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Altered fractionation outcomes for hypoxic head and neck cancer using the *HYP-RT* Monte Carlo model

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Objective: Altered fractionation radiotherapy is simulated on a set of virtual tumours to assess the total doses required for tumour control compared with clinical head and neck data and the doses required to control hypoxic vs well-oxygenated tumours with different radiobiological properties.

Methods: The *HYP-RT* model is utilised to explore the impact of tumour oxygenation and the onset times of accelerated repopulation (AR) and reoxygenation (ROx) during radiotherapy. A biological effective dose analysis is used to rank the schedules based on their relative normal tissue toxicities.

Results: Altering the onset times of AR and ROx has a large impact on the doses required to achieve tumour control. Immediate onset of ROx and 2-week onset time of AR produce results closely predicting average human outcomes in terms of the total prescription doses

in clinical trials. Modifying oxygen enhancement ratio curves based on dose/fraction significantly reduces the dose (5–10 Gy) required for tumour control for hyperfractionated schedules. *HYP-RT* predicts 10×1.1 Gy per week to be most beneficial, whereas the conventional schedule is predicted as beneficial for early toxicity but has average–poor late toxicity.

Conclusion: *HYP-RT* predicts that altered radiotherapy schedules increase the therapeutic ratio and may be used to make predictions about the prescription doses required to achieve tumour control for tumours with different oxygenation levels and treatment responses.

Advances in knowledge: Oxidic and hypoxic tumours have large differences in total radiation dose requirements, affected by AR and ROx onset times by up to 15–25 Gy for the same fractionation schedule.

HYPOXIA AND RADIOTHERAPY FRACTIONATION

Hypoxia is a well-known problem in radiotherapy owing to the increased radioresistance of hypoxic cells. Upon diagnosis, head and neck cancers, and more specifically head and neck squamous cell carcinoma (HNSCC), commonly exhibit significant regions of tumour hypoxia. Often described in terms of partial pressure of oxygen (pO_2) in units of mmHg, tissues are commonly described as hypoxic when the pO_2 level falls below 10 mmHg, although 5.0 and 2.5 mmHg thresholds are also utilised in the literature [1].

The increased radioresistance of hypoxic tumours has been verified in multiple clinical trials, which report on the relatively poor radiotherapy local control (LC) rates of tumours with low oxygenation [1,2]. Moreover, the variability of the hypoxic subvolume and severity of hypoxia between patients can be very large [1]. Consequently, predicting the response of an individual tumour to conventional or altered fractionation radiotherapy is extremely difficult.

Altered fractionation has been shown to improve LC rates of HNSCC in a number of randomised Phase II and III clinical trials (Schedules 2–8 in Table 1). However, specific benefits in terms of either tumour control probability (TCP) or normal tissue complication probability for individual patients are difficult to deduce because of the large number of tumours in the trials. Moreover, there is difficulty in stratifying clinical trial patients into smaller groups with similar tumour properties while maintaining the numbers required for statistical significance. Important radiobiological properties that may vary among tumours in a patient cohort include the level of hypoxia, the onset time of accelerated repopulation (AR) and the timing of reoxygenation (ROx).

MODELLING HYPOXIC TUMOUR RESPONSE

HYP-RT is a Monte Carlo (MC) model developed to propagate a virtual head and neck tumour, starting from one infinitely dividing stem cell, up to 100 million cells. The model incorporates a realistic epithelial cell hierarchy, tumour hypoxia and mimics the effects of AR and ROx during radiotherapy-induced tumour shrinkage [3–5].

Models utilising MC methods to simulate cell propagation and the effects of oxygenation of radiotherapy

response began in the 1980s [6,7]. A stochastic cell-based spatial and/or temporal model in the current era is being perused by researchers such as Stamatakis, Dionysiou, Borkenstein, Harting, Daşu and Titz [8–13]. Many models now focus on spatial-based cell parameterisation and the placement of cells within a “lattice” defining the tumour volume. The *HYP-RT* temporal model differs from these models through the assumption of random cell placement within the tumour, providing efficient simulation of up to 10^8 individual cells on an off-the-shelf computer. Spatial coordinates are extremely draining on computational resources, and so although cell placement is important for some tumour models, it was not considered necessary for the current work considering the random nature of oxygen allocation to cells and the absence of cell–cell interactions in *HYP-RT*.

HYP-RT utilises a continuous pO_2 histogram to define the initial tumour oxygenation status while modelling realistic epithelial cell hierarchy and incorporating both the dynamic processes of AR and ROx induced by treatment. This combination of features makes the model unique in terms of speed and the simple use and interpretation of temporal tumour and treatment-based parameters.

AIMS

To model head and neck tumour cell kill during conventional 5×2 Gy per week radiotherapy and altered fractionation radiotherapy to predict:

- the total doses required to achieve tumour control (total cell kill) for tumours responding with different AR and ROx onset times
- the increased doses required to control hypoxic compared with well-oxygenated (oxic) tumours
- the impact of the oxygen enhancement ratio (OER) curve utilised in simulations when adjusted according to the dose per fraction delivered in each schedule
- the efficacy of each of the schedules, using total dose results and calculated biological effective doses (BEDs) for average early (acute) and late responding normal tissues, by estimating those with the least toxicity for the same chance of tumour control, *i.e.* those having the highest therapeutic ratio.

