum for a considerable time, leading to higher tumor cell killing efficiency when combined with radiotherapy. In particular, the number of DNA breaks induced by radiation appeared to be much higher when applying the hydrogel in comparison to the free drug.

Another advantage of soft carriers is that they can also deliver mixtures of drugs. Au et al. demonstrated how these properties can be fully exploited when combining two drugs which influence each other’s effectivity.\[^{[44]}\] The authors encapsulated wortmannin and docetaxel in block copolymer nanocapsules (Figure 2). Docetaxel is a well-known chemotherapeutic while also a radiosensitizer since it can arrest cells in the G2/M phase which is much more sensitive to radiation.\[^{[45]}\] Wortmannin is a steroid metabolite of Penicillium funiculosum fungus that inhibits important DNA protein kinases blocking in this way DNA repair.\[^{[46]}\] Wortmannin was shown to increase both radiation and chemotherapeutics efficacy when given as pre-treatment, and not as co-treatment. The polymeric nanocarriers prepared in this work appeared to release wortmannin faster than docetaxel such that a pre-treatment was achieved. In vivo testing indicated that the doubly loaded nanocapsules resulted in the best tumor growth control when combined with radiation, realizing almost complete remission when applied to mice xenografted with a prostate cancer cell line. Another study with wortmannin was carried out by Caster et al. They investigated whether the size of the particles affects chemo–radiotherapy, evaluating three sizes of methoxypoly(ethylene glycol)-block-poly(lactic-co-glycolic acid) (mPEG-PLGA) nanocarriers in terms of therapeutic efficacy and toxicity.\[^{[47]}\] It is often thought that smaller particles are better due to their longer blood circulation and better tumor distribution. However, the authors pointed out that medium sized particles (100 nm) gave the best results, even though the smaller particles indeed showed more homogenous tumor uptake. In terms of tumor growth, the 100 nm diameter particles were slightly better than those with 50 or 150 nm diameters, but the most toxic effects to healthy tissue were observed for the smaller particles. This phenomenon was maybe caused by enhanced permeability of normal tissue blood vessels as result of radiation,\[^{[48]}\] which

![Figure 2. a) Synthesis and characterization of single- and dual-drug-loaded PEG–PLGA NPs and b) corresponding size distributions as obtained using transmission electron microscopy (TEM). The inserts in (a) show the chemical structure, lipophilicity and the molecular weight of the drugs used while inserts in (b) show the number size distribution obtained from the TEM images. Adapted with permission.][44]

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Figure 3. a) Schematic drawing of the mesoporous silica nanocarriers and the proposed mechanism of targeting to the nucleus, b) their structure as obtained by Scanning Electron Microscopy (SEM), and c) element mappings revealing the different elements (i.e., Y, F, Yb, and Gd) enclosed in the nanostructures. Adapted with permission.[50] Copyright 2015, The Royal Society of Chemistry.

appeared to increase diffusion of smaller particles, causing higher intestinal toxicity. These results clearly show that particle design can play an essential role in achieving the best treatment.

2.3. Silica Nanocarriers

Silica particles and in particular mesoporous silica are a favorite platform for drug delivery due to their large loading capacity and relative ease of preparation.[49] However, there are just a couple of studies where silica NP were employed in combination with radiotherapy, nevertheless showing the potential of these nanosystems. Fan et al. designed small silica nanocarriers which were loaded with mitomycin C (MMC), a drug used to attack hypoxic tumors. These particles also had magnetic and upconversion luminescence properties due to the addition of various lanthanides.[50] MMC acts as an inhibitor of DNA synthesis triggered by the reducing environment of hypoxic cells, but it also has a radiosensitizing function.[51] To achieve an optimal effect, MMC needs to be in the cell nucleus, therefore these nanocarriers were functionalized with a ligand targeting this organelle. In Figure 3, the proposed mechanism as well as the structure and element composition of the particles are shown. Facilitated by the luminescence of the particles, the in vitro results showed an accumulation in the nucleus, while the magnetic properties of the particles were found to be very valuable in vivo biodistribution studies of the NPs using magnetic resonance imaging (MRI). When examined in tumor bearing mice, significant decrease in tumor volume in comparison with untargeted particles was observed which was ascribed to the intranuclear radiosensitization. The added value of radiotherapy was especially clear for low MMC concentrations showing that combined therapies could decrease the concentration of drugs needed for the same or better outcome.

