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Mechanistic modelling of Radium-223 treatment of bone metastases

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Short Title: Modelling of Radium-223 treatment

CONFLICT OF INTEREST

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Author Contributions

Conception and design: JOS, KMP, SJM; **Data collection, analysis and interpretation:** HM, FGL, SJM;

Manuscript writing: HM, FGL, SJM; **Final approval of manuscript:** All authors

ACCEPTED MANUSCRIPT

Mechanistic modelling of Radium-223 treatment of bone metastases

Short Title: Modelling of Radium-223 treatment

ACCEPTED MANUSCRIPT

Summary:

Despite the reported effectiveness of $^{223}\text{RaCl}_2$ for treatment of bone metastases, many questions remain regarding its dosimetry and pharmacodynamics. This study models three different $^{223}\text{RaCl}_2$ uptake scenarios, comparing their predictions of time to first symptomatic skeletal event to published clinical data. Our results suggest that approaches which assume a uniform biodistribution of ^{223}Ra throughout metastatic sites do not accurately predict biological effects of α -radionuclide therapies, with exposure of small sub-populations providing superior agreement with clinical data.

ABSTRACT:

Introduction: Despite the effectiveness of $^{223}\text{RaCl}_2$ for treating patients with symptomatic bone metastatic disease, its mechanisms of action are still unclear. Even established dosimetric approaches differ considerably in their conclusions. *In silico* tumour models bring a new perspective to this as they can quantitatively simulate the interaction of α -particles with the target(s). Here, we investigated three different mathematical models of tumour growth that take into account the radiation effect of Radium-223 treatments and compare the results to clinical data.

Methods: The well-established Gompertz growth model was applied to simulate metastatic tumour burden. Based on published measurements of Radium-223 uptake, we have incorporated the radiation effect of α -particles into the model and investigated three radium distribution scenarios – uniform exposure, exposure of only an outer layer, and exposure of a constant volume of the tumour. For each scenario, the times for various tumour stages to progress to the first symptomatic skeletal event were calculated.

Results: Uniform and outer-layer exposure scenarios showed very poor agreement with the Kaplan-Meier patient curves from clinical data. However, the constant volume effect predicted very similar outcomes to the observed clinical results, suggesting, depending on dose-rate, that relatively small fractions of the cell population see damage from Radium-223.

Conclusions: The commonly-used assumption of uniform Radium-223 distribution does not accurately reflect clinical responses. The suggestion that only a sub-population of the tumour might be affected by Radium-223 shows that there is a pressing need to further study the tumour and drug kinetics in order to schedule more effective treatments in the future.

Key words: Computational simulation, Metastasis, Radium-223, Tumour control

INTRODUCTION

Prostate cancer is the most common cancer in men, being a major public health concern.¹ The standard-of-care for patients with advanced prostate cancer is androgen deprivation therapy.² After prolonged androgen deprivation, the disease invariably progresses to a castration-resistant stage, an ultimately fatal condition.² At this lethal stage, a large percentage of patients with castration resistant prostate cancer (CRPC) (65%-90%) develop bone metastases.^{1,3} That often leads to severe pain and symptomatic skeletal events (SSE), that include spinal-cord compression and symptomatic pathological fractures.⁴ Additionally, bone metastases are also a major complication of several other solid cancers, such as breast, lung, kidney, thyroid cancers as well as multiple myeloma.⁵

Treatment of bone metastases in patients with CRPC may involve bisphosphonates, denosumab and β^- emitting radiopharmaceuticals, which reduce pain levels and the incidence of SSE, but fail to prolong survival. Fortunately, the number of therapeutic approaches is increasing and new promising modalities have been approved based on evidence of prolonged survival. These include the use of chemotherapy (docetaxel, cabazitaxel), androgen receptor directed therapy (e.g. Abiraterone and Enzalutamide) and radionuclide systemic therapy (Radium-223).⁶ The latter has emerged as one of the most important modalities for cancer management for patients with multiple skeletal metastases.

Radium-223 (^{223}Ra) is a calcium-mimetic and complexes with hydroxyapatite crystals in osteoblastic bone metastases. It has a physical half-life of 11.4 days and each ^{223}Ra decay results in the emission of 4 α -particles in the primary decay chain (Figure 1).⁷ In treatments for bone metastatic patients, ^{223}Ra has shown a mean effective half-life of 8.2 days, based on the radiopharmaceutical biokinetics that take into account its biological clearance rate.⁸

Figure 1 - Schematic representation of the ^{223}Ra decay chain. The energy of each emitted α -particle is shown in brackets.