Note that for one of the modelled hyperfractionated schedules (Schedule 11 in Table 1), a 2-week

unplanned treatment break was considered to obtain quantitative predictions about the corresponding increases in the dose and time required to account for the break.

METHODS

Modelling *in vivo* cell division and hypoxia

The *HYP-RT* temporal tumour growth algorithm has been reported and justified in detail [4,5], and therefore the description of the modelling methods in the current report is brief. This has previously included explanations about the values and ranges of cell and environment-based default model parameters and how these are allocated using random number generation and stored for each cell.

Epithelial tumour cell kinetics is modelled in *HYP-RT* based on set probabilities of division for stem (S) and transit (limited generation number) (T) cells. Differentiating (D1 or D2) and fully differentiated (D3) cells are also considered as well as cells in a temporary state of quiescence induced by extreme hypoxia (≤ 1 mmHg). Modelled stem cells always divide into two daughter cells, with one daughter cell a stem cell and the other a stem, transit or differentiating cell (3:87:10 probability ratio) (Figure 1).

In *HYP-RT*, stem cells, transit cells and first generation differentiating cells are referred to as “basal” cells, as they would normally reside in the basal layers of healthy epithelial tissue. These cells have a chance of repopulating the tumour either because of their inherent proliferative capacity or owing to possible mutation and de-differentiation into stem-like cells under stressed hypoxic environmental conditions. As such, many of the results presented in the current work relate to the killing of all basal plus all hypoxic cells rather than presenting results obtained after total cell death.

Tumour oxygenation (hypoxic or oxic) is modelled using continuous probability distributions based on human HNSCC Eppendorf pO_2 histogram data [14,15]. When a cell divides, one daughter cell is assigned a pO_2 value equal to that of the mother cell. If a second daughter cell survives mitosis, it is randomly allocated a new pO_2 value from the distribution. The pO_2 value allocated can alter the cell cycle time by a factor of up to 3, with a final maximum cell cycle time

of 60 h. The allocated pO_2 value of a cell stays fixed during a cell's lifetime unless affected by ROx during treatment.

In the model, cells have a set probability of becoming quiescent if their pO_2 level is ≤ 1 mmHg. Hypoxic quiescent cells die owing to necrosis with a half-life of 4 days unless reoxygenated.

Modelling fractionated radiotherapy

The *HYP-RT* radiotherapy algorithm was developed to simulate the effects of fractionated therapy, assuming that a uniform dose is delivered to all tumour cells. The linear quadratic (LQ) theory of cell survival is used to calculate average cell survival probability for cycling oxic cells. The surviving fraction is then converted to a cell death probability between 0.0 and 1.0. This is calculated for each cell, for each dose fraction in the schedule. The default α and β values used are 0.3 and 0.03, respectively ($\alpha/\beta = 10$ Gy). For a standard 2 Gy treatment schedule, the default surviving fraction at 2 Gy (SF_2) (for oxic cycling cells) value applied is 48.7%. This value was fixed for the current study to allow for schedule comparisons; however, it may be varied or allocated a range by the user to study inter-patient radiosensitivity variation effects.

Cells are assessed individually and chronologically to determine whether they die or survive each treatment fraction. For cycling cells, the final cell death probability is determined using the LQ theory surviving fraction after d Gy (SF_d) factor and an OER factor (Figure 2; [16,17]). The OER factor is applied in terms of a “reduction in death probability” factor based on the cell's pO_2 level and use of normalised OER curves. The three different OER curves shown in Figure 2 were normalised by scaling them to have maximal values of 1.0 at 60 mmHg [4]. The final death probability of a cell could then be calculated as $(1 - SF_d)$ multiplied by the OER factor.

A total of 11 fractionation schedules are simulated in this work to investigate the potential of the *HYP-RT* model for predicting the total doses required to achieve complete basal cell kill (LC) for HNSCC virtual tumours (Table 1). 7 of these 11 schedules (2–8) are based on successful altered clinical trials that have been proven to increase the therapeutic ratio, while one is the conventional schedule (1), two are non-successful schedules

Table 1. The 11 head and neck squamous cell carcinoma clinical trial schedules simulated with the *HYP-RT* model

