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Review paper

Approaches to combat hypoxia in cancer therapy and the potential for *in silico* models in their evaluation

Jake C. Forster[a,b], Loredana G. Marcu[c,d,*], Eva Bezak[b,d]

[a] SA Medical Imaging, Department of Nuclear Medicine, The Queen Elizabeth Hospital, Woodville South, SA 5011, Australia
[b] Department of Physics, University of Adelaide, North Terrace, Adelaide SA 5005, Australia
[c] Faculty of Science, University of Oradea, Oradea 410087, Romania
[d] Cancer Research Institute and School of Health Sciences, University of South Australia, Adelaide SA 5001, Australia

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ABSTRACT

Aim: The negative impact of tumour hypoxia on cancer treatment outcome has been long-known, yet there has been little success combating it. This paper investigates the potential role of *in silico* modelling to help test emerging hypoxia-targeting treatments in cancer therapy.
Methods: A Medline search was undertaken on the current landscape of *in silico* models that simulate cancer therapy and evaluate their ability to test hypoxia-targeting treatments. Techniques and treatments to combat tumour hypoxia and their current challenges are also presented.
Results: Hypoxia-targeting treatments include tumour reoxygenation, hypoxic cell radiosensitization with nitroimidazoles, hypoxia-activated prodrugs and molecular targeting. Their main challenges are toxicity and not achieving adequate delivery to hypoxic regions of the tumour. There is promising research toward combining two or more of these techniques. Different types of *in silico* therapy models have been developed ranging from temporal to spatial and from stochastic to deterministic models. Numerous models have compared the effectiveness of different radiotherapy fractionation schedules for controlling hypoxic tumours. Similarly, models could help identify and optimize new treatments for overcoming hypoxia that utilize novel hypoxia-targeting technology.
Conclusion: Current therapy models should attempt to incorporate more sophisticated modelling of tumour angiogenesis/vasculature and vessel perfusion in order to become more useful for testing hypoxia-targeting treatments, which typically rely upon the tumour vasculature for delivery of additional oxygen, (pro)drugs and nanoparticles.

1. Introduction

Tumour hypoxia, or the lack of adequate oxygenation in the cellular microenvironment, is common for many cancer types and has long been known as one of the determining factors of tumour response to radiation [1]. Over the decades, hypoxia has raised great attention, becoming "the most cited biological topic in translational radiation oncology" [2]. Yet, there has been little success in combating tumour hypoxia in the clinic and it remains a major challenge for radiotherapy, chemotherapy and immunotherapy.

Several factors contribute to the hypoxic microenvironment and they include: an imbalance of angiogenic regulators in cancer cells leading to the development of abnormal vessel structure and chaotic vasculature organisation, the increased metabolic demand of malignant cells, and high interstitial pressures that compress tumour vessels [3–5]. As a result, tumours can contain: (1) chronic hypoxia located farthest from blood vessels (diffusion-limited or consumption-limited hypoxia), (2) acute hypoxia located adjacent to tumour vessels that are temporarily without blood flow.

Clinical studies have found that tumour hypoxia has prognostic value as well as predictive value to both radio- and chemotherapy [6–8].

Methods to detect hypoxic cells have come a long way, from the invasive polarographic electrode to the clinically feasible use of endogenous and exogenous markers. Functional imaging-based biomarkers also offer quantification of hypoxia, allowing for image-guided treatment adaptation and personalization [9].

Several approaches to combat tumour hypoxia have been tested or are being pursued, including attempts to reoxygenate the tumour, efforts to radiosensitize hypoxic cells with nitroimidazoles, the use of

* Corresponding author at: Faculty of Science, University of Oradea, 410087, Romania.
E-mail address: loredana@marcunet.com (L.G. Marcu).

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hypoxia-activated prodrugs, and molecular targeting of hypoxia. Presently, each of these approaches shows promise but has its challenges and limitations. The only notable progress toward hypoxia-targeting (hypoxia modifying) in the clinic was made in Denmark in 1990, when nimorazole, a nitroimidazole and hypoxic cell radiosensitizer, was implemented as part of standard treatment with radiotherapy for head and neck cancer [10,11]. As a result, there remains much room for improvement in terms of the efficacy of hypoxia-targeting treatments.

Over the years, *in silico* models gained more importance in cancer research, owing to their ability to assist with treatment optimisation and individualised therapy in a prompt, economical and non-invasive manner. The term *in silico,* which suggests the use of silicon for computer chips, denotes an operation performed via computer. Thus, *in silico* models refer to simulations executed by computers, which in the context of the current paper encompasses biophysical processes (such as the interaction of radiation with cells) and patient data (biomarkers of response to therapy), with the aim to evaluate the effect of hypoxia within these processes and to find solutions to counteract it.

While not without limitations, computational models present with several advantages as compared to the more traditional, bio-clinical approaches. From this perspective, models allow:

- inclusion of various kinetic and dynamic parameters to simulate complex processes (such as the cell cycle duration, the labelling index and the partial oxygen pressure);
- quantitative evaluation of input factors that impact treatment outcome (such as the fraction of cellular subtypes, the ratio of symmetrically dividing cancer stem cells, the fraction of recruited cells, etc.)
- assessment of biological processes in tumour development and response to therapy (like reoxygenation and accelerated repopulation);
- simulations of extreme values for biologically meaningful parameters to determine their lower and upper bounds for biologically indorsed results (for instance, very small and very high values of partial oxygen pressure);
- identification of research gaps (for instance, the lack of quantification of certain biological parameters) to be pursued by experimental studies.

With regards to hypoxia, researchers have used varied approaches to modelling. Deterministic as well as stochastic modelling techniques have been used to evaluate the temporal or spatial development of hypoxic tumours and their behaviour under treatment. Due to the probabilistic nature of radiation-cell interaction, stochastic models seem to be more representative of the radiobiological processes taking place in tumours and have gained more popularity. Phenomena such as acute hypoxia, reoxygenation, formation of the angiogenic network and regulation of hypoxia-related signalling pathways are also well-suited to stochastic modelling.

This paper reviews two important aspects related to the challenges imposed by the lack of oxygenation in tumours: (1) hypoxia-targeting treatments from past to the future and (2) the potential for *in silico* modelling to help identify effective hypoxia-targeting approaches.

2. Methods

A Medline search was undertaken on the current landscape of *in silico* models that simulate cancer therapy and their ability to test hypoxia-targeting treatments was evaluated. Search of literature published in English was conducted using the following keywords: (computational OR "*in silico*") AND cancer AND (therapy OR radiotherapy OR chemotherapy OR immunotherapy) AND hypoxia. Additional articles were retrieved via pearling of identified literature. Manuscripts were reviewed, discussed and the most significant ones summarized in tabulated format.