α -particles are characterized by their high linear energy transfer (LET) in the order of $100 \text{ keV}/\mu\text{m}$ in soft tissue.⁹ Their high LET and short penetration range ($<80 \mu\text{m}$ in water) allow for a treatment that can be highly selective, with high levels of complex DNA damage to target tissues and negligible collateral damage to the surrounding cells and tissues.^{10–12}

The ALSYMPCA trial was an important milestone for the proof of concept of ^{223}Ra dichloride ($^{223}\text{RaCl}_2$) therapy, as $^{223}\text{RaCl}_2$ led not only to a prolonged time to the first SSE (5.8 months) but also to a significant positive effect on overall survival (3.6 months), without evidence of long-term toxicity.^{13–15} Yet, questions remain regarding pharmacodynamics and dosing for optimized patients' benefit.

It is widely accepted that α -emitting radionuclides are a promising treatment for small tumours. Nevertheless, relatively few studies to date have fully explored ^{223}Ra uptake kinetics and their impact on the dosimetry involved in such therapies.^{8,16} This is also important as new α -particle targeted approaches are starting to be utilized clinically.^{17–19} The application of radiobiological models to better understand the mechanisms of action of high LET radiation has already demonstrated its value in other areas of radiation therapy. These models are an essential component of better techniques to maximize benefits and reduce side effects for targeted radiotherapy.²⁰

This study aims to simulate different ^{223}Ra treatment scenarios and calculate the time necessary for a simulated patient group to reach the first SSE, to compare with clinical data and provide insight into the underlying mechanisms of ^{223}Ra uptake.

METHODS

Growth Model

The well-established Gompertz growth model was chosen to simulate bone metastatic site growth. This growth model describes an initial rapid growth phase which later slows, reflecting factors such as competition for nutrients, limited space availability, etc. It has been shown to have one of the best fits to experimental data for solid tumours^{21–23}. The Gompertz equation is:

$$N(t) = K \times \left(\frac{N_0}{K}\right)^{e^{-A_{growth} \times t}} \quad (1)$$

where N_0 is the initial number of cells, A_{growth} determines the growth rate and K is the maximum supportable number of cells, known as the carrying capacity. The Gompertz growth rate equation of our tumour model is then given by the following expression:

$$\frac{dN(t)}{dt} = A_{growth} \times N(t) \times \ln\left(\frac{K}{N(t)}\right) \quad (2)$$

Choosing a growth model

Since a validated model for bone metastatic growth is not yet available, Gompertz model parameters can be estimated based on tumour growth kinetics from various publications, which can be converted into a standard expression in terms of A_{growth} and K . A list of published data and corresponding parameters is presented in Table S1.

In addition to these published datasets, we also calculated growth kinetics based on mice xenograft experiments performed in our laboratory using bone metastatic cells derived from a prostate cancer patient (PC-3, ATCC, Manassas). We extrapolated the growth parameter A_{growth} by fitting equation 1 to the tumour volume growth data (shown in Figure S1). The maximum metastatic volume was set equal to the volume of a sphere whose diameter matched the maximum metastasis diameter reported in a clinical study (40 mm).²⁴ The maximum cell number, K , was then calculated assuming spherical tumour cells of radius 5 μm , packed with a cell volume occupation fraction of 60%, corresponding approximately to a random close packing.

To test the plausibility of these different parameter sets, we calculated metastatic growth predictions in the absence of radiation, for two different starting volumes, $N_0 = 0.1\% K$ and $N_0 = 10\% K$, corresponding to undetectable and detectable metastatic tumours, respectively. The resulting curves comparing tumour

growth curves can be seen in Figure S2. The growth kinetics for published primary tumour models showed that for both initial tumour volumes, an untreated patient would reach 90% of the maximum volume within less than 2.5 months. Consequently, this tumour growth appears to be too rapid when compared to the observed progression of untreated metastases. By contrast, a model based on liver metastasis data was shown to grow too slowly, taking three or more years to progress.