Trial number	Clinical trial simulated	Fractionation category	Fractionation scheme											
			M	T	W	Th	F	S	Su	M	T	W	Th	F
1	Conventional	Standard 2 Gy per fraction	I	I	I	I	I			I	I	I	I	I
2	RTOG: Fu et al [18]	Hyperfractionated	II	II	II	II	II			II	II	II	II	II
3	Pinto et al [19]	Hyperfractionated	II	II	II	II	II			II	II	II	II	II
4	Hiliniak et al [20]	Accelerated	I	I	I	II	I			I	I	I	II	I
5	DAHANCA: Overgaard et al [21]	Accelerated	I	I	I	I	I	I		I	I	I	I	I
6	GORTEC: Bourhis et al [22]	Hyperaccelerated	II	II	II	II	II			II	II	II	II	II
7	UK (CHART): Dische et al [23]	Continuous hyperaccelerated	III	III	III	III	III	III	III	III	III	III	III	III
8	RTOG: Fu et al [18]	Concomitant Boost (after 5 weeks)	I	I	I	I	I			I	I	I	I	I
			II	II	II	II	II			II	II	II	II	II
9	EORTC split course 22851, Horiot et al [24]	Split course hyperaccelerated (2-week break after 8 days)	III	III	III	III	III			III				
			III	III	III	III	III			III	III	III	III	III
10	Polish (CAIR): Skladowski et al [25]	Continuous accelerated	I	I	I	I	I	I	I	I	I	I	I	I
11	RTOG: with an unplanned treatment break	Split course hyperaccelerated (2-week break after 2 weeks)	II	II	II	II	II			II	II	II	II	II

#, fraction of radiotherapy; BED₃, calculated value of the biological effective dose using an α/β value of 3 Gy and the clinical trial prescription total dose and schedule; CAIR, continuous accelerated irradiation; CHART, continuous hyperfractionated accelerated radiotherapy; DAHANCA, Danish Head and Neck Cancer Group; EORTC, European Organisation for Research and Treatment of Cancer; GORTEC, French Head and Neck Oncology and Radiotherapy Group; LC, local control; RT, radiotherapy; RTOG, Radiation Therapy Oncology Group.

Schedules 2–8 are from therapeutically beneficial trials, Schedules 9 and 10 are from trials with no therapeutic gain, and number 11 is a schedule with an unscheduled 2-week break to mimic patient recovery time when acute side effects become temporarily too severe to proceed with treatment.

(9 and 10) and one is an altered schedule (11) with an unplanned 2-week treatment break.

The methods used to implement AR through loss of asymmetrical stem cell division as well as the gradual daily increase of oxygenation levels to cells to simulate

ROx have been outlined in detail previously [3]. Two different AR and ROx onset times are simulated for each schedule (0 weeks and 2 weeks) as well as no AR or ROx effects, with model outcomes averaged over nine different simulations using unique random number seeds (three radiotherapy runs on three virtual tumours).

Table 1. Continued

S	Su	Repeating	Dose/# (Gy)	#'s/ day	Days/ week	Total dose (Gy)	Total #	Total time (weeks)	Trial LC (%) at 2–5 years (vs conventional RT)	Late toxicity compared with the conventional schedule arm of the trial, BED _{late}
			2.0	1	1	70.0	35	7.0	49 (conventional average)	N/A BED ₃ = 70 Gy
			1.2	2	5	81.6	68	6.8	54 (vs 46)	Late—slight increase, not significant, BED ₃ =114 Gy
			1.1	2	5	70.4	64	6.4	62 (vs 52)	Late—no increase, BED ₃ =96 Gy
			2.0	1 or 2	5	66.0	33	5.5	81 (vs 85)	Late—no increase, BED ₃ =110 Gy
I			2.0	1	6	66.0	33	6.5	76 (vs 64)	Late—no increase, BED ₃ =110 Gy
			1.8	2	5	63.0	35	3.3	58 (vs 34)	Late—no increase, BED ₃ =101 Gy
			1.5	3	7	54.0	36	1.7	52 (vs same)	Late—reduced, BED ₃ =81 Gy
		For 3.6 weeks	1.8	1	5	72.0	4.2	6	55 (vs 46)	Late—no increase, BED ₃ =113 Gy
			1.5 Gy	2 during boosts						
		1.8-week break	1.6	3	5	72.0	45	5.8	59 (vs 46)	Late—increased, BED ₃ =110 Gy
I	I		2.0	1	7	64.0	32	4.6	82 (vs 35)	Late—slight increase, but not significant, BED ₃ =107 Gy
		Break	1.2	2	5	81.6	68	8.8	N/A	N/A

An analysis was performed to determine the AR and ROx onset time combination with total dose results (required to control the virtual tumours) best matching clinical trial prescriptions. This involved calculating the percentage difference in total doses between the simulations and the clinical trials, where the differences were summed for each AR and ROx combination (considering all trial schedules and considering only the “successful” trial schedules) and the summed value closest to zero represented the best matching AR and ROx onset time pair. For oxyc tumour simulations,

a similar methodology was used, but with no ROx considered.

BED calculations for normal tissues

Although non-tumour cells are not currently simulated in *HYP-RT*, BED equations have been utilised to predict relative early and late reacting normal tissue toxicities for the 11 schedules. It was assumed that the normal tissues received 100% of the dose required to control the tumour. In reality, normal tissue doses may be lower than this; however, using the 100% dose allowed

Figure 1. Epithelial cell kinetics in *HYP-RT* simulations, involving stem (S), transit (T), differentiating (D1 and D2) and fully differentiated (D3) cells.

for a non-biased comparison of the schedules. Further, changing this percentage to a value $<100\%$ would only change the relative differences in BED results (and the corresponding BED rankings) among the different schedules.