Mesoporous silica particles were also exploited in the delivery of the radiosensitizer tert-butyl hydroperoxide (TBHP) and the generation of CO gas in combination with radiotherapy.[52] The idea behind this concept was that X-rays can break the O–O bonds of TBHP generating hydroxyl radicals which are not only capable of damaging the DNA of cells but can also break the bonds in metallic carbonyls leading to gas release. CO gas is able to cause mitochondria exhaustion and cell death. These silica particles could also be radiolabelled with $^{64}$Cu, allowing biodistribution data to be collected using positron emission tomography imaging. Around 7% ID (Injected dose) per g appeared to end in the tumor upon intravenous injection. This facilitated therapeutics studies, revealing that the combination of gas therapy and radiotherapy using these nanostructures led to tumor regression, not observed with either gas or radiotherapy alone. Silica nanocarriers were also used to encapsulate selenocystine, a drug typically used to induce oxidative stress leading to cell death.[53] Selenocystine is a powerful drug although its potential has not yet been fully explored mostly due to its low solubility. Encapsulating this substance in mesoporous silica
3. Enhanced Targeting or Tumor Uptake in Concurrent Radiotherapy and Drug Delivery

In concurrent treatments, therapeutic outcome is sometimes improved by factors not related to the therapy itself. For instance, various authors reported that radiation can affect the interaction between nanocarriers and tumor tissue (e.g., uptake), influencing the efficacy of the cure. The phenomenon of radiation enhanced delivery of nanocarriers was reviewed by Stapleton et al., who indicated that radiation-induced changes in the tumor vasculature, tumor interstitium as well as the interstitial fluid pressure (IFP) are most likely responsible for the observed effects. A study by Yi et al. focusing on passive targeting investigated the effect of radiation on the uptake of different types of nanocarriers (CuS, Au, Si, and liposomes) as well as their efflux when cells were irradiated by X-rays at different doses. The results revealed increased uptake and decreased expulsion of the NPs after radiation treatment (Figure 4), which appeared to be independent of the cell type. The authors studied the mechanism behind these observations and showed that the cell cycle changes as result of radiation as well as the presence of Caveolin-1, a protein involved in the endocytosis of nanocarriers, appeared to be significantly increased. These positive results were used to evaluate the radiosensitizing effect of CuS@Melanin-PEG particles containing DOX. In vitro studies showed that much better cell killing efficiency was obtained in the case of combined treatments when compared with free DOX plus X-rays or bare CuS@Melanin-PEG and X-ray exposure. This superior cell killing potential was also confirmed in animal studies. Several other pre-clinical reports supported this favorable increased

![Figure 4](image_url)
uptake for other particles such as nanocarriers composed of poly(N-(2-hydroxypropyl)methacrylamide (HPMA) polymers, solid lipid nanocarriers and liposomes. These radiation effects were also proven to occur in humans as different clinical trials indicated higher tumor uptake of liposomes when combined with fractionated radiotherapy. Besides passive targeting, ionizing radiation can also influence active targeting approaches leading to over-expression of certain proteins such as β3 integrin involved in cell signaling, and consequently to higher uptake. Indeed, Lee et al. showed that radiation influences the expression of various gastric cancer related proteins, which allowed for the selection of peptides recognizing these receptors. Conjugation of these peptides to liposomes led to the enhanced uptake of DOX in gastric cancer tumors, even at a radiation dose of just 2 Gy.

4. Enhancing the Effect of Ionizing Radiation

High Z-materials and in particular metallic NPs are claimed to act as radiosensitizers by increasing the radiation dose deposition. The mechanism behind this radiosensitization is not entirely understood but claimed to be primarily caused by the emission of secondary electrons such as photo-, Compton, and/or Auger electrons upon interaction with high Z materials. Auger electrons have typically low energy and would travel short distances but if emitted near the nucleus they can induce direct damage to the DNA leading to double strand breaks which are hard to repair. In principle, the higher the Z of the material and the lower the energy of the X-rays, the higher the chance of direct interaction with the nanocarriers. Since clinical beams are highly energetic such an interaction and production of secondary electrons is much less likely to occur, but different successful studies are nevertheless reported. The application of gold and other inorganic radiosensitizers in combination with chemical effects medicated by the same particles will be discussed here.