The most suitable model appears to be that based on bone metastasis models in mice. With both initial volumes N_0 , an untreated metastasis would reach 90% of the maximum volume within 10 and 7 months, respectively. Therefore, we have chosen this growth model for subsequent calculations of the ^{223}Ra treatment effect, giving Gompertz parameters of $A_{\text{growth}}=0.0129 \text{ day}^{-1}$ and $K=3.84 \times 10^{10}$ cells.

Dosimetric effect of ^{223}Ra treatment in bone tissue

When assessing radiation dose effects from radiopharmaceuticals to target tissues it is important to take into account the radiation mean absorbed dose. Based on literature, we know that the dose per delivered activity, D_{act} , of this ^{223}Ra treatment in bone is 0.76 Gy/MBq. This is based on estimates of deposited dose from SPECT imaging of patients treated with ^{223}Ra ^{25,26}. We can then calculate the absorbed dose in the bone endosteum tissue for a 70 kg patient, with a typical treatment schedule of 55 kBq/kg, as:

$$D_{\text{act}} \times A_0 = 0.76 \left(\frac{\text{Gy}}{\text{MBq}} \right) \times \left[0.055 \left(\frac{\text{MBq}}{\text{kg}} \right) \times 70 (\text{kg}) \right] = 2.93 \text{ Gy} \quad (3)$$

where A_0 is the initial activity of a single ^{223}Ra treatment fraction injection.

Since the dose deposited in bone metastasis is unclear and should vary significantly for different patients and tissue locations, we assumed the bone metastatic mean absorbed dose to be the same as the absorbed dose for the bone endosteum tissue, as an approximation.

The instantaneous dose rate, $\dot{D}$, can then be calculated assuming an exponential decay of the primary ^{223}Ra atoms, as their half-life is significantly longer than that of the daughter isotopes. Thus, by assuming that the dose rate is directly proportional to the activity, the instantaneous dose-rate can be specified as:

$$\dot{D}(t) = \dot{D}_0 \times e^{-\lambda_{\text{eff}} t} \quad (4)$$

where λ_{eff} is the ^{223}Ra effective decay rate calculated from the ^{223}Ra effective half-life ($T_{1/2\text{eff}} = 8.2 \text{ days}$ ⁸), and $\dot{D}_0$ is the initial dose rate. The total dose delivered by a single treatment can then be calculated as:

$$D = \int_0^{\infty} \dot{D}(t) dt = \int_0^{\infty} \dot{D}_0 \times e^{-\lambda_{eff} t} dt = \frac{\dot{D}_0}{\lambda_{eff}} \quad (5)$$

For a treatment delivering a dose $D = 2.93$ Gy with an effective decay rate of $3.52 \times 10^{-3} \text{ h}^{-1}$, $\dot{D}_0$ can be calculated to be 1.03×10^{-2} Gy/h. As the ^{223}Ra treatment is typically delivered as a series of 6 fractions, the dose rate from each individual fraction is summed to give the total dose rate at any given time during the therapy.

Radiation effects in the tumour volume

The radiation effect on tumour survival was evaluated for the ^{223}Ra treatment using the linear-quadratic model, based on literature data describing α -particle effects on *in vitro* experiments using bone metastatic cells derived from a prostate cancer patient (PC-3, ATCC, Manassas). Here, the radiosensitivity parameter of cells to α -particles (α_1) was found to be 1.82 Gy^{-1} .²⁷ As cell death results mainly from single alpha-particle interaction events, the relationship between the surviving fraction (SF) and the cell absorbed dose (D) is approximate to a log-linear model.¹² Thus, the survival fraction curve, which resulted from clonogenic assays using an external α -source, followed the linear equation parameters:

$$SF_{\alpha}(D) = e^{-\alpha_1 D} \quad (6)$$

If there is no tumour growth, the radiation effect on the tumour volume is described by:

$$\Delta N(t) = -N(t) \times (1 - e^{-\alpha_1 \dot{D} \Delta t}) \quad (7)$$

where $N(t)$ is the number of cells at the time t . By applying the Taylor expansion method for small timesteps Δt , we can simplify the equation to:

$$\frac{dN(t)}{dt} = -N(t) \times \alpha_1 \times \dot{D}(t) \quad (8)$$

where $\dot{D}(t)$ is the absorbed dose rate in bone at a given time t . Taking into account the Gompertz growth behavior of the tumour metastasis, together with the radiation effect, we can describe the ^{223}Ra treatment model on bone metastasis growth as:

$$\frac{dN(t)}{dt} = A_{growth} \times N(t) \times \ln\left(\frac{K}{N(t)}\right) - N(t) \times \alpha_1 \times \dot{D}(t) \quad (9)$$

²²³Ra treatment exposure scenarios

The previously described mathematical model illustrates a uniform effect, assuming that all cells are equally affected by the radiation dose. This assumption may not be accurate in practice, so we have also tested two other radiation distribution scenarios, assuming that only a sub-population of cells, $N_p(t)$, are affected.