3. Hypoxia-targeting treatments and their limitations

3.1. Tumour reoxygenation

There are several techniques for improving tumour oxygenation. It is known from the "Rs of radiotherapy" that one reason to deliver radiation in multiple dose fractions is to enable tumour reoxygenation in-between fractions [12]. Tumour shrinkage over the course of radiotherapy allows blood vessels to reach previously distant, thus hypoxic cells (reoxygenation of chronically hypoxic cells). To a certain extent, acutely hypoxic tumour areas can also be managed with fractionation, due to their dynamic nature. However, the longer the treatment time, the more accelerated repopulation there is to contend with.

Carbogen breathing (95% O_2 and 5% CO_2) could help reoxygenate the tumour by increasing the blood oxygenation. However, a prospective, randomized clinical trial found no improvement in the efficacy of radiotherapy for head and neck cancer (HNC) [13], indicating that the deficiencies of the tumour vasculature were not overcome. Hyperbaric oxygen (HBO) involves 100% O_2 breathing at an increased pressure (typically 3 or 4 atmospheres). For HNCs, the use of HBO with radiotherapy showed some benefit over radiotherapy alone but still left a lot to be desired, most likely because of poorly perfused tumour vessels. There are also concerns that HBO has the potential to enhance metastatic tumour growth, aggravate acute radiation sickness and induce oxygen toxicity [14].

Perfluorocarbons (PFCs) can be used as additional O_2 carriers in the blood [15–17]. Not only do PFCs provide additional carrying capacity for O_2, critically they hold O_2 in the dissolved state, whereas about 98% of O_2 in blood is not dissolved but reversibly bound to haemoglobin. The pO_2 is greater for dissolved O_2 than O_2 in the gas state [18,19]. Since O_2 diffusion from vessel to tissue is driven by a pO_2 gradient [19], PFCs provide enhanced distribution of O_2 to tissue compared to blood. There are stable PFC emulsions with droplet diameters less than about 200 nm [15,18], allowing them to pass through even the narrowest of capillaries (with diameters of ~5 μm), unlike red blood cells. While the relationship between oxygen content and pO_2 is sigmoidal for blood, it is linear for PFCs [18], indicating that PFCs may possibly be combined with carbogen breathing [20] or HBO [21,22] to great effect. Aside from PFCs, microbubbles can also be used as additional O_2 carriers [17]. The development of nanoparticles (NPs) containing PFCs and systems for triggering the release of O_2 from PFCs and microbubbles are active areas of research [23–26].

A separate approach to achieve tumour reoxygenation uses Albumin-Manganese dioxide (MnO_2) NPs. Cancer cells favour anaerobic glycolysis (the Warburg effect) which creates a low pH in the tumour. This effect is even more pronounced under hypoxic conditions. There is also a high concentration of H_2O_2 in hypoxic regions, produced by macrophages. MnO_2 NPs react with H_2O_2 under acidic conditions to release O_2, thus achieving targeted release of O_2 in the tumour and particularly in hypoxic regions [17,27,28]. A challenge is that these NPs have a negative surface charge which can bind to the extracellular matrix (ECM) and impede their diffusion to hypoxic regions [27]. There is research into using NPs to degrade the ECM [27]. Not only could this improve the delivery of Albumin-MnO_2 NPs to hypoxic regions, it could also reduce the tumour's interstitial pressure, in turn increasing tumour vessel perfusion and helping tumour reoxygenation.

Another way to increase tumour oxygenation is to decrease the metabolic O_2 consumption rates (JO_2) of cancer and stromal cells [28]. Theoretical modelling by Secomb et al. [29] indicated that decreasing JO_2 would be far more effective at reoxygenating the tumour than increasing blood oxygenation or flow rate.

3.2. Hypoxia-activated prodrugs

Hypoxia-activated prodrugs (HAPs) are chemicals that can be metabolized by enzymatic reduction under hypoxic conditions to release

an active drug [30,31]. The nitroimidazoles (e.g. misonidazole, pimonidazole and nimorazole) are examples of HAPs. They are more cytotoxic to hypoxic cells than normoxic cells through the mechanism of bioreduction [31]. However, nitroimidazoles also have a radiosensitising effect on hypoxic cells, which is due to a mechanism which is similar to that exhibited by oxygen [30–32]. Nitroimidazoles are electron-affinic and can react with radiation-induced DNA radicals to enhance DNA strand breaks in the same way as oxygen [30,33,34], hence they are often described as "oxygen-mimetic". They do not radiosensitise well-oxygenated cells because oxygen out-competes nitroimidazoles for reaction with DNA radicals.

The challenge with nitroimidazoles is their toxicity in terms of effectiveness at tolerable doses. The use of nimorazole with conventional fractionated radiotherapy for HNC showed benefit over radiotherapy alone (at tolerable doses of the drug) in a phase III trial (DAHANCA 5). As a result of this trial, nimorazole is now part of standard treatment for HNC in Denmark [10,11,35]. A phase III trial (NIMRAD) recently started in the United Kingdom will evaluate the effectiveness of nimorazole for HNC when used with contemporary radiotherapy techniques (intensity-modulated radiotherapy) [36]. Clinical trials evaluating the use of several other nitroimidazoles with radiotherapy, including misonidazole, pimonidazole, metronidazole and etanidazole, did not show benefit (tolerable drug doses were ineffective) [30,37].

Next to nitroimidazoles, there are several other nitroaromatic HAPs (e.g. nitrotriazoles) and aromatic N-oxides (e.g. tirapazamine). These may not radiosensitize hypoxic cells to the same degree as oxygen or nitroimidazoles, rather their success may come from their hypoxia-selective bioreduction into an active drug (e.g. a cytotoxin) [30,31]. The addition of Sanazol, a nitrotriazole, to radiotherapy for advanced uterine cervix squamous cell carcinoma showed significant benefit in a prospective, randomized trial [38]. Unfortunately, clinical trials for tirapazamine and 5 other HAPs have been discontinued due to their ineffectiveness at tolerable doses [28,37].

A challenge facing the delivery of HAPs to hypoxic regions is the aberrant tumour vasculature, which often prevents the agent from reaching the desired target. One way to improve HAP delivery to hypoxic regions is by their incorporation into nanoparticles [27,39]. In mouse models, NPs selectively accumulate in the tumour by the enhanced permeation and retention effect. This is where macromolecules with a molecular weight greater than 40 kDa are unable to extravasate from normal vessels but they do escape from the highly permeable/leaky tumour vessels. Furthermore, due to their large size, they are more likely to be trapped in the tumour tissue rather than re-enter the blood [27]. Therefore, NP diameters from 20 to 100 nm are typically used. With increasing NP size, the NP has greater uptake but decreased depth of tissue penetration. If a HAP is packaged into a NP, it may have a better chance of diffusing long distances from vessels to reach hypoxic regions of the tumour. NPs of a biocompatible heavy metal, such as gold, could be used to provide additional radiosensitization of hypoxic cells. Unfortunately, the success of NP-based drug delivery systems in mouse studies has not translated well to patients, possibly due to a diminished permeation and retention effect in humans compared to mice [40,41].