4.1. Gold Nanocarriers

Gold nanocarriers find various applications in medicine due to their biocompatibility, easy synthesis and range of possible morphologies. Radioisotopic labeling using gold nanocarriers was reported for low as well as high energy beams based on in vitro studies and in vivo studies. These results indicated that the morphology of gold nanocarriers and the presence of a targeting agent such as RGD (αvβ3 integrin) would affect tumor uptake. For this purpose, spherical and rod-shaped gold nanocarriers were covered with a thin layer of polydopamine in which iodine-125 was encapsulated and subsequently cisplatin was added. Iodine-125 permitted single photon emission computed tomography (SPECT) evaluation in vivo, revealing that active targeting does lead to somewhat higher tumor uptake and that the rod-shaped particles are better in tumor angiogenesis targeting. This improved uptake is probably due to the shape of the NPs since rod-like structures are able to more efficiently evade macrophages leading to longer blood circulation. In addition, the rod-shaped particles diffused much faster and could penetrate deeper into the tumor interstitials. Not surprisingly, the cisplatin-loaded rods showed a much enhanced therapeutic effect when combined with radiation in comparison to their spherical equivalents.

4.2. Selenium Nanocarriers

Selenium (Se) is a trace element that is required for important physiological functions in the human body. Se-containing materials are widely researched as pharmaceuticals because of their supposedly low toxicity and excellent biocompatibility.
past decades, a great number of studies demonstrated that Se species are promising agents for cancer therapeutics, due to their capability of generating reactive oxygen species (ROS), which can induce apoptosis in tumor cells.\(^{[88,89]}\) Interestingly, Se-containing compounds combined with radiotherapy appeared to specifically enhance the radiosensitivity of cancer cells resulting in a wave of selenium related publications.\(^{[90–92]}\) It needs to be mentioned that the mechanism of action of selenium is still not well-understood. An increase in ROS levels was observed when combining Se compounds with radiotherapy, which is not necessarily only related to the production of secondary electrons, but may have a different origin. Therefore, the combination of selenium and radiotherapy is often referred to as chemo-radiotherapy,\(^{[93]}\) although selenium compounds are not strictly speaking acting as chemotherapeutics.

An example of the use of Se NPs and X-rays is the work of Chen et al.\(^{[94]}\) who demonstrated that the morphology of the synthesized NPs changes upon X-ray exposure even at small radiation dose. This shape transition in turn leads to the conversion of elemental selenium (Se\(^0\)) to selenites which are proven to be much more toxic. Cell survival studies showed combined therapy to be more effective, which could be due to several factors such as the arrestment of the radiation sensitive G2/M phase, increase in autophagy (i.e., cell degradation and recycling process) and higher ROS production. The exact mechanism of Se-NP radiosensitization, however, remained undiscussed.

A similar approach was followed in two other studies in which a brachytherapy source, \(^{125}\)I, was used, and compared to X-ray irradiation. The Se-NPs were functionalized with various agents such as transferrin to improve cell uptake.\(^{[95,96]}\) Here it was found as well that Se leads to cell cycle arrest and apoptosis where \(^{125}\)I worked much more efficiently than X-rays. The mechanism of action was ascribed to the produced Compton electrons as a result of the interaction of ionizing radiation with Se leading to higher ROS production.

Rather than using selenium alone, the combination with elements having radiosensitizing properties was also investigated. In this respect Chang et al. designed a core-shell nanosystem, having gold as the core and selenium as the shell as shown in Figure 6.\(^{[97]}\) The idea behind this concept was to increase the radiotherapeutic dose at the tumor via the gold and boost the production of ROS via selenium. In vitro as well as in vivo evaluation confirmed the synergy of the two elements leading to much better control of the tumor growth without toxic effects to healthy
Figure 6. A) Schematic Illustration of the synthesis of gold-selenium particles, showing the covering of the gold rod by a selenium shell, subsequent layer of chitosan (CS) and the conjugation of tumor targeting peptides RGD and ACPP. B) TEM images of the golden rods and C) TEM of the gold-selenium particles including the size distribution as shown in the insert and D) the zeta potential of the bare gold and selenium particles and Au@Se nanoparticles having either RGF and ACPP peptides. Adapted with permission. [97] Copyright 2017, American Chemical Society.

tissue. The cell studies showed that the expression level of the p53 gene increased, which is known to trigger cell apoptosis and therefore, plays an important role in tumor regression.