The first scenario is an outer layer effect, where only the surface of the metastatic volume is exposed to radiation. The number of affected cells $N_p(t)$ is modelled by the number of cells present in a layer at the surface of the metastases, described by a thickness layer (T_{layer}). Here, $N_p(t)$ was calculated assuming a cell radius of 5 μm in a tumour sphere with a cell volume occupation fraction of 60%, as for the calculation of K above.

The second modeled scenario is a constant volume exposure, where $N_p(t)$ is constant, regardless of the tumour growth stage, except when $N(t) < N_{constant}$, as described by the equation system:

$$N_p(t) = \begin{cases} N(t) & , N(t) < N_{constant} \\ N_{constant} & , N(t) \geq N_{constant} \end{cases} \quad (10)$$

For both of these scenarios, the differential equation that describes the tumour growth is then:

$$\frac{dN(t)}{dt} = A_{growth} \times N(t) \times \ln\left(\frac{K}{N(t)}\right) - N_p(t) \times \alpha_1 \times \dot{D}(t) \quad (11)$$

Figure 2 shows a schematic representation of all the treatment exposure scenarios considered at different stages of tumour growth.

Figure 2 - Schematic representation of the radiation exposed (orange) and non-exposed (blue) tumour volumes for the uniform (A), outer layer (B) and constant volume (C) exposure scenarios.

These three model scenarios were then solved using Matlab 2016b (Mathworks, Inc., Natick, MA) for

different assumptions of initial tumour volume and radiotherapy treatment delivery. For the uniform effect model we initially assumed a fixed an initial dose rate $\dot{D}_0$ of 1.03×10^{-2} Gy/h, corresponding to the dose rate calculated for bone endosteum. In addition, we also optimized the differential equation parameters for the three model scenarios using a least-squares fit, given the placebo and treatment data from clinical observations described below. The variable parameters were $\dot{D}_0$ for the uniform effect, T_{layer} for the outer layer effect and $N_{constant}$ for the constant volume effect. The resulting best fit parameters, together with the corresponding uncertainties, were calculated from the least-squares fit function in Matlab.

The resulting analysis allowed us to predict the tumour growth delays for each of the assumed exposure scenarios, which can then be compared to clinical observations.

Clinical trial data

To provide a test for this model, published results for time until 1st SSE were obtained from the ALSYMPCA trial for placebo and ^{223}Ra treated groups.¹³ Based on the assumption that skeletal events resulted from a particular level of metastatic burden, we assumed that skeletal events occurred when $N(t)$ reaches 80% of the maximum number of cells (K). The ratio between the number of cells that correspond to an SSE (N_{Met}) and the maximum number of cells (K) is here defined as S_{Met} :

$$S_{Met} = \frac{N_{Met}}{K} = 0.8 \quad (12)$$

This value was chosen empirically, based on biological and mathematical rationale. Biologically, it is plausible that S_{Met} should be close to 1, or in other words N_{Met} being close to K , as this represents the stage when the metastases are in significant competition for resources with normal tissue. This stage would be expected to be associated with significant symptomatic events. This is also mathematically reasonable, as if S_{Met} is very small, the metastatic growth rate will be too rapid, while if S_{Met} is very close to 1, no metastatic growth would be seen. We could then simulate the growth of metastases from different initial volumes to determine the time taken for a skeletal event to occur either with or without ^{223}Ra treatment.

Assuming that the control and treated populations were identical, a ‘virtual patient population’ was generated with a range of different initial tumour volumes which reproduced the observed time to failure in the control, untreated population, representing 123 first SSE in 307 patients. We then simulated the effects of ^{223}Ra treatment in this population using each of the radiation models, to predict responses in the treated population.