The BED study was designed to discover which of the schedules could achieve 100% TCP while having relatively low BED and hence low toxicity for normal tissues. Normal tissue BED calculations (Equations 1 and 2, [26]) utilised the total doses required for tumour control as predicted by the model as well as the total treatment time and fraction number:

Figure 2. Oxygen enhancement ratio (OER) curves (before normalisation) implemented in the model for adjusting the radiosensitivity of cells during radiotherapy based on cellular pO_2 and dose per fraction. #, fraction of radiotherapy.

$$BED_{late} = nd \left(1 + \frac{d}{\alpha/\beta} \right) \quad (1)$$

$$BED_{early} = nd \left(1 + \frac{d}{\alpha/\beta} \right) - \frac{\ln(2)(t - T_k)}{\alpha T_p} \quad (2)$$

where d is the dose per fraction, n is the number of fractions, T_k is the repopulation kick-off time, T_p is the potential doubling time (2.5 days) and α/β is the alpha-beta ratio for normal tissues, e.g. early oral mucosa effects (10 Gy) or late normal tissue effects (3 Gy) [27]. Note that complete repair between fractions was assumed for all fractionation schedules in this work and the minimum time between fractions was 6 h.

Based on BED outcomes (and normalisation of BED values to “scores” between 0.0 and 10.0 for each AR and ROx onset time combination), the 11 schedules were ordered (ranked) from most to least beneficial in terms of toxicity for the same chance of tumour control. The normalisation process utilised the following formula:

$$BED_SCORE = 10 \times \frac{(BED - BED_{min})}{(BED_{max} - BED_{min})} \quad (3)$$

where BED_{min} is the minimum BED value in the data set and BED_{max} is the maximum BED value in the data set. This ordering was performed by summing the BED scores for all nine AR and ROx combinations

modelled for each schedule. This process allowed for cross-comparisons of schedules by summing results in a manner that was not biased by the particular schedule's total prescription dose (assuming that each AR and ROx onset time combination is clinically valid and equally likely). A low score represented low toxicity and a high score represented high toxicity.

The validity of the *HYP-RT* model compared with the standard LQ model (for oxic and hypoxic tumours) and the known limitations and assumptions of the cell growth and radiotherapy algorithms have been previously reported [3–5]. Statistical uncertainty in the model results arising from the use of different random number seeds continued to be in the range of 4–6 Gy in the current work [3]. For

figures referenced in the following Results section, error margins (1 standard deviation) of this dose level apply; however, they have been purposely removed from some figures owing to the large amount of data displayed to allow for clearer visualisation of the results.

RESULTS

Tumour simulation outcomes

Model outcomes that compare the doses required for 100% tumour control (for “moderately hypoxic” tumours [3,4]) are presented in Figure 3a,b for seven AR and ROx onset time combinations, illustrating the full range of dose outcomes arising owing to the onset time variations, for each of the 11 schedules. Figure 3a shows that the ranges in results caused by altering AR

Figure 3. The total doses delivered in simulations to achieve 100% tumour control probability (TCP) in (a) hypoxic tumours and (b) oxic tumours, for 11 fractionation schedules (Table 1), compared with the radiation doses delivered in reported clinical trials. AR, accelerated repopulation; ROx, reoxygenation.

and ROx onset times for hypoxic tumours are relatively large (32 ± 12 Gy averaged over all schedules). Figure 3b shows similar but slightly reduced range results for oxic tumour simulations (20 ± 8 Gy averaged over all schedules).

Based on hypoxic tumour simulations, the combination of ROx and AR onset times best matching clinical prescriptions is 0 weeks and 2 weeks, respectively. For oxic tumours, onset of AR at 2 weeks also provides the best overall match to clinical data. Modelled cell survival curves for this 0- and 2-weeks onset time combination (Figure 4) allows the differences in cell kill throughout the duration of the treatment to be visualised. From these curves, the effect of the weekend treatment gaps is also evident, where applicable. Note that all results (refer to Table 1 schedule numbers) and their simulation end points were the elimination of all cells that could potentially grow back the tumour.

Oxic vs hypoxic tumour simulation outcomes

A direct comparison of model outcomes for oxic vs moderately hypoxic tumours is shown in Figure 5a (for no AR in the simulations) and Figure 5b (for onset of AR at 2 weeks). For well-oxygenated tumours, a close match between the model and the clinical data was not expected because it is likely that tumours in clinical trials have at least some degree of hypoxia present at the start of treatment. Note that no ROx was simulated for the data shown in Figure 5.

With no AR simulated, oxic tumours had an approximate 10 ± 6 Gy advantage over hypoxic tumours (Figure 5a). However, with AR onset at 2 weeks, the difference between oxic and hypoxic tumours was reduced and was frequently within the statistical uncertainty of the model (4–6 Gy), e.g. for Schedules 2, 5, 6, 9, 10 and 11 (Figure 5b). This was attributed to the effects of AR and the slightly higher percentage of proliferating stem cells in simulated oxic tumours compared with hypoxic tumours. Further, the simulated hypoxic tumours were comprised of a proportion of quiescent stem cells with extremely low oxygenation levels when no ROx was modelled. For the conventional schedule (Schedule 1) specifically, it took on average an extra 16 ± 4 Gy to control hypoxic tumours for the case of AR onset at 2 weeks and ROx onset at 0 weeks, as previously reported [3].