Selenium nanocarriers were also used in combination with biomaterials. Liu et al. used red blood cell membrane (RBC) to cover selenium nanocarriers and bevacizumab (Avastin, Av), an antibody targeting angiogenic factors and inhibiting angiogenesis.[98] The obtained RBCs@Se/Av nanocarriers exhibited a synergistic effect in the presence of X-rays based on the result of isobologram analysis on melanoma and HUVEC cells and the inhibitory effect on the cell survival as determined by colony assays. The mechanism of the enhanced therapeutic outcome was mainly due to the increased generation of ROS which could damage DNA and the activation of the p53 gene. Moreover, the antibody Avastin inhibited cell migration and hindered blood cell formation, reducing even further the growth of tumors.

4.3. Other Nanocarriers

Besides gold, other high Z materials have also received some attention. Bismuth, having radiosensitization and good attenuation of X-rays, is a promising candidate,[99] provided that no toxic issues arise. An interesting study was performed by Hu et al. who aimed at preparing hollow bismuth subcarbonate nanotubes (Figure 7). Their shape would ensure kidney clearance and less accumulation in liver and spleen, and therefore diminished toxicity.[100] These nanotubes were successfully loaded with DOX and as they appeared to degrade at acidic pH, they were able to release encapsulated drugs. The pharmacokinetics of the bismuth nanotubes were compared to those of bismuth nanoclusters, revealing significantly higher tumor uptake of the elongated structures and improved renal clearance and minimal accumulation in healthy organs. The higher tumor uptake was assigned to the improved penetration abilities of elongated samples in leaky tumors, that is, enhanced EPR effect as also suggested by others.[101] Further therapeutic evaluation showed an excellent tumor reduction and much lower toxic side effects in comparison to free DOX, indicating that encapsulation of the drug indeed reduced its harmful properties.

A rather different approach was followed by Ma et al. who designed Bi₅S₇ nanocarriers coated by a mesoporous silica layer which could be applied for the encapsulation of DOX or/and P-32 colloids used in systemic radionuclide therapy.[102] The authors examined in vitro the combination of drugs and radionuclide therapy confirming improved cell killing efficiency of the combined system. In vivo studies appeared to be performed without the addition of DOX for unreported reasons. External therapy with X-rays was shown to be less efficient than P-32 radionuclide therapy in tumor reduction. However, the radiation dose given by P-32 was not provided, therefore a fair comparison between the two treatments cannot be made. In both cases a clear benefit of the use of Bi was observed, likely serving as a radiation dose enhancer.

Other examples include elements such as tantalum, zinc, and iron, which were also applied as nanocarriers in concurrent chemo- and radiotherapy. Tantalum (Ta), an element with a high atomic number (Z = 73), is expected to act as radio enhancer by producing Compton and Auger electrons upon X-ray irradiation. The potential of Ta was explored by Song et al. who developed a TaOₓ-based hollow nanoplatform using a one-pot template-free synthesis. The hollow morphology of the prepared TaOₓ allowed for excellent loading of the chemotherapeutic drug, SN-38, an inhibitor of DNA topoisomerase. Additionally, the partly deproto- nated tantalum hydroxyl group on the surface of the nanocarriers were capable of binding various metal ions, such as ⁹⁹ᵐTc⁴⁺, Fe³⁺, or Mn²⁺, enabling biodistribution studies based on SPECT imaging or MRI. Therapeutic evaluation in tumor bearing mice clearly demonstrated the much better treatment efficiency of the synergistic approach.[103]
Subsequent studies of the same system aimed at overcoming tumor hypoxia by encapsulating catalase, an enzyme that can catalyze the reaction of H₂O₂ to H₂O and O₂, into the same TaOₓ nanoplateform. Figure 8 shows a schematic illustration of the preparation of the nanocarriers and their characterization using TEM. Although encapsulation weakens the activity of catalase, the TaOₓ@Cat-PEG nanocarriers still possessed a superior ability to kill tumor cells when exposed to X-rays, compared to particles not carrying the enzyme. Additionally, compared to free catalase which was destroyed within 3 h, catalase encapsulated in TaOₓ was capable of producing a longer lasting effect as the enzyme activity was only slightly compromised over time. The same group continued this approach by investigating direct delivery of oxygen by combining the nanocarriers with perfluorocarbon, which functions as an oxygen reservoir releasing it slowly to the system. The results revealed the tumor oxygenation increased from 10% to 37% after the injection of TaOₓ@PFC-PEG@O₂, while TaOₓ-PEG alone had only a tiny effect on oxygen levels. In vivo evaluation of TaOₓ@PFC-PEG@O₂ showed evident enhancement of radiotherapy and good tumor control.