Specifically, for a placebo patient who failed at a given time $t_{placebo}$, at this timepoint their metastatic volume must be N_{Met} , corresponding to the first SSE occurrence. By substituting these values into equation 1, we can calculate a patient specific initial disease volume, N_0^p . We then model how this patient would have responded to treatment by simulating the growth of this tumour using the kinetics described in equation 1 for 6 cycles of ^{223}Ra treatment, beginning with an initial volume of N_0^p at $t=0$. This equation was solved using a differential equation solver method in Matlab (ODE45) and simulations were continued until the patient disease burden reached N_{Met} . By calculating this time for each patient in the population, a new simulated Kaplan-Meier curve for the treated population could be generated for each set of model assumptions. These were then compared to the actual clinical data of treated populations to evaluate how well they reproduced the treatment response. This comparison was based on the fitting quality parameter R^2 between their predicted time to SSE and the corresponding clinical observations.

Sensitivity analysis to the tumour growth model parameters

We have also conducted an extensive sensitivity analysis of the influence of the growth parameters K , A_{growth} , and S_{Met} on the best fit variables for all the three tumour models analysed ($\dot{D}_0$ for the uniform effect, T_{layer} for the outer layer effect and $N_{constant}$ for the constant volume effect). For simplicity, during the analysis of the constant volume model, we used the normalization parameter $C_{Fraction}$, which corresponds to the ratio between the constant volume of radiation affected cells ($N_{Constant}$) and the tumour maximum number of cells (K).

Although the outer layer and constant volume effect models also depend on the value of $\dot{D}_0$, initial fitting found $\dot{D}_0$ was highly covariant with the model-specific parameter T_{layer} . As a result, $\dot{D}_0$ was treated as a constant in the main analysis to enable robust fitting, which did not significantly affect the overall fit quality. Further information on the effect of varying the initial dose rate $\dot{D}_0$ on the different models, particularly the constant volume model, is also presented in the supplementary information.

RESULTS

Modeling the radiation effect on the tumour growth

Tumour evolution with time was calculated for the three radiation exposure models previously mentioned - uniform, outer layer and constant volume scenarios. For the three models, treatments were simulated on a 'virtual patient population' based on placebo-treated patients who would have a first skeletal event between 0 to 13 months from the treatment start date. Figure 3 shows the comparison between tumour growth with time, for the three models with and without the radiation treatment, starting at two illustrative times before the placebo patient would experience a skeletal event.

When these growth models were fit to the ALSYMPCA data, we found best-fit parameters of $\dot{D}_0 = 1.14 \times 10^{-4} \pm 0.19 \times 10^{-4}$ Gy/h for the uniform effect, $T_{layer} = 65.8 \pm 6.1$ μm for the outer layer effect and $N_{constant} = 2.02 \times 10^8 \pm 0.02 \times 10^8$ cells for the constant volume effect scenario. Kaplan Meier curves for the simple uniform model based on bone endosteum and the best-fit version of each model are presented in Figure 4, together with the treatment and placebo data from the ALSYMPCA trial.

Figure 3 Illustrative tumour growth curves for the uniform (A), outer-layer (B) and constant volume (C) exposure scenarios. The curves represent the progression of untreated/placebo (blue) metastases and progression when treated with ²²³Ra at different growth stages (orange).

Figure 4 - Kaplan-Meier curves for the clinical data of placebo and treatment groups from the ALSYMPCA trial data¹³, comparing to the uniform model fit with initial dose rate estimation ($\dot{D}_0 = 0.0103$ Gy/h). The curves for the uniform, outer layer and constant volume effect model scenarios with best-fit parameters are also shown.

Dependence of constant volume model on $\dot{D}_0$

Previously, we fixed $\dot{D}_0 = 1.03 \times 10^{-2}$ Gy/h. However, as this is necessarily an approximation due to inter- and intra-patient variation, we have studied the effect of varying $\dot{D}_0$ in the constant volume model. The dependence of $C_{Fraction}$ (the ratio between $N_{constant}$ and K) on $\dot{D}_0$ is illustrated in Figure 5A, showing a linear dependence between these terms at dose rates from 10^{-4} to 10^{-1} Gy/h. This is confirmed by Figure 5B, showing the best-fitting $\dot{D}_0 \times C_{Fraction}$ is constant for a wide range of initial dose rates. Thus, dosimetric uncertainties translate into significant uncertainties in the value of $C_{Fraction}$, but have no significant impact on fit quality.