3.3. Molecular targeting of hypoxia

Another technique to combat hypoxic cells is to target/inhibit hypoxia-responsive signalling pathways. The focus has mainly been on hypoxia-inducible factor (HIF), the unfolded protein response (UPR) and mTOR pathways [28,31]. Molecular targeting is challenging because the pathways are complex and it is not clear where the main vulnerabilities lie. Drugs for molecular targeting of hypoxia can be included in the active drug of a HAP to improve the selectivity of the molecular targeting drug for hypoxic cells. For example, Xu et al. developed Pt(IV) prodrugs that release a HIF-1α inhibitor plus cisplatin (a cytotoxin) [42].

Again, there may be challenges with molecular targeting drugs reaching hypoxic regions of the tumour. This could possibly be improved by incorporating the drug into a NP [43], and a heavy metal NP could provide additional radiosensitization [44].

3.4. Hyperthermia

Hyperthermia can take the form of local or whole-body heating from 39 to 45 °C for as long as 6 h, or ablative heating at 60 to 100 °C. There are several promising antitumour effects from hyperthermia, including a cytotoxic effect that increases with decreasing pH, inhibition of DSB repair and an immune response driven in part by heat shock proteins [45,46]. The addition of hyperthermia to radiotherapy has shown benefit in many clinical trials [47]. Hyperthermia may be particularly beneficial for combatting hypoxic tumours [48]. It may cause vessels to dilate and reduce the interstitial pressure, thus improving vessel perfusion and tissue oxygenation [49,50]. Also, the pH-dependent cytotoxic effect is enhanced under hypoxia. Hyperthermia has the potential to harm normal tissue too, so targeted delivery using NP technology is worthwhile pursuing.

3.5. High-LET radiation

The presence or absence of oxygen makes less of a difference to the amount of radiation-induced cell killing with high-LET radiation. The decrease in oxygen enhancement ratio (OER) with increasing LET is due to the increased clustering of DNA damage, which makes the additional DNA strand breaks facilitated by oxygen less necessary for the production of DSBs [51]. High-LET radiation can be delivered via external beam or internal radionuclides, but in each case there is limited treatment availability and the stakes are much higher around conforming dose to the tumour.

Hypoxia can be targeted with low-LET radiation using a spatially heterogeneous dose distribution, with higher dose delivered to hypoxic regions of the tumour ("dose-painting"). Temporal changes in hypoxia are the main obstacle to success with this approach.

3.6. Other innovative directions for hypoxia-targeting

- Chen et al. [52] coated a hypoxia-targeting bacteria (VNP20009) with a photothermal agent (melanin-like polydopamine) for combined biotherapy and near-infrared laser-driven photothermal therapy of hypoxic cells.
- NPs can be incorporated into cells that normally migrate to hypoxic regions via chemotaxis [27], e.g. monocytes [53,54] and macrophages. The advantage of this approach is that it does not rely upon the NPs diffusing from tumour vessels to reach the hypoxic cells.
- Since tumours and especially their hypoxic regions are acidic, the outer layer of a NP can be a pH-sensitive molecule that destabilises and releases the contents (e.g. a cytotoxic drug, molecular inhibitor or metal NP [55]) in low-pH [27].
- Nitric oxide (NO) may be an even more efficient radiosensitizer than oxygen, while endogenous NO levels may be prognostic and predictive, like pO_2 [30,33]. Thus, hypoxic cells can be radiosensitized by delivering NO or generating it *in situ*. Fan et al. [56] developed a NP that enters the cell cytoplasm and contains a NO donor, SNO. Tumour irradiation with x-rays induced cleavage of the S-N bond for controlled, dose-dependent NO release.

A summary of the various approaches to combat tumour hypoxia is presented in Table 1.

Table 1. Hypoxia-targeting treatments in cancer therapy

4. *In silico* modelling to test treatments to hypoxic tumours

Various models have been developed to predict the outcome of a

Table 1
Hypoxia-targeting treatments in cancer therapy.

Hypoxia-targeting category	Hypoxia-targeting technique/agent	Observations
Tumour reoxygenation	Radiation dose fractionation	Most common, as no additional agent is needed. Both conventional and altered fractionation schedules contribute to tumour reoxygenation over the course of radiotherapy (the Rs of radiotherapy) [12]
	Carbogen breathing	Clinically used in the last decades without significant success [13]
	Hyperbaric oxygen	Clinically used in the last decades with limited benefits [14]
	Perfluorocarbon (PFC)	The high oxygen dissolving ability of PFC led to its incorporation into bio-nanomaterials to modulate the hypoxic microenvironment inside tumours [23,25]
	Albumin-MnO_2 nanoparticles (MnO_2 NPs)	Additional O_2 is created due to the redox activity of MnO_2 NPs toward H_2O_2 that is present in tumours. The method showed efficiency both in combination with radiation and photodynamic therapy [17,27,28].
Hypoxia-activated prodrugs	Nitroimidazoles	Are oxygen-mimetic radiosensitisers. Clinical utility is limited to certain agents (nimorazole), as others did not meet the criteria for routine clinical implementation [30,37]
	Aromatic N-oxides	These agents undergo hypoxia-selective bioreduction into an active cytotoxin. The most representative drug is Tirapazamine, which showed more promise *in vitro* than *in vivo* [28]. SN30000 is an analogue of tirapazamine but reaches the hypoxic tumour in higher concentration; the prodrug is currently undergoing clinical trial.
Molecular targeting	HIF-targeting agents	Highly challenging methods due to complex signaling pathways and unclear targets [28]. Prodrugs are being developed that release HIF-1α inhibitors [42].
	PI3K/mTOR inhibitors	
Other methods	Photothermal therapy	A laser-driven eradication of hypoxic cells using hypoxia-targeting bacteria coated with photothermal agent [52].
	Nitric oxide (NO)	Highly efficient radiosensitizer due to its ability to diffuse across cell membrane, and its pleiotropic effects on cell signalling, tumour perfusion, inhibition of DNA damage repair [30]. Lately NO is incorporated in NPs for more specific targeting [56].
	Hyperthermia	Several antitumour effects: direct cytotoxic effect that increases with decreasing pH, inhibition of DSB repair, immune response driven by heat shock proteins [45,46].
	High LET radiation	It is justified by the decrease in oxygen enhancement ratio with increasing LET. Thus, cellular radiosensitivity to high LET radiation is less dependent on oxygen tension as compared to low LET radiation.

therapeutic treatment to a tumour. Such models ("therapy models" hereafter) include different effects of tumour hypoxia, such as increased radioresistance, longer cell cycle times, cell arrest and the release of pro-angiogenic growth factors. Modelling different therapeutic procedures is a valuable tool for indicating which treatment strategies are most likely to increase tumour control in a hypoxic environment and therefore should be considered for clinical trials.