Apart from TaOₓ nanocarriers, another Ta-containing species, Ta₂O₅, also exhibits excellent radiation enhancement. The exploration of Ta₂O₅ nanocarriers in radiotherapy was firstly carried out in 2013 by Brown et al. and later their full potential in chemo-radiotherapy was explored by Chen et al. In this later work, mesoporous Ta₂O₅ nanocarriers were prepared to encapsulate and deliver DOX in synergistic chemo-radiotherapy. The Ta₂O₅-PEG/DOX nanocarriers exhibited an improved therapeutic efficacy under X-ray irradiation, and also possessed lower toxicity compared to free DOX.
Some semiconductor materials consisting of lighter elements, such as Ti or Zn, also appear to have radioenhancement ability based on photo–electrochemical reactions in which the semiconductors can convert ionizing energy to electrochemical energy.\[108\] ZnO nanocarriers were first explored as a radioenhancer by Zhang et al.\[109\] TfR Ab/ DOX /ZnO nanocomposites were fabricated by conjugating transferrin receptor antibody (TfR Ab) serving as a targeting agent to the ZnO and subsequently loading the nanocarriers with DOX. The combined system exhibited higher cell uptake of DOX than that of free DOX and therefore likely much better cell killing ability under X-ray exposure. In another study, a core-shell platform was fabricated with a Fe₃O₄ core functioning as MRI contrast agent and a ZnO shell acting as both a radiosensitizer and a platform to load DOX. Also here, ZnO induced radiation dose enhancement and improved inhibition of tumor growth. The authors hypothesized that besides the possible conversion of X-rays to photons of lower energy (in the UV spectrum), Auger electrons were created due to the interaction between the X-rays and Zn, leading to the generation of ROS.\[110\]

An interesting study was performed by Kang et al. who developed an iron-based nanoplatform for chemo–proton therapy by loading paclitaxel on the surface of superparamagnetic iron oxide nanocarriers (SPION) conjugated with folic acid (FA).\[111\] The advantage of this system was the reduced toxicity of paclitaxel due to the FA conjugation and enhanced radiosensitization of iron, leading to high killing efficiency of brain tumor cells with few side effects. Similarly, ultra-small superparamagnetic iron oxide nanocarriers (USPIOs) were applied in MRI/PAI (Photoacoustic imaging)-guided radio-chemotherapy.\[112\] There, poly (acrylic acid)-coated USPIOs were first synthesized, and then loaded with cisplatin as the chemotherapeutic agent. After that the particles were coated with polydopamine and PEG, and GE11 peptide was conjugated as the targeting agent. The obtained GE11-PDA-Pt@USPIOs displayed excellent therapeutic activity when combined with ionizing radiation. Interestingly, USPIOs nanocarriers appeared to have an additional advantage since they triggered the decomposition of H₂O₂ by Fenton-like reactions, resulting in the oxygenation of the tumors.

5. Radiation Triggered Release

The best way to reduce chemotherapeutic drug side effects is to ensure their release at the tumor site only. In the case of radiotherapy combined with drug delivery such a release can be achieved by using radiation as a trigger. Two approaches are mostly utilized: 1) implementing high Z materials to interact with radiation resulting in indirect production of ROS which can destroy chemical bonds, 2) designing radiation sensitive chemical bonds in soft matter systems that are either broken or modified when interacting with radicals and other reactive species formed by ionizing radiation. These approaches can also be combined.