Figure 5 - Effect of initial dose rate ($\dot{D}_0$) on the best-fitting C_{Fraction} (A) and the product of $\dot{D}_0$ and C_{Fraction} (B). These datasets were fitted with $A_{\text{growth}}=0.0129 \text{ days}^{-1}$, $S_{\text{met}}=0.8$, and $K=3.84 \times 10^{10}$ cells. Points are coloured according to the fit quality (R^2) of the model to the ALSYMPCA treatment data. C_{Fraction} is the ratio between the constant volume of radiation affected cells (N_{constant}) and the tumour maximum number of cells (K).

However, some constraints can be placed on these values based on fit quality. Once the initial dose rate becomes significantly less than $1 \times 10^{-3} \text{ Gy/h}$, the quality of the fit degrades significantly, suggesting that these models with high coverage ($C_{\text{Fraction}} > 10\%$) and low dose rate provide a less accurate representation of clinical data than higher dose rates with only small portions of the disease being exposed.

Sensitivity analysis of tumour growth model parameters

As noted above, there are no robust data on bone metastatic growth kinetics or failure conditions. Thus, in order to fully understand the effect of A_{growth} , S_{Met} and K on tumour kinetics, we studied model predictions in a range of alternative parameter sets, presented in the Supplementary Information and summarized briefly below.

A_{growth} and S_{Met} choices can be constrained based on the time to failure of untreated tumours – when A_{growth} was very large ($>0.02 \text{ day}^{-1}$) or small ($<0.001 \text{ day}^{-1}$), the tumour growth kinetics were not compatible with experimental observations (failing too quickly or too slowly, respectively). Similarly, larger S_{Met} values were associated with longer times to failure, and vice-versa (Figure S3).

Best-fit parameters depended on both A_{growth} and S_{Met} , with faster growth requiring higher doses to offset increased proliferation, while K had no significant impact (Figure S4). However, the constant volume model provided the best fit independent of parameter choice (Table S2, Figure S5), and the inter-relation of $\dot{D}_0$ and C_{Fraction} was also independent of growth parameters (Figure S6). The overall best fit performance was found for model parameters similar to those derived from the mouse data (A_{growth} values ranging from 0.09 to 0.013

day⁻¹, and for S_{Met} values between 0.75 and 0.8, Figure S7), although a range of parameters produced similar fits.

DISCUSSION

Here, we report the results from a mathematical modelling approach to evaluate the treatment outcomes of ²²³Ra on bones metastases. During this process, we analysed tumour growth kinetics, following a Gompertz model, with the effects of α -particle radiation from ²²³Ra. The time to the first SSE was then compared for each tested model with the clinical data available from the ALSYMPCA trial. An extensive sensitivity analysis was also conducted to further explore the effects of all the tumour growth model parameters involved.

The addition of the ²²³Ra effect to the PC-3 based Gompertz tumour growth showed different results for the three exposure scenarios investigated in this work. The uniform effect, where the radiation affects the whole tumour volume, gave over-optimistic results, when using the initial dose rate estimate ($\dot{D}_0 = 0.0103$ Gy/h). This was noticeable in Figure 3 as 12 and 6 month growth delays in reaching N_{Met} were observed when compared to the placebo curves, for late and early tumour stages respectively. When comparing these results with the clinical data, it further proves to be an unrealistic model scenario (Figure 4). Even if taking a lower initial dose rate, the best-fit model still results in a different Kaplan-Meier curve compared to the clinical data, over-estimating the effects on patients with high disease burden and under-estimating the effects on patients with lower disease burden. The sensitivity analysis has also shown that this is the case regardless of the growth model parameters used (Figure S5). These results lead us to conclude that the metastatic tumour cells cannot be experiencing a uniform dose exposure. This is particularly relevant since the assumption that there is a uniform radiopharmaceutical activity distribution in bone metastatic volume sites is frequently used for bone absorption radiation dose calculations and response modelling.^{8,28–31}

Regarding the outer layer effect scenario, we observed growth delays of 3.0 and 5.0 months compared to the placebo curve, for early and late tumour stages respectively on Figure 3. However, these growth delays show that the model still predicts cell killing rates which are too high for early tumour growth stages and too low for later tumour growth stages. This is due to the changing fraction of cells affected by the penetration range of ²²³Ra. This inadequacy of the model is further confirmed in Figure 4, where the best-fit model shows poor agreement with clinical data, which again is true for a range of growth parameters (Figure S5).