In their development, models have pursued two different approaches to calculate predictions of treatment outcome. One approach, referred to as "stochastic", uses real-world statistical data collected from years of observation and implements a probabilistic view towards the modelled processes. While it may seem like a crude approach, the end results emerge holistically without needing an exact analytical description of each process involved. The other approach is based on exact analytical descriptions of every single process involved. This suggests a more accurate solution, but on the downside, given the complexity of the human body, it is very difficult to analytically describe all the mechanisms that occur during treatment. This means that most models will focus on the main biological processes and disregard those that are expected to have a negligible effect on treatment outcome.

Based on the current scientific literature, the sections below present both stochastic and deterministic models developed to test treatments to hypoxic tumours.

4.1. Stochastic/Monte Carlo models

4.1.1. Temporal models

Temporal models explicitly simulate each tumour cell and their progression in time, with non-quiescent cells passing through a cell cycle. Prime examples are the HYP-RT model by Harriss-Phillips et al. [57–60] and the model by Marcu and Marcu [61–63]. These models have included a hierarchy of cell types, from cancer stem cells (CSCs) to differentiated cells. Cell pO_2s were randomly sampled from a distribution. Radiotherapy dose fractions were typically simulated using the Linear Quadratic (LQ) model, which was modified so that hypoxic cells were more radioresistant. For each cell, the LQ expression was evaluated to obtain the probability of cell survival, then a random number was sampled to determine if the cell survived radiation insult. The HYP-RT model also included longer cell cycle times for hypoxic cells, tumour reoxygenation in-between dose fractions and accelerated repopulation (after a kick-off time) via increased CSC symmetric division.

The model by Harriss-Phillips et al. has been used to simulate HNC radiotherapy. For conventional radiotherapy (2 Gy per weekday), on average an additional 16 ± 6 Gy was required to control moderately hypoxic tumours of HNC compared to well-oxygenated tumours [58]. Hypoxic tumours consistently required an extra 15 to 25 Gy for various altered fractionation schedules, with a hyperfractionated schedule of 1.1 Gy twice per weekday found to be the most beneficial, considering both early and late effects [57]. This model was also used to simulate stereotactic body radiotherapy (SBRT) schedules and found that biologically effective doses (BED_{10}) of 125–135 Gy were required to control small hypoxic tumours, which exceed the recommended BED_{10} limit of 100 Gy for SBRT [57].

With the aim of managing hypoxia in SBRT, some authors suggest an inter-fraction interval ≥72 h to facilitate reoxygenation [64]. This is because the shorter overall treatment time in SBRT reduces the chance of reoxygenation in tumours with chronic hypoxia, an outcome that is more pronounced in larger tumour volumes. SBRT also causes vascular damages that could make tumours more hypoxic if cure is not achieved. Kelada et al. showed via hypoxia imaging with ^{18}F-MISO that high single doses of radiation may induce high levels and even persistent states of hypoxia in lung tumours [65].

Marcu et al. also applied their model to HNC and showed that hyperfractionation with 1.2 Gy twice per weekday was superior to the conventional schedule for a moderately hypoxic tumour (mean pO_2 of 6 mmHg) [62], but that only well-oxygenated and mildly hypoxic tumours (the latter with a mean pO_2 of 9 mmHg) are likely to be controlled by hyperfractionated radiotherapy alone [63] (Fig. 1). Otherwise, tumour control is achievable when radiotherapy is administered in combination with other cytotoxic therapies. The group also simulated hyperfractionated radiotherapy combined with the anti-CSC drug all-trans-retinoic acid (ATRA) a differentiating agent which increased asymmetric division of CSCs, cell arrest and apoptosis [61]. For moderately hypoxic tumours, ATRA was beneficial if treatment triggered the recruitment of quiescent CSCs to the cell cycle [62].

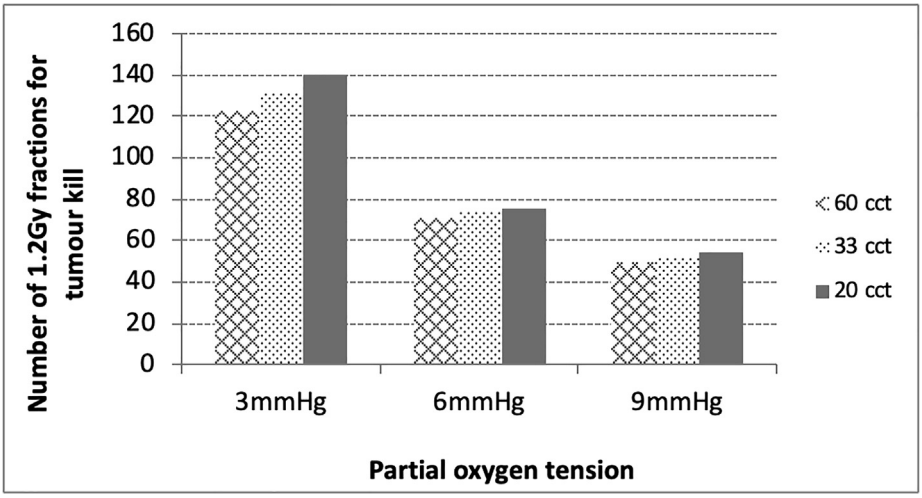

Fig. 1. HNC response as a function of growth kinetics and hypoxia. Tumour hypoxia has been modelled for three scenarios: severely hypoxic (3 mmHg), moderately hypoxic (6 mmHg) and mildly hypoxic tumours (9 mmHg) for three HNC groups with different growth kinetics (20 h, 33 h and 60 h cell cycle time (cct)) [63].

4.1.2. Spatial models

A spatial component may be beneficial for the simulation of tumour hypoxia. If a spatial network of blood vessels is modelled, cell or tissue pO_2 can be assigned in spatial relation to the vessels. Dasu and Toma-Dasu developed a spatial therapy model, which originated with two-dimensional (2D) modelling of vessel units and tissue pO_2 [66–70]. A cross section of tumour tissue was simulated. The tissue contained randomly scattered vessel units, such that a certain distribution of intervascular distances was satisfied. A 2D pO_2 map was then obtained by numerically solving an oxygen diffusion equation (a partial differential equation):

$$-\nabla(D\nabla p) + q(p) = 0$$

where p is the partial oxygen tension (pO_2), D is the diffusion coefficient and $q(p)$ is a function describing the local consumption of oxygen.