5.1. High Z Nanocarrier Systems

An example of the first approach is the work carried out by Deng et al. who designed a liposome system containing gold nanocarriers and the photosensitizer verteporfin (VP), combined with X-ray radiotherapy.\[113\] The gold nanocarriers (3–5 nm) were chosen to ensure interaction with X-rays and the generation of ROS. The ROS on their turn were applied to oxidize unsaturated lipids, destabilizing the liposomes and leading to drug release. In addition, gold was claimed to transfer the X-rays to energies able to activate the photosensitizer VP, leading to the formation of O₂ which other ROS which could further decompose the lipid membrane. The authors showed that the concentration of O₂ increased both in the case of using gold alone or with VP, but the combination of the two entities generated the highest levels of ROS. The release ability of the combined system was evaluated using Calcein as a probe, revealing a reasonable amount freed when exposed to radiation of 4 Gy. In vivo experiments carried out using DOX as
a drug model showed that the X-ray triggered system resulted in significant tumor regression when compared to controls.

A somewhat similar approach was followed by Zhou et al. who developed polymeric nanovesicles (80–90 nm) composed of ROS responsive poly(propylene sulfide)-poly(ethylene glycol) (PPS-PEG) amphiphilic polymers and hydrophobic AuNPs tethered with X-ray labile linoleic acid hydroperoxide (LAHP) molecules.[114] DOX was encapsulated in the vesicle lumen serving as a model drug. The main goal of this paper was to generate OH radicals via the heterolysis of the hydroperoxide bond in LAHP when exposed to X-ray irradiation. This reaction can then lead to oxidation of the PPS membrane, its degradation and subsequent release, as schematically shown in Figure 9. Gold served here primarily to increase radiation dose deposition. Indeed, upon X-ray radiation of 8 Gy a burst release of DOX was observed within 30 min, while the untreated samples showed no leakage of the drug. In vivo evaluation revealed an enhanced effect of the radiation sensitive system leading to almost complete tumor inhibition when exposed to X-rays. Interestingly, the nanocarriers without X-ray treatment also exhibited tumor reduction, probably due to the long retention of the particles at the tumor and the slow release of DOX.

Su et al. explored a rather different approach by fabricating silica-covered gold nanocarriers (AuNP@SiO₂) to which different molecules were conjugated (6-carboxyfluorescein, cisplatin and green fluorescent protein) through 12 mer single-stranded DNA (ssDNA) linkers that are sensitive to ROS.[115] The gold nanocarriers in this work served to generate secondary electrons when interacting with X-rays, leading to ROS formation by the radiolysis of water and subsequent release of the conjugated reporters as demonstrated in Figure 10. In this way the authors developed a general method to release molecules from the surface of a particle using ionizing radiation. They managed to experimentally demonstrate that AuNP@SiO₂ particles and the methods (ICPMS and Fluorimetry) used to detect release when triggered by X-rays. Adapted with permission.[115] Copyright 2018, American Chemical Society.

![Figure 9. Schematic illustration of the release of drugs from PPS-PEG vesicles. The vesicles are composed of the hydrophobic Au-LAHP and the oxidation prone amphiphilic PPS-PEG. Upon irradiation, the formed OH radicals oxidize the PPS and make the block copolymer hydrophilic leading to disassembly and release of DOX. Adapted with permission.[114] Copyright 2018, Wiley-VCH.](Image)

![Figure 10. The conjugation of different reporters (6-Carboxyfluorescein (6-FAM), cisplatin, and green fluorescent protein) to the surface of AuNP@SiO₂ particles and the methods (ICPMS and Fluorimetry) used to detect release when triggered by X-rays. Adapted with permission.[115] Copyright 2018, American Chemical Society.](Image)
conjugated with cell penetrating peptides (CPPs) for nuclear accumulation and nitroimidazole for the release of nitrite ions upon irradiation. Nitrite ions can interact with hydrogen peroxide formed by the radiolysis of water, consequently leading to the creation of RNS. The incentive to use gold was to convert high energy X-rays to electrons with lower energy which could be used to decompose the nitroimidazole to nitrite ions. Cell viability assays under hypoxic conditions were applied to determine the cell killing ability of the NPs, which as expected revealed that fewer cells survive when these particles were applied in comparison to simple gold nanocarriers or PEG–AuNP not conjugated with CPP. The authors ascribed these positive results to the formation of both RNS and ROS. However, it needs to be mentioned that the higher killing efficiency of the NPs accumulating in the nucleus could also be due to damage induced by Auger electrons, which are able to directly destroy DNA molecules.