The impact of dose per fraction-dependent OERs

To mimic the phenomenon of hypoxic cell partial resensitisation for small fraction sizes, three different OER curves were modelled based on the dose per fraction required in the schedule (Figure 2). The use of a reduced (shallower) OER curve had the most impact on hyperfractionated schedules (Schedules 2 and 3) utilising doses per fraction <1.25 Gy and correspondingly having the largest decrease in maximal OER from 3.0 to 2.0 (Figure 6). These hyperfractionated schedules had total dose differences of 5–10 Gy using the

Figure 4. Cell survival curves for moderately hypoxic tumours undergoing simulated altered fractionation schedules, determined from simulations of eight of the clinical schedules outlined in Table 1, with onset of accelerated repopulation at 2 weeks and onset of reoxygenation at 0 weeks (immediately). DAHANCA, Danish Head and Neck Cancer Group; CHART, continuous hyperfractionated accelerated radiotherapy; GORTEC, French Head and Neck Oncology and Radiotherapy Group; RTOG, Radiation Therapy Oncology Group; wk, week.

Figure 5. The total doses required in the model to achieve 100% tumour control probability for oxyc vs moderately hypoxic tumours for 11 schedules (Table 1) compared with the doses delivered in clinical trials; (a) without the simulation of accelerated repopulation (AR) and (b) with onset of AR at 2 weeks. ROx, reoxygenation.

standard vs reduced OER curves (for simulations involving AR).

For schedules with a dose per fraction <1.75 Gy and ≥ 1.25 Gy, there were no significant differences in tumour control doses when using the standard or a more shallow OER curve. Hence, no extra benefit was predicted for Schedule 7 [UK CHART (continuous hyperfractionated accelerated radiotherapy), 1.5 Gy per fraction] for combating hypoxic cell radioresistance. Note that for simplicity, Figures 4 and 6 have neglected the schedules that have been reported to decrease the therapeutic ratio.

Ordering schedules based on normal tissue toxicity

BED results for each AR and ROx onset time combination were normalised to values between 0.0 and 10.0. These results or scores were then summed

for each schedule. Results for the 11 schedules are shown in Table 2 (for AR onset at 0 and 2 weeks or not at all) for oxyc tumours and in Table 3 for hypoxic tumours (for a subset of seven different combinations of AR and ROx onset times). In Tables 2 and 3, note that simulated treatments with the lowest BED scores, *i.e.* predicted beneficial schedules with the lowest doses to normal tissues and hence the least toxicity, are shown in bold italic (≤ 3.0), while simulated treatments with the highest BED score, *i.e.* the highest normal tissue dose and hence the highest toxicity, are shown in italics (≥ 7.0). Intermediately scoring schedules are shown in bold.

Normal tissue BED predictions from oxyc tumour simulations (Table 2) indicate that:

- Schedules 3, 8 and 2 have relatively low toxicity for early effects, whereas Schedules 4, 7, 6 and 10 are the most toxic

Figure 6. The total dose required for hypoxic tumour control for simulations utilising fixed vs dose per fraction dependent oxygen enhancement ratio (OER) curves in simulations for various onset times of accelerated repopulation (AR) and reoxygenation (ROx). Error bars of $\pm 6\%$ (4–6 Gy) are neglected to simplify the figure.

- Schedules 3, 7, 2 and 9 have relatively low toxicity for late effects, whereas Schedules 1, 4 and 5 are the most toxic
- when considering both early and late effects, the overall most beneficial schedule is clearly Schedule 3 followed by Schedules 2 and 8, whereas the 2.0 Gy per fraction schedules (4, 10, 1 and 5) have high toxicity scores
- when considering the onset time of AR of 2 weeks alone, the overall most beneficial schedules are Schedules 3 and 8, whereas Schedules 4 and 7 are clearly the most toxic.

Normal tissue BED predictions from hypoxic tumour simulations (Table 3) indicate that:

- Schedules 3, 2 and 1 have relatively low toxicity for early effects, whereas Schedules 4, 7 and 9 are the most toxic
- Schedules 3, 2 and 7 have relatively low predicted toxicity for late effects, whereas Schedules 1, 4 and 5 are the most toxic
- when considering both early and late effects, the overall most beneficial schedules are Schedules 3 and 2, whereas Schedule 4 is clearly the most toxic followed by Schedules 10 and 1
- when considering the onset time of AR of 2 weeks and ROx of 0 weeks alone, the overall most beneficial schedules are Schedules 3, 9 and 2, whereas Schedule 4 is clearly the most toxic.