Using this method, contributions to pO_2 were obtained from multiple nearby vessel units. They typically used a mean vessel pO_2 of 40 mmHg, an oxygen diffusion coefficient of 2×10^{-5} $cm^2 s^{-1}$ and a maximum oxygen consumption rate of 15 mmHg s^{-1}. Acute hypoxia was simulated by disabling a fraction of the vessel units.

This model by Dasu and Toma-Dasu was then extended to three-dimensions (3D) (though without implementing a connected vasculature) [71]. Voxelized 3D tumours are modelled with a typical voxel size of 200 μm [72]). The number of cells in each voxel and the pO_2 in each voxel are tracked. The surviving fraction after a radiotherapy dose fraction was calculated on a per voxel basis. The LQ model was typically used, modified for the voxel pO_2. Tumour reoxygenation was simulated in-between fractions in the form of changes to the voxel pO_2 map (local oxygenation changes). While this model did not explicitly simulate the progression of virtual time, temporal processes such as intra-fraction repair and accelerated repopulation were accounted for in further studies by including additional terms in the LQ expression [73,74]. Dasu et al. have also used different radiosensitivities for chronically and acutely hypoxic cells, based on the premise that prolonged oxygen deprivation associated with chronic hypoxia may lead to depleted energy levels and impair the cell's ability to repair DNA damage [75]. Thus, the LQ model was modified to include inducible repair in acutely but not chronically hypoxic cells.

The results from Dasu and Toma-Dasu's model showed that fractionation was superior to single-fraction treatment for hypoxic metastasis-like radiosurgery targets [76]. This was also the case for hypoxic tumours of non-small cell lung cancer (NSCLC) [77]. Hypofractionation with 3 to 5 fractions was optimal for NSCLC [64]. Another study found that hypoxic tumours of cervical cancer were controlled by external beam radiotherapy plus pulsed dose-rate brachytherapy (using ^{192}Ir), but not by external beam alone (assuming fast reoxygenation) [73]. This model has also been used to simulate heterogenous/segmented spatial dose distributions, with higher doses delivered to hypoxic regions [71], and carbon ion radiotherapy [72,78].

4.1.3. Spatio-temporal models

Spatio-temporal therapy models are hybrid models that include spatial information about the tumour and, at the same time, simulate the progression of virtual time. Some spatio-temporal models simulate individual cell cycling. In these models, cells typically occupy positions on a 3D lattice. Examples are the works by Kocher et al. [79–81] and Harting et al. [82–84]. When a cell divides, it pushes other cells outward in the lattice and the removal of a cell causes other cells to fall inward, thus achieving tumour growth and tumour shrinkage. Vessel units are simulated at some of the lattice positions, but they are scattered and do not connect to form continuous vessels. Kocher et al. allocated cell pO_2 based on distance to nearest vessel unit [79], whereas Harting et al. summed contributions from multiple vessel units, assuming independent radial sources [82]. Angiogenesis, in the form of placing an additional vessel unit, was triggered in the model by Kocher et al. if the vessel density became too low [79], while in the model by Harting et al. angiogenesis occurred in response to the accumulation of "tumour angiogenesis factors", which are released by hypoxic cells [82,83]. Both models simulated dose fractions using a pO_2-modified LQ expression evaluated for each cell. Kocher et al. also simulated vessel damages. Both models indicated that hyperfractionation may be beneficial over conventional fractionation for hypoxic tumours [80,84].

Another spatio-temporal model by Kempf et al. [85] simulated the growth of a tumour spheroid by cycling cells that were *not* constrained to lattice positions. However, the simulated tumour was avascular and angiogenesis was not simulated (cell pO_2 was calculated from a fixed pO_2 at the spheroid surface). There are also spatio-temporal cellular automaton models by Powathil et al. [86–89] and Paul-Gilloteaux et al. [90]. These models are powerful but have some potential limitations: they grow tumours in only two dimensions, vessel units are stationary (no angiogenesis), and individual cell movement is either not simulated or not tracked (in the former case, this also means cell division is limited to when there is space available nearby).

A different approach taken by spatio-temporal models is to simulate a voxelized tumour wherein each voxel ("geometric cell") contains a variable number of cells. Each voxel has a set of properties describing its state, including the number of cells it contains. Rather than simulating individual cell cycling, the voxels' state properties are evolved in

virtual time steps. Importantly, these models simulate the transfer of cells between voxels, so that tumours can spatially grow and shrink. Examples include the models by Stamatakos et al. (In Silico Oncology Group, National Technical University of Athens) [91–94] - which was built upon a previous model by Antipas et al. [95–98], and Espinoza et al. [99,100]. A compartmental-style model developed by Jeong et al. [101–103], has the tumour composed of independent and homogenous tumourlets, with exchanges between compartments of the tumourlet.

In their work, Antipas et al. included the oxygen enhancement ratio (OER, defined as the ratio of the dose under hypoxic conditions to the dose under oxic conditions for an isoeffect), with different OERs for the α and β parameters of the linear quadratic model applied to glioblastoma. Tumour response was simulated using both conventional and altered fractionation schedules for OER values of 1, 2.5 and 3 [95]. Regarding response to therapy, the model indicated that hyperfractionation may be superior to conventional fractionation [95].

Regarding the separation of OER into linear and quadratic components in the framework of the LQ model, radiobiological studies have shown that DNA lesions associated with the single-hit killing (α component) express a lower OER (1.7–1.8) while cellular lesions associated with the quadratic mechanism (β component) present with higher OERs (3.0–3.5) [104]. In a local tumour control model of prostate cancer, Nahum et al. [105] assumed an $OER_\alpha = 1.75$ and $OER_\beta = 3.25$, while based on patient survival data analysis for cervix cancer Carlson et al. estimated an average $OER_\alpha = OER_\beta = 1.5$ [106]. It should be noted that the OER depends on dose and dose rate, and values obtained from *in vitro* studies may not translate well to in vivo.

Espinoza et al. simulated dose painting by numbers (i.e. by voxel pO_2 and cell density) and found benefit with just a few treatment optimizations [91]. Jeong et al. found that conventional fractionation is usually optimal for HNC and that tumour hypoxia increases the total dose required to achieve local tumour control by about 30% [103]. They also demonstrated over- and under-treatment with a range of fractionation schedules [102].