5.2. Systems Based on ROS-Sensitive Bonds

The utilization of radiation sensitive bonds is more common as a release strategy, and was also applied to liberate NO radicals. W. Fan et al. speculated that the weak S—N bond (about 150 kJ mol⁻¹) in SNO molecules can be damaged by X ray radiation leading to NO release. To demonstrate this concept, the authors constructed a nanotheranostic system that contains monodisperse NaYF₄:Yb/Er upconversion nanocarriers (UCNPs) covered by silica. The UCNP core could be used for luminescent imaging, while silica functioned as platform to conjugate PEG and SNO groups. Upon irradiation with X-rays at a dose of 5 to 20 Gy, SNO decomposed and released NO even under hypoxic conditions. The nanocarriers were further investigated using zebrafish having a tumor burden, revealing a substantial tumor growth delay than controls, attributed to the radiation-induced release of NO.

A different approach was followed by Tanabe et al. who prepared DNA amphiphilic molecules (DAMs) composed of hydrophilic oligodeoxynucleotides (ODNs) and hydrophobic alkyl chains, linked by a radiation-sensitive disulfide bond. These molecules were shown to self-assemble into small aggregates in which hydrophobic drugs and dyes could be encapsulated. When exposed to X-ray radiation, release was achieved by selectively reducing the disulfide bond. However, a radiation dose much larger than applied in radiotherapy was needed to achieve sufficient destruction of the self-assembled nanocarriers. The groups of Xu and Zhang proposed a strategy to design radiation sensitive polymers based on chemical bonds involving Se or Te to reduce the radiation dose necessary to break bonds. The Se-Se bond has lower energy (172 kJ mol⁻¹) when compared to the S–S bond (240 kJ mol⁻¹), and in addition Se is known to have a redox response in the presence of either oxidants or reductants. This approach was demonstrated using an amphiphilic tri-block copolymer having two PEG blocks and a hydrophobic block linked to PEG through a Se-Se bond. The amphiphilic block copolymer could assemble into spherical micelles of around 60 nm in which DOX could be encapsulated. Upon γ-irradiation of 5 Gy, 40% of the DOX was released, which was suggested to occur due to the oxidation of the selenide to selenium leading to disassembly of the micelles (Figure 11). To demonstrate the role of the Se-Se bond, control experiments were carried out using analogous polymer particles that did not contain a diselenide bond. These nanocarriers failed to release any DOX molecules even when irradiated up to 500 Gy.

To further decrease the radiation dose, the same group attempted to utilize Te–Te bonds, since it was shown by Cao et al. that the oxidative potential of telluride compounds was approximately 0.5 V lower than that of selenide indicating that these bonds will be more sensitive to oxidation by ROS. For tellurium-containing polymer micelles, gamma-irradiation of just 2 Gy resulted in a size increase of the micelles from 35 to 250 nm. NMR studies showed that Te bond was oxidized already in the presence of just 100 μM H₂O₂ leading to the hydration of the polymer and subsequent swelling of the micelles. Release assays were unfortunately not performed.

Te was further used to synthesize a side-chain tellurium-containing amphiphilic block polymer, PEG-b-PAA-g-Te, which could self-assemble into nanocarriers. Cisplatin was successfully encapsulated into the self-assemblies due to a favorable interaction between Pt and Te. Similar to the earlier study, Te was shown to oxidize upon 2 Gy of γ-radiation, making the hydrophobic block hydrophilic and leading to disassembly. Release of cisplatin could be achieved at somewhat higher dose (5 Gy). Cytotoxic assays showed that the tellurium containing nanocarriers lead to cell death without X-rays or cisplatin incorporation. Interestingly, the coordination of cisplatin with Te reduced the toxic
effects of the Te-NP but upon gamma irradiation cell death was once again achieved.

Table 1 shows an overview of the different radiation sensitive bonds, their bonding strength and the necessary radiation dose to have an effect according to the literature.