When considering a population of oxic as well as hypoxic tumours, the model predicts that hyperfractionated

Schedules 2 and then 3 are most beneficial for early effects and Schedules 4 and then Schedule 7 are most toxic. Further, the conventional schedule has the highest predicted rate of late toxicity but low to average early toxicity. This may indicate that, if altered schedules can maintain the same level of early normal tissue effects clinically as the conventional treatment schedule (judged as low to medium level effects in this work), they could have an advantage and improve the therapeutic ratio as their predicted late toxicities are reduced.

Schedule 11 with the 2-week unplanned break after 2 weeks of treatment, as expected, was predicted to have early effect benefits for normal tissues; however, the extension in overall time required to control simulated tumours meant that it had a mid-range score for late toxicity for both oxic and hypoxic tumours.

DISCUSSION

When varying AR and ROx onset times after the beginning of treatment, total dose results from the model had a large range, even when considering the same fractionation schedule (Figure 3). The average range in dose required to control a tumour was 18 ± 9 (average of 40 Gy), averaging across all 11 schedules. Consequently, relatively few individual simulation dose results agree well with clinical trial prescriptions. However, for all but one extremely hyperfractionated schedule (7: UK CHART), clinical trial prescriptions were within the total dose range predicted by the model.

Table 2. Acute and late responding normal tissue biological effective dose (BED) score (0.0–10.0) results used to compare the predicted benefits of 11 different fractionation schedules used during oxic tumour simulations, with onset of accelerated repopulation (AR) and reoxygenation (ROx) modelled at 0 weeks, 2 weeks or not at all

Acute normal tissue effects: BED formula— $T_k=7$ days, $\alpha=0.35\text{ Gy}^{-1}$, $\alpha/\beta=10\text{ Gy}$, $T_p=2.5$ days							
Tumour type	Schedule number	Dose/fraction of radiotherapy	No AR, no ROx	AR (0 weeks)	AR (2 weeks)	Summed BED scores	Summed BED order
Oxic tumours	1	2.0	3.0	5.0	5.1	13.1	5
	2	1.2	3.0	5.1	4.5	12.6	4
	3	1.1	0.4	0.0	0.0	<u>0.4</u>	<u>1</u>
	4	2.0	5.2	6.8	<i>10.0</i>	22.1	8
	5	2.0	4.7	<i>7.9</i>	5.2	17.8	6
	6	1.8	<i>7.9</i>	<i>8.5</i>	6.0	22.5	10
	7	1.5	<i>10.0</i>	<i>8.5</i>	<i>8.1</i>	<u>26.6</u>	<u>11</u>
	8	1.8	3.0	3.8	3.3	10.1	3
	9	1.6	5.9	<i>8.8</i>	3.7	18.4	7
	10	2.0	<i>7.5</i>	<i>10.0</i>	5.0	22.4	9
	11	1.2	0.0	4.3	3.9	8.1	2
Late normal tissue effects: BED formula— $\alpha/\beta=3\text{ Gy}$							
Tumour type	Schedule number	Dose/fraction of radiotherapy	No AR, no ROx	AR (0 weeks)	AR (2 weeks)	Summed BED scores	Summed BED order
Oxic tumours	1	2.0	<i>7.6</i>	<i>10.0</i>	<i>9.6</i>	<u>27.2</u>	<u>11</u>
	2	1.2	2.0	4.5	3.3	9.8	3
	3	1.1	0.0	2.4	0.1	<u>2.4</u>	<u>1</u>
	4	2.0	<i>8.4</i>	<i>8.1</i>	<i>10.0</i>	26.5	10
	5	2.0	<i>7.3</i>	<i>9.3</i>	5.9	22.4	9
	6	1.8	6.8	4.1	1.7	12.6	5
	7	1.5	4.7	0.0	0.0	4.7	2
	8	1.8	6.6	<i>5.5</i>	4.6	16.7	6
	9	1.6	5.7	5.4	0.7	11.9	4
	10	2.0	<i>10.0</i>	<i>8.9</i>	3.3	22.2	8
	11	1.2	3.6	<i>8.9</i>	<i>8.0</i>	20.5	7

T_k , repopulation kick-off time; T_p , potential doubling time.

A high score indicates a non-beneficial schedule (high toxicity—italics), an intermediate score represents a mid-range schedule (intermediate toxicity—bold) and a low score indicates a beneficial schedule (low toxicity—bold italic). The summed BED scores are added per schedule, with the underlined text representing the schedule with the lowest and highest values.

The range in model results (for the same tumour and treatment schedule but different AR and ROx onset times) could indicate the potential benefits of treatment individualisation in terms of specific patient/tumour prescriptions if the kinetics of AR and ROx could be measured pre (and perhaps even mid) treatment through imaging and cell assay studies. For mid-treatment tumour measurements, the model could be used to resimulate the tumour under new

conditions or with new parameters and an updated optimal prescription recomputed. This prescription could be compared with the original model prediction or clinical prescription. Further, the model could be used to simulate the original fractionation schedule and a change in the schedule mid treatment (if desired by the clinician to account any newly discovered information) to predict how the change in fractionation could affect the time/dose needed to bring the tumour under control.