A work in progress is the spatio-temporal stochastic model developed by Forster et al. that evaluates the impact of tumour angiogenesis and hypoxia on tumour growth with further aim to assess response to both low- and high-LET therapy [107,108]. In the model, cells are randomly positioned in 3D space without overlap, while blood vessels consist of strings of vessel units branching outwards to achieve the required vascular volume. Main model parameters include the relative vascular volume, blood oxygenation, distance from vessels to onset of necrosis and probability of cancer stem cells to undergo symmetrical division. According to the model results, the pre-treatment tumour volume doubling time was shown to vary from 44.5 ± 0.8 days for oxic tumours to 129 ± 16 days for severely hypoxic tumours. By its design, the model allows spatial assessment of tumour kinetics as a function of hypoxia-related parameters, preparing the ground for radiotherapy simulations using Monte Carlo track structure techniques.

4.2. Numerical/deterministic models

As mentioned above, deterministic (a.k.a. analytical, mathematical or numerical) models of therapy, unlike stochastic simulations, use partial differential equations to model biological phenomena. This deterministic approach typically uses numerical methods to solve problems.

Several groups followed the deterministic path for mathematical modelling of hypoxic tumours and their response to therapy. Carlson et al. developed a hypoxic tumour model that was treated with radiation by means of a temporal pattern of dose delivery [109]. An oxygen distribution was generated reflecting an arrangement of straight capillaries surrounded by tumour cells. The oxygen partial pressure *p(r)* was determined as a function of radial distance (*r*) from the vessel wall:

$$p(r) = p_0 \frac{R_{max}^2}{R_0^2}\left(2\ln\frac{R_{max}}{r} - 1 + \frac{r^2}{R_{max}^2}\right)$$

where p_0 represents the partial oxygen pressure next to the vessel wall, R_{max} is the oxygen diffusion limit in tissue and R_0 is a constant related to the rate of oxygen diffusion and consumption. Both conventional and hypofractionated radiotherapy schedules were simulated. The oxygen partial pressure was expressed as a function of radial distance from the capillary wall. A decrease in pO_2 resulted in a decrease of the lethal damage through one-track processes by a constant and through two-track processes by the square of this constant. Aside from hypoxia, the model considered intrafraction double-strand break repair and clonogen (accelerated) repopulation [109]. The results indicated that hypofractionation is less effective at controlling hypoxic tumours than conventional fractionation. Tumour hypoxia had the largest negative impact on treatment efficacy for treatments with less than 10 fractions.

In a model developed by Strigari et al. [110] to investigate stereotactic hypofractionation outcomes, tumour hypoxia was considered in a Poisson model of tumour control probability (TCP):

$$TCP = e^{-N_0(P_0+P_h)}$$

where P_0 and P_h are the fractions of oxic and hypoxic cells, respectively, after treatment completion. These two parameters were dependent on repopulation during treatment and reoxygenation after every dose fraction. The model allowed clinical outcome prediction associated with SBRT considering both direct killing as well as indirect vascular damage.

Another mathematical model that compared hypofractionated with conventional radiotherapy was designed by Chvetsov et al. for non-small cell lung cancer [111]. This model also compared a uniform dose distribution with a non-uniform dose distribution that targeted regions of the tumour containing hypoxia at the beginning of treatment. Tumour response to radiotherapy considered the major components that dictate the clinical endpoint: hypoxia, radiosensitivity and cell proliferation. Sensitivity studies were performed on the numerical simulations for several parameters, including the volume proportion of hypoxia and the rate of reoxygenation. The non-uniform hypoxia-targeting dose distribution was found to be more effective than the uniform dose distribution for a hypofractionated regimen, but the opposite was found for conventional radiotherapy [111].

Numerical simulations and mean-field analysis of a tumour model based on age-dependent stochastic processes was explored in a study aiming to analyse the behaviour of heterogeneous, multi-scale cellular population [112]. The model considers oxygen-dependent progression through the cell cycle in order to explore the dynamics of the oxygen-regulated G1/S transition. This aspect helps understanding the impact of cycle-specific therapy on tumour control as well as the occurrence of drug resistance. Quiescent sub-populations are also considered to evaluate the effect of cell recruitment (i.e. the process of re-entering the cell cycle) on resistance to therapy.

A mathematical framework for adaptive treatment planning on the basis of functional hypoxia images or cell density images was developed by Saberian et al. [113]. They simulated the evolution of hypoxia to generate images which were fed into the adaptive planning system. The approximation algorithm developed in their work – the certainty equivalent control - calls for the solution of a sequence of convex programs over the treatment course. Two versions of the certainty equivalent control method were implemented: one using hypoxia images obtained from simulating the spatio-temporal evolution of hypoxia and the other using cell density images which were derived from the hypoxia images. Numerical simulations of spatio-temporal evolution of hypoxia in head and neck cancers were conducted using a first-order vector autoregressive process, leading to a lognormal distribution of pO_2. The approach chosen by the authors is not without challenges, as the implementation of this framework demands exact mathematical definitions of (1) the tumour state dynamics, (2) the normal tissue state

dynamics (BED constraints for serial and parallel organs), (3) the feasible control variables and (4) the measure of treatment effectiveness. Furthermore, adaptive planning strongly depends on the accuracy of the available functional images. The results suggest that dynamic planning by adapting the fluence-map before every dose fraction based on a functional hypoxia image or cell density image could greatly improve the planning process and further guide the treatment [113].

Population-based response models are another valuable model category as they investigate the behaviour of a virtual population of cancer patients to identify trends among individuals as opposed to examining a single subject. In line with this, Avanzo et al. included in a population-based model a correction factor for a fraction of patients with more hypoxic tumours [114]. Patients were treated for nasopharyngeal cancer with intensity modulated radiotherapy combined with chemotherapy. In the model, patients were divided into two groups (fractions): one fraction (H) with tumours having more severe hypoxia and another fraction (1-H) encompassing the remaining patients with low hypoxia levels. The population TCP was expressed as:

$$TCP = TCP_h(H) + TCP_a(1 - H)$$

where TCP_h is calculated with OER factors ($OER_\alpha = OER_\beta = 1.5$), and TCP_a is determined without OER. The model estimation of tumour control (average TCP = 90.9%) showed good correlation with the local control rate (86.7%) reported in a clinical study from the authors' institution.

Various schedules of proton and heavy ion therapy for hypoxic tumours were simulated via a microdosimetric kinetic model by Strigari et al, in an attempt to incorporate the oxygen enhancement ratio in treatment planning for ion beam therapy [115]. The model considers the specific biophysical characteristics of ion therapy in terms of LET spectra, tissue type and fractionation regimes, supported by *in vitro* data on different cell lines under both oxic and hypoxic conditions. Treatment simulations were conducted on a planning system designed for ion beam therapy with active scanning developed by INFN (Istituto Nazionale di Fisica Nucleare) that includes beamlet superposition and secondary interactions within tissues [116]. The model showed that hypoxia has a strong impact on treatment outcome and indicated that higher tumour control can be achieved with high-LET ion beams. For acute hypoxia, the optimal treatment regimen was found to be hypofractionated high-LET therapy. Further model developments aim to employ hypoxia distribution maps toward biological adaptive treatments.