### Table 1. Summary of the bonding energies of radiation sensitive chemical bonds.

| Chemical bond | Energy of the bonds [kJ/mol] | Minimum dose for an observed effect [Gy] | Reference |
|---------------|------------------------------|-----------------------------------------|-----------|
| –SNO          | 150                          | 5                                       | 117       |
| S=S           | 240                          | 30                                      | 118       |
| Se-Se         | 172                          | 5                                       | 120       |
| Te            | –                            | 2                                       | 121       |

6. Clinical Perspective

In this review paper we examined the role that different nanocarriers could play in combined radiotherapy and systemic drug administration based on four different strategies. According to the discussed papers, nanocarriers could be beneficial in increasing efficacy as well as reducing side effects. However, before these formulations can be implemented in the clinic several challenges would need to be overcome. One of the main hurdles that nanomedicines face in general is sufficient accumulation at the tumor. The EPR effect that is always claimed to be responsible for the build-up of nanoentities in malignant tissues is well demonstrated in lab animals but is more heterogeneous in humans, that is, the same tumor type may or may not show uptake of NPs in different patients. This heterogeneous nature of tumors leads to unpredictable uptake and often failure of passive targeting in patients. [123] Therefore, one of the main challenges is understanding the principles governing uptake of nanocarriers in human cancers so that strategies could be developed to exploit or even modify the microenvironment of tumors to ensure better delivery. [124] However, particularly in radiotherapy, microscopic and macroscopic changes of tumor tissue can occur that will greatly influence the optimal time as well as the frequency of nanocarriers administration. Naturally, radiation induced effects may also differ per tumor and stage of the disease. Ideally, the EPR effect should be studied under the relevant conditions, but may often not be possible. Perhaps, imaging techniques such as SPECT or MRI might help to assess the likeliness of nanocarriers accumulation in patients prior to the treatment, helping to properly select cohorts that could benefit from nanocarriers mediated therapies. [125]

Determining the most optimal time to deliver the nanocarriers according to the best uptake moment might however, not be sufficient for the best therapeutic outcome. Many drugs act as radiosensitizers and should actually be provided before the radiation treatment. Therefore, combined treatment relying on nanocarriers might require more complex treatment schedule. In external beam therapy the radiation dose is typically fractionated over many days, and will allow for applying different administration strategies. In radionuclide therapy, however, treatments are often done only once and it will be much more problematic to find the best time of nanocarriers administration.

When combined with radiotherapy, the size and shape of the nanocarriers may also have an effect different than observed in conventional systemic chemotherapy mediated by nanocarriers. For instance, induction of leaky vessels in healthy tissue due to radiation may result in side effects strongly linked to the morphology of the particles. At the same time radiation dose enhancement based on high Z-material will also depend on size and shape. The right therapeutic window would need to be obtained in these cases and would clearly differ per cancer type and even location of the tumor. These issues indicate the essential of using orthotropic tumor models to fully evaluate the potential of these combined therapies.

It is well known that radiotherapy can cause late toxic effects but in combination with chemotherapy, [126] large toxicity studies are still lacking to be able to provide reliable information. [127] One can anticipate that toxicity due to the drugs themselves would be reduced when using nanocarriers as it was demonstrated for liposomes. [128] However, the nanoentities themselves might induce toxic effects. Currently, there is very little information about possible toxicity of nanocarriers particular in the long run, but it can be anticipated that type and morphology, as well as the route of administration, will influence the chance of adverse effects. [129] In addition, non-degradable nanocarriers would require more attention since they might induce inflammatory effects especially at a later stage. [130] So far the studies discussed in this paper focused primarily on acute toxicity or were limited to a few weeks. It is therefore essential for clinical translation to carefully evaluate long term toxicity effects.

Finally, to enable a translation to the clinic, the synthesis of the nanocarriers should be easy, cheap, and scalable. At the moment many of the investigated systems are far too complex, requiring many synthetic steps, to make it to the hospital. In this respect, simplicity is preferred.

Table 2 summarizes the most important challenges and opportunities in radiotherapy combined by chemical effects mediated by nanocarriers.

Although many challenges still remain, nanocarriers do provide means of reducing drug toxicity, enhancing tumor...
reduction, or combining more than one drug in a single carrier that can have great consequences in cancer therapy. Moreover, nanocarriers hold potential in other therapies such as immunotherapy, which would open a whole world of new possibilities especially in combination with radiotherapy.\[13]  In all cases for successful implementation in the clinic it is wise to start with simple nanocarriers, which may lead to suboptimal improvements but will help to understand basic factors affecting such combined therapies. Based on knowledge obtained from these studies, better systems can be designed to fully exploit the potential of nanocarriers in combined therapies.

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Conflict of Interest

The authors declare no conflict of interest.

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