Table 3. Acute and late responding normal tissue biological effective dose (BED) score results (0.0-10.0) used to compare the predicted benefits of 11 different fractionation schedules used during hypoxic tumour simulations, with onset of accelerated repopulation (AR) and reoxygenation (ROx) modelled at 0 weeks, 2 weeks or not at all

Acute normal tissue effects: BED formula— $T_k=7$ days, $\alpha=0.35\text{ Gy}^{-1}$, $\alpha/\beta=10\text{ Gy}$, $T_p=2.5$ days												
Tumour type	Schedule number	Dose/fraction of radiotherapy	No AR, no ROx	AR (0 weeks)	AR (2 weeks)	ROx (0 weeks)	ROx (0 weeks) + AR (0 weeks)	ROx (2 weeks) + AR (0 weeks)	ROx (0 weeks) + AR (2 weeks)	ROx (2 weeks) + AR (2 weeks)	Summed BED scores	Summed BED order
Hypoxic tumours	1	2.0	3.0	3.2	3.6	3.0	2.6	1.0	2.3	1.9	23.3	4
	2	1.2	3.5	1.2	1.0	3.7	3.4	0.0	0.9	1.8	15.8	3
	3	1.1	2.2	0.1	1.0	2.8	1.7	0.7	0.1	0.0	9.4	2
	4	2.0	10.0	10.0	10.0	9.9	9.2	10.0	10.0	10.0	89.2	11
	5	2.0	4.8	3.7	3.7	5.0	4.5	3.1	3.2	3.7	35.3	6
	6	1.8	7.2	3.8	4.2	7.7	7.5	4.5	5.7	3.8	49.4	8
	7	1.5	9.7	4.5	5.6	10.0	10.0	3.3	8.1	5.9	65.3	10
	8	1.8	4.0	3.7	3.6	4.5	4.1	2.7	3.3	4.3	33.7	5
	9	1.5	8.8	5.8	4.7	8.4	7.7	7.9	7.4	4.3	60.2	9
	10	2.0	6.5	3.9	4.6	6.1	6.0	3.7	4.0	4.6	44.3	7
	11	1.2	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.4	1.2	1
Late normal tissue effects: BED formula— $\alpha/\beta=3\text{ Gy}$												
Tumour type	Schedule number	Dose/fraction of radiotherapy	No AR, no ROx	AR (0 weeks)	AR (2 weeks)	ROx (0 weeks)	ROx (0 weeks) + AR (0 weeks)	ROx (2 weeks) + AR (0 weeks)	ROx (0 weeks) + AR (2 weeks)	ROx (2 weeks) + AR (2 weeks)	Summed BED scores	Summed BED order
Hypoxic tumours	1	2.0	7.1	10.0	10.0	8.4	8.2	10.0	10.0	10.0	83.7	11
	2	1.2	1.9	2.6	0.9	0.7	3.2	3.8	3.4	0.6	17.0	2
	3	1.1	0.0	2.3	2.0	0.0	0.0	5.9	1.9	2.9	15.8	1
	4	2.0	10.0	7.9	7.7	10.0	10.0	8.2	9.0	6.6	75.6	10
	5	2.0	7.6	7.2	6.4	9.3	8.7	8.8	8.6	7.1	69.9	9
	6	1.8	6.2	2.6	2.1	6.7	8.5	4.8	2.2	2.8	38.5	5
	7	1.5	5.2	0.0	0.0	3.7	7.1	0.0	0.3	1.5	17.8	3
	8	1.8	5.0	5.7	5.0	7.9	7.4	7.2	8.7	5.8	57.4	7
	9	1.5	6.0	2.6	0.5	3.0	4.9	5.3	0.0	0.0	23.5	4
	10	2.0	9.2	5.4	5.5	8.2	9.6	7.1	7.2	6.2	62.8	8
	11	1.2	0.1	4.7	3.6	0.3	2.1	9.3	5.0	6.0	38.9	6

T_k , repopulation kick-off time; T_p , potential doubling time.
A high score indicates a non-beneficial schedule (high toxicity—italics), an intermediate score represents a mid-range schedule (intermediate toxicity—bold) and a low score indicates a beneficial schedule (low toxicity—bold italic). The summed BED scores are added per schedule, with the underlined text representing the schedule with the lowest and highest values.

Besides AR and ROx effects, the average higher doses required in simulations (to achieve tumour control) *vs* clinical trial prescriptions (to obtain improved local tumour control) could be attributed to the inclusion of well-oxygenated tumours in the clinical trials, as opposed to the specific oxygenation levels (pO_2 histograms) implemented in the model. The higher model doses could also be attributed to the tumour control end point used. There were two choices for this end point: (1) control all tumours and report the total doses required or (2) deliver the dose required to achieve the same level of cell kill (according to Poisson theory) based on reported LC rates from clinical trials.