The third tested model scenario showed the best predictions of treatment effect. The treatment group

results for the constant volume exposure scenario predicted very similar outcomes to the clinical data, as shown in Figure 4, especially until 10 months after treatment initiation (first ten months $R^2 = 0.989$). The results from this model seem to indicate that the ^{223}Ra treatment saturates, affecting only a constant number of cells regardless of the tumour growth once its volume is above N_{constant} . The exact number of cells affected depends on the assumed dose rate in the metastatic volumes, with N_{constant} ranging from 5% to 0.05% of the maximum metastatic burden (K) for initial dose rates $\dot{D}_0$ varying from 0.001 to 0.1 Gy/h, respectively (Figure S6). For these cases, the fitting quality of the model is still high ($R^2 > 0.96$), as shown in Figure 5 and Figure S7. This further supports the idea of a non-uniform ^{223}Ra biodistribution at the tumour microenvironment, with effects that saturate rapidly with tumour volume, which may possibly be related to poor blood vessel perfusion in different metastatic sites.³² This is in agreement with some evidence seen in the literature of inhomogeneous distributions of β and α -particle emitting radionuclides in pre-clinical models, where some of the target cells received no radiation.^{33–36}

However, it should be noted that these models remain an idealized description of the metastatic tumour burden, and a number of refinements to this model and its limitations will help building more accurate and predictive descriptions of bone metastases radionuclide therapy. Some of these limitations are related to the mathematical models of tumour growth kinetics. While the Gompertz model is well-established for primary tumours and a conceptually reasonable model of metastatic burden, it may be inadequate to simulate metastatic growth at earlier stages. Unfortunately, there is very limited growth data for human bone metastases in the literature. Analysis of a range of published clinical data in various tumour types produced unrealistic growth kinetics (Table S1, Figure S2). As a result, in this work we made use of parameters based on a human prostate cell line derived from a bone metastatic site, which showed the most realistic tumour growth predictions and was therefore chosen to be applied in our mathematical model. The lack of detailed, quantitative clinical data on the progression of metastatic burden in these patients leads to significant uncertainty in the A_{growth} , K and S_{Met} parameters which had to be estimated indirectly based on tumour studies in mice. While the model's general conclusions appear to be robust against variation in these parameters (see supplementary information), more accurate growth data could significantly improve the confidence in specific fitted parameter values.

An additional challenge regarding some of the growth models analyzed was the extrapolation of growth parameters from tumour size data in literature, as in many studies data were either incomplete or reported in a format which made it difficult to fully characterize tumour growth kinetics.

It is also important to understand that different tumour microenvironments will have different sub-populations of cells, as quiescent cells. These sub-populations may have a significant impact in tumour eradication and should be considered in future model optimizations.^{37–39} Important indirect radiation effects, such as bystander effects, should also be taken into account in future treatment model simulations. Despite the fact that it is difficult to fully characterize this effect in bone metastases, it clearly has a significant role in cell death, especially using high LET particles such as α -particles.^{12,40–42} Further studies of radiation indirect effects and bone metastases development in humans, particularly tracking multiple independent metastases, are needed to develop more realistic models of metastatic growth.

Furthermore, the data from the clinical treatment observations used in this work would also benefit from increased statistical power. After 10 months of treatment, the number of SSE is low (average below 7 SSE/month), which increases statistical uncertainties. In addition, this model involves an extrapolation from SSE to a single value of ‘tumour burden’, while in reality patients will likely have a number of metastatic sites that may have different sizes and growth rates. We have addressed part of this issue by analyzing the effect of different model parameters to the tumour growth, as shown in the parameter sensitivity analysis section. However, model refinements are still needed. Obtaining additional data at the level of individual metastases, from imaging studies, would enable the development of more accurate treatment models.

CONCLUSION

Modelling the growth and radiation response of bone metastases in patients treated with ^{223}Ra has been shown to be able to accurately reproduce clinical responses, but only when assuming that a relatively low constant number of cells are exposed to α -particles, depending on the treatment dose rate for each bone metastatic site. These observations support that uniform conventional dosimetric approaches are not valid to accurately predict the biological effects of α -particle radionuclide therapies. Further *in vivo* and *in vitro* studies regarding metastatic growth, tumour microenvironment and ^{223}Ra uptake mechanisms are necessary in order to plan more effective treatments in the future.

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