Simulated treatments to hypoxic tumours from the wide range of therapy models discussed above are summarised in Table 2.

Table 2. Insights that have been gained from *in silico* modelling on ways to combat/overcome tumour hypoxia.

5. Limitations of current therapy models for testing hypoxia-targeting treatments

The hypoxia-targeting methods that models have evaluated to date are mainly dose fractionation, high-LET radiation and dose-painting. As discussed above, most novel hypoxia-targeting treatments (e.g. PFCs, HAPs and molecular targeting drugs) rely upon the tumour vasculature for their delivery. Accordingly, comprehensive modelling of tumour vasculature, tumour angiogenesis and vessel perfusion could be important for therapy models that test hypoxia-targeting treatments. Thus far, therapy models have generally simulated discrete, scattered blood vessel units. It could be advantageous to instead model a connected network of whole blood vessels. For instance, Espinoza et al. simulated parallel, linear vessels [117], but ultimately only to obtain pO_2 distributions in "geometric cells" [99,100]. Modelling a connected network of blood vessels would provide realistic microscopic distributions of pO_2 in relation to the vessels, enable suitable consideration of vessel damages and help to simulate the delivery of drugs, nanoparticles, and other agents. 3D models of connected tumour vasculature and angiogenesis exist outside of therapy models [118–125].

Therapy models have almost always simulated radiotherapy using the pO_2-dependent LQ model. It may be beneficial to instead simulate the radiation effect starting from Monte Carlo track structure (MCTS), generated from a radiobiological, step-by-step MCTS code such as Geant4-DNA [126–128], PARTRAC [129–132], RITRACKS [133,134] or KUBEC [135]. Spatial distributions of DNA damage can be obtained from track segments through cell nuclei, then stochastic modelling of DNA repair and misrepair can be simulated to predict cell death or survival, as has been demonstrated in single cell simulations [136–138]. The radiochemical oxygen effect can be modelled mechanistically by increasing the conversion of radiation-induced DNA radicals to strand breaks with increasing cellular pO_2 [51]. Zhang et al. [139] developed a therapy model that uses MCTS, though not to the degree described above; it uses fast MCTS to predict the yields of simple and complex DSBs, which are then input into the deterministic two-lesion kinetic model. Nevertheless, it signifies potential interest in using MCTS in therapy models.

6. Conclusion and future perspectives

The current challenges facing hypoxia-targeting treatments are toxicity and inefficient delivery of additional O_2, (pro)drugs or NPs to hypoxic regions of the tumour. There is ongoing research into the development of technologies and treatments that target hypoxia in two or more different ways, which appear promising.

As more effective hypoxia-targeting treatments are developed, implementing them in the clinic as part of standard treatment will be a separate challenge of considerable magnitude. The main difficulty lies in the impracticality of identifying and quantifying tumour hypoxia in each individual patient with the current technology, which will be required to stratify patients by hypoxia status, assuming hypoxia-targeting treatments will be selectively administered to patients with hypoxia.

The aim of this work was to present the current landscape of *in silico* models of tumour hypoxia and response to treatment, and to identify modelling trends and future clinical needs for an improved management of radiobiological hypoxia.

Various types of *in silico* therapy models have been used to compare the effectiveness of different radiotherapy fractionation schedules for controlling hypoxic tumours. This demonstrates the potential for *in silico* modelling to help identify effective treatment strategies for dealing with hypoxic tumours, which could include treatments that make use of the emerging and rapidly developing hypoxia-targeting techniques. *In silico* models could play a complementary role to preclinical animal models, helping to justify which hypoxia-targeting treatments should go to clinical trials and indicating optimal treatment details. Current therapy models should attempt to incorporate more sophisticated modelling of tumour vasculature, tumour angiogenesis and vessel perfusion in order to become more useful for testing hypoxia-targeting treatments, which typically rely upon the tumour vasculature for delivery of additional O_2, (pro)drugs and/or NPs. Future *in silico* models should allow for even better incorporation of patient-specific values in order to optimise the treatment path for each individual patient.

In silico models are a continuously evolving tool that allow fast and non-invasive simulations of various treatment strategies. They started as a novelty who's use in clinical setups was questioned by many, to become an accepted and valuable asset in the clinician's toolbox. The exponential growth of computing power and the establishment of dedicated teams that develop models incorporating areas of artificial intelligence, like machine learning and deep learning, has led to *in silico* models being used in most high-level treatment facilities around the world. Advancements in radiomics and radiogenomics may soon provide a breakthrough in the war against hypoxia by making it possible to obtain detailed information about tumour oxygenation status from the routinely acquired diagnostic images.

Table 2
Insights that have been gained from *in silico* modelling on ways to combat/overcome tumour hypoxia.