Option 1 was chosen in the current study to reduce bias in the assessment of relative differences in the schedules and because of the uncertainties relating to Poisson theory at the single cell level. Effects at the single-cell level may include immune system responses and cell death owing to necrosis, which are not accounted for in Poisson theory as it presumes every tumour will regrow if one clonogenic cell survives treatment. Further, the sharp gradient of the Poisson function was considered inappropriate for converting cell kill outcomes from this stochastic model into TCP, as statistical fluctuations in model results are approximately 5 Gy on average. When investigating the simulation of total doses that fluctuate at this level, very large ranges in Poisson-based TCP (~ 0 to 100%) arose. Based on the *HYP-RT* model, it has been reported previously [3] that the difference between killing all basal cells that could potentially regrow the tumour, compared with killing all but last 1 cell or all but the last 5 cells, has an approximately 1–6 Gy total dose reduction effect. This may be the more likely clinical scenario based on reported LC percentages averaging around 60%. Note that a 60% LC rate equates to an average of 0.5 cells remaining in a group of tumours, according to Poisson theory.

Figure 5 indicates that some schedules required more dose to achieve tumour control than commonly prescribed for human tumours. For example, approximately 9 weeks of 2 Gy per fraction radiotherapy (90 Gy) is required for hypoxic tumours using Schedule 1. However, these particular results predict the total doses required for 100% tumour control based on the elimination of all basal cells while also simulating AR. Owing to the rapid mid-treatment growth of these tumours, these high tumour control doses are not inconceivable for patients experiencing AR.

The low overall toxicity predicted for Schedules 2 and 3 (hyperfractionated) are in agreement with clinical trials reporting the benefits of b.i.d. schedules for treatment of HNSCC [18]. In this work, Schedule 11, with the 2-week break after the initial 2 weeks of treatment, was beneficial for early normal tissue effects because of the extended treatment time, and it had average BED scores for late normal tissues, with no AR considered. With AR considered for Schedule 11, the total treatment time lengthened to the extent that the increased total dose resulted in a high rate of late toxicity. This high toxicity was accounted for in simulations because of the extra stem cell proliferation detected during the 2-week break and the consequential increase in treatment time required to control the tumour (approximately one extra treatment week or 10×1.2 Gy).

Schedule 1 (conventional) and Schedule 4 (6×2 Gy per week), followed by Schedules 2, 3, 5 and 11, had the largest differences in total dose outcomes for hypoxic *vs* oxic tumours. Note that, for these findings, the OER functions used in the model were affected by dose per fraction, causing the impact of hypoxic cell resistance to become less severe for smaller doses per fraction. Despite the reduced impact of hypoxia on hyperfractionated schedules, Schedules 2 and 3 still had large total dose differences for oxic *vs* hypoxic tumours. This finding should further encourage individualisation of radiotherapy planning based on tumour oxygenation, *e.g.* positron emission tomography scans using hypoxic markers such as F-18 misonidazole (F-MISO).

CONCLUSIONS

Modelling tumour growth and treatment response could have the potential to assist in the ongoing pursuit of patient individualised radiotherapy planning. In this study, the stochastic HNSCC model, *HYP-RT*, was utilised and validated by simulating altered fractionation radiotherapy schedules derived from clinical trials, to compare the resultant tumour control doses with clinical prescriptions and to highlight the significant differences in dose requirements for head and neck tumours exhibiting different repopulation and ROx characteristics during treatment. Further, oxic as well as hypoxic tumours were simulated using continuous oxygenation probability distributions to describe tumour oxygenation status.

On average, the total doses required to model tumour control using multiple altered fractionation schedules were in good agreement with clinical trial prescription doses. However, AR and ROx caused significant variations in outcomes, even for the same schedule.

Schedule 1 (conventional) and Schedule 4 (6×2 Gy per week), followed by Schedules 2, 3 (hyperfractionated) and 5 (accelerated), had the largest differences in tumour control doses for hypoxic vs oxic tumours. The modelled OER curves that were utilised based on dose per fraction caused hypoxic cell radioresistance to be significantly reduced for small doses per fraction schedules. These findings should encourage research into devising individualised tumour predictive assays, for example, assays investigating tumour hypoxia and the likelihood of early ROx.

Normal tissue BED outcomes for the modelled schedules compared well with reported clinical toxicity data for early (mucosal effects, if available) and late normal tissue effects, with the small dose per fraction schedules proving most beneficial for the therapeutic ratio.

The points below summarise the key outcomes for the schedules modelled:

- oxic and hypoxic tumours have large differences in total dose requirements, which are significantly affected by AR and ROx onset times (by up to 15–25 Gy for the same schedule)
- tumours with immediate (0 weeks) onset times of ROx and 2-week onset times of AR require total doses closely matching “average” human tumour HNSCC doses from clinical trials
- *HYP-RT* predicts Schedule 3 (10×1.1 Gy per week) to be the most beneficial fractionation schedule based on high TCP as well as relatively low early and late responding normal tissue effects, with Schedule 2 (10×1.2 Gy per week) the next most beneficial schedule
- the least beneficial (most toxic) schedules for late normal tissue effects are Schedules 4, 5 and 10, which are all 2.0 Gy per fraction accelerated schedules in agreement with average clinical trial data
- the conventional schedule is relatively beneficial in terms of early normal tissue effects but has one of the poorest normal tissue toxicities for late effects, indicating that altered schedules have the potential to decrease late toxicity for most combinations of onset times of AR and ROx.

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