Reference	Type of model	Method of simulating hypoxia	Aim	Conclusions
HYP-RT model by Harriss-Phillips et al. [58,60]	Stochastic temporal, cellular	Cell pO_2 values were sampled from a hypoxic distribution.	Simulate conventional fractionated HNC radiotherapy, including the effects of accelerated repopulation and reoxygenation.	Hypoxic HNC tumours required require an extra 16 ± 6 Gy to control than well-oxygenated tumours.
[59]			Compare various fractionation schedules for HNC radiotherapy.	Hyperfractionation with 10 × 1.1 Gy per week is optimal irrespective of oxygen status considering TCP + early and late toxicity.
[57]			Compare hypofractionated and SBRT-like schedules for HNC radiotherapy.	A BED_{10} of 125 to 135 Gy may be required to control small hypoxic HNSCC tumours, compared to a BED_{10} of ~80 Gy for well-oxygenated tumours.
Model by Marcu & Marcu [63]	Stochastic temporal, cellular	Variable values of mean pO_2 were considered.	Simulate hyperfractionated radiotherapy with 10 × 1.2 Gy per week.	Moderately and severely hypoxic HNC tumours are unlikely to be controlled by radiotherapy alone.
[61,62]			Explore the effects of ATRA administered with hyperfractionated radiotherapy (10 × 1.2 Gy per week) for HNC.	ATRA could almost halve the radiation dose required to control a moderately hypoxic tumour.
Model by Dasu & Toma-Dasu et al. [68]	Stochastic spatial + deterministic temporal, tumour composed of voxels that each contain multiple cells	Scattered blood vessel units, a differential equation describing oxygen diffusion and consumption is solved numerically to generate a 3D pO_2 map.	Explore the benefit of spatially non-uniform dose distributions that target hypoxia.	A segmented dose distribution according to initial regions of hypoxia was superior to a uniform dose distribution. A finely heterogenous distribution should be avoided due to oxygen changes occurring during the course of treatment.
[76]			Compare single-fraction vs fractionation for metastasis-like radiosurgery targets	Fractionation with 3 to 5 fractions is superior to single-fraction treatment for hypoxic tumours, especially large ones of diameter ~ 3 cm (assuming reoxygenation).
[72]			Explore the effect of carbon ion radiotherapy on hypoxic tumours	Reoxygenation is still important for carbon ions, so extreme hypofractionation as well as hyperfractionation should be pursued with caution.
[78]			Simulate carbon-ion therapy for HNC	The benefit of C-ion therapy over conventional fractionated x-rays is greater for hypoxic tumours than well-oxygenated tumours if the C-ion therapy is delivered in more than 12 fractions.
[74]			Compare fractionation schedules for NSCLC	SBRT-like hypofractionated schedules might be optimal for hypoxic tumours.
[73]			Explore the effect of HDR brachytherapy in addition to EBRT for hypoxic cervical cancer	EBRT + brachytherapy has a similar effect on hypoxic tumours as EBRT alone has on well-oxygenated tumours (assuming fast reoxygenation).
Kocher et al. [80]	Stochastic spatio-temporal, cellular, cell positions on a lattice	Discrete blood vessel units scattered on a lattice, initial uniform vessel density of 350 vessel units/mm^3, cells located further than 100 μm from a vessel unit were hypoxic, angiogenesis occurs if the local vessel density drops below 90% of the normal density.	Compare fractionation schedules for a hypoxic tumour, considering cell cycle times of 2 and 5 days	Hyperfractionation with 10 × 1.6 Gy per week gave 40–80% higher tumour control than conventional fractionation for a CCT of 2 days
Kempf et al. [85]	Stochastic spatio-temporal, cellular, cell positions not confined to a lattice	Nutrients including O_2 diffuse into a growing avascular tumour spheroid from the surrounding normal tissue.	To study the spatio-temporal dynamics of tumour hypoxia during radiotherapy, including for fractionation schedules of 1 × 2 Gy per 24 h or 1 × 4 Gy per 48 h	There can be fast and drastic changes to chronic diffusion-limited hypoxia. Sustained tumour reoxygenation was only achieved in the absence of hypoxic cell radioresistance (which could be achieved by a hypoxic radiosensitiser).
Paul-Gilloteaux et al. [90]	Stochastic spatio (2D)-temporal, cellular automaton	Vessel units were randomly scattered. The vascular density was set to 3.8% of the voxels. A 2D pO_2 map was obtained by applying a Gaussian filter to the vessel map, resulting in 1% of cells with $O_2 < 0.2\%$ and an average O_2 of 3%. Radiation-induced vessel damages are simulated.	Compare fractionation schedules.	The optimal dose per fraction for hypofractionation was 8 Gy. At this dose, there was enhancement of vessel perfusion and minimal blood vessel death, which resulted in some tumour reoxygenation.
Jeong et al. [103]	Stochastic temporal, tumour composed of tumourlets	One of the three compartments of a tumourlet is the hypoxic compartment, which contains hypoxic cells.	Compare radiotherapy fractionation schedules.	The dose for 50% TCP was smaller for conventional fractionation (2–2.4 Gy) than 4–5 Gy per fraction. Hypoxic tumours required ~30% more dose for control.

(*continued on next page*)

Table 2 (*continued*)

Reference	Type of model	Method of simulating hypoxia	Aim	Conclusions
Espinoza et al. [99]	Stochastic spatio-temporal, tumour composed of voxels that contain variable numbers of cells	The proliferation of capillary cells in each voxel is evolved analytically in virtual time steps. The vascular fraction in the voxel is used to derive the pO_2 distribution therein.	Simulate conventional fractionated HNC radiotherapy	The total dose to achieve 50% TCP was 71.0 Gy for a hypoxic tumour and 53.6 Gy for a well-oxygenated tumour (vascular fractions of 3.6 and 7.2%, respectively).
[100]			Explore the benefit of dose painting by numbers (DPBN) with conventional fractionated HNC radiotherapy. The spatial dose distribution was optimized to minimise either the number of surviving cells or the spatial heterogeneity of surviving cells.	DPBN was more beneficial for tumours that exhibited reoxygenation. Optimisation minimising cell survival was the more effective approach. A total of three optimisations over the course of treatment provided a similar amount of benefit as optimising before every fraction.
Forster et al. [107,108]	Stochastic spatio-temporal (4D), cellular	pO_2 modelled dynamically as a function of distance from nearest vessel; angiogenesis reflects a connected and chaotic vasculature with vessels represented by discrete units.	To evaluate tumour growth kinetics and to provide the foundation for a 4D cellular radiotherapy simulation tool.	Tumour volume doubling time for HNSCC varied by a factor of 3 from oxic to severely hypoxic tumours; symmetric division probability of cancer stem cells strongly impacts on doubling times. The model sets the background for radiotherapy simulation of both low- and high-LET beams with Monte Carlo track structures.
Carlson et al. [109]	Numerical	pO_2 modelled as a function of radial distance from capillary wall.	Compare radiotherapy fractionation schedules for HNC and prostate cancer.	The effectiveness of hypofractionation is greatly reduced by tumour hypoxia. Conventional fractionation is usually superior to hypofractionation for hypoxic tumours.
Saberian et al. [113]	Numerical	Stochastic spatio-temporal evolution of hypoxia is modelled using a first-order vector autoregressive process.	For HNC radiotherapy, assess the benefit of adapting the fluence-map before every dose fraction based on a functional hypoxia image or cell density image. Note that after a few fractions, regions of high cell density should correspond to hypoxia. In the absence of images, the evolution of hypoxia and cell density were simulated.	Adaptive hypoxia-targeted dosimetry is just as beneficial whether using functional hypoxia images or cell density images.
Chvetsov et al. [111]	Numerical	Initial hypoxic volume of 20%	Assess the benefit of a non-uniform dose distribution targeting the initial spatial distribution of hypoxia for conventional and hypofractionated radiotherapy of NSCLC.	A non-uniform dose distribution (non-adaptive) was more beneficial for hypofractionated schedules than for conventional fractionation due to reoxygenation effects.
Avanzo et al. [114]	Numerical, population-based response model	Hypoxia was incorporated in TCP calculation via a correction factor for a fraction of patients with more hypoxic tumours	To correlate tumour control probability with local control rate given by clinical studies.	Hypoxia considerations must be incorporated in TCP estimations to obtain correlations with clinical data (local control).
Strigari et al. [115]	Numerical, interfaced with a treatment planning system for ion therapy	A cubic volume of uniform variable pO_2 is considered inside the planning target volume generated by the treatment planning system.	Simulate proton and heavy ion therapy for hypoxic tumours and compare fractionation schedules.	High-LET ions and/or hypofractionation improve control of hypoxic tumours.

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