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ORIGINAL ARTICLE

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A two-drug combination simulation study for metastatic castrate resistant prostate cancer

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Background: Prostate cancer often evolves resistance to androgen deprivation therapy leading to a lethal metastatic castrate-resistant form. Besides androgen independence, subpopulations of the tumor are genetically heterogeneous. With the advent of tumor genome sequencing we asked which has the greater influence on reducing tumor size: genetic background, heterogeneity, or drug potency?

Methods: A previously developed theoretical evolutionary dynamics model of stochastic branching processes is applied to compute the probability of tumor eradication with two targeted drugs. Publicly available data sets were surveyed to parameterize the model.

Results: Our calculations reveal that the greatest influence on successful treatment is the genetic background including the number of mutations overcoming resistance. Another important criteria is the tumor size at which it is still possible to achieve tumor eradication, for example, 2-4 cm large tumors have at best a 10% probability to be eradicated when 50 mutations can confer resistance to each drug.

Conclusion: Overall, this study finds that genetic background and tumor heterogeneity are more important than drug potency in treating mCRPC. It also points toward identifying metastatic sites early using biochemical assays and/or dPET.

KEYWORDS
dPET, drug combinations, evolutionary dynamics, mCRPC, overcoming resistance, PET, tumor heterogeneity

1 | INTRODUCTION

An unmet need for metastatic resistant prostate cancer (mCRPC) is development of effective therapy eradicating tumors. Despite advances in drug development resistance emerges.[1] Generally there are two possibilities how resistance emerges in a tumor: (i) during drug treatment the tumor mutates and becomes resistance due to genomic instability (acquired resistance) or (ii) prior to treatment there are already pre-existing clones that are resistant (innate resistance). When treatment commences these pre-existing clones thrive due to their drug resistance (increased fitness) under the selection pressure of the drugs. Due to the large number of cells per tumor ($\sim 10^9$ cells) it is likely that one or more clones already possess a resistance mechanism. Mechanistically, resistance in mCRPC can emerge due to androgen receptor (AR) splice variants, increased activation or expression of AR, co-activation of AR via non-hormonal

entities, activation of glucocorticoid receptor, increased drug efflux, and β-tubulin or cell growth pathways might be dysregulated.[2] Within a mCRPC patient multiple of these resistance mechanisms might be found due to the heterogeneity within large tumors and across multiple metastatic sites.[1,3] Many resistance mechanisms manifest themselves on the genomic level due to genomic instability found within cancer cells.[4–6] Hence, identification of mechanisms of escape from treatment remain a priority.[7] Ultimately combination therapies targeting multiple escape mechanism will be developed as is already the case for HIV, TB, and some cancers.[8]

In order to gauge the influence of genetic background, tumor heterogeneity, and drug potency on plausible eradication of mCRPC we used a computational simulation approach. In particular, we used theoretical evolutionary dynamics which provides insights into how prostate cancer evolves and evades resistance.[9] Here, we apply a recently developed stochastic branching process model[10] to estimate the probability that resistance will emerge to two-drug therapy.

2 | METHOD

To compute a probability of eradication, P_{erad}, the model determines four independent events:

$$P_{erad} = P_{1b}P_{1t}P_{2b}P_{2t} \quad (1)$$

where P_{1b} is the probability that no 1-step resistant lineage arises before treatment, P_{1t} is the probability that no 1-step lineages arise during treatment, P_{2b} is the probability that no 2-step lineages arise before treatment, and P_{2t} is the probability that no 2-step lineages arise during treatment. Each is determined as follows:

$$P_{1b} = \exp(-\mathrm{Mun}_{12}) \quad (2)$$

$$P_{1t} = \exp\left(-\mathrm{Mun}_{12}\frac{s}{s'}\right) \quad (3)$$

$$P_{2b} = \exp\left[\mathrm{Mu}^2\frac{s'-s}{ss'}\left(n_1(n_2+n_{12})\ln\left(\frac{1}{sM}+u(n_2+n_{12})\frac{s'-s}{ss'}\right)+n_2(n_1+n_{12})\ln\left(\frac{1}{sM}+u(n_2+n_{12})\frac{s'-s}{ss'}\right)\right)\right] \quad (4)$$

$$P_{2t} = \exp\left(-\mathrm{Mu}^2(2n_1n_2+n_{12}(n_1+n_2))\frac{s}{s^{2\prime}}\right) \quad (5)$$

where M is the number of cells in the tumor, u is the mutation rate, $s = 1 - d/b$, where b and d are birth and death rates, s' is the survival rate of sensitive cells, n_1 and n_2 are the number of mutations conferring resistance to drugs 1 and 2, and n_{12} is the number of mutations conferring cross-resistance to both drugs. Parameter values were determined from literature curation and estimation as described in the section 2. Derivation of model equations is given in the supplementary information to the original publication.

2.1 | Parameterization of equations

To parameterize model Equations 1-4 we used publicly available patient data that was carefully compiled by the cancer research community across multiple research studies, see Table 1. Tumor sizes of mCRPC derive from radiography.[11] The number of tumor cells per cubic cm was obtained from Lutz et al.[12] Growth rates were obtained from median values reported by Wilkerson et al. from a meta-analysis using the median growth rate of resistant tumors (see Figure 3a and sensitive Figure 3b).[13] There are approximately 50 known mechanisms of resistance to androgen deprivation therapy and approximately 50 mechanisms for other targeted inhibitors.[10] Point mutation rates were assumed to follow the typical value for human cancers of 1×10^{-9} per base pair per division.[14] Conversion between tumor population and tumor volume assumes spherical tumor with a density of 2×10^8 cells/cm^3.[12]

3 | RESULTS

In order to gauge the influence of genetic background, tumor heterogeneity, and drug potency on plausible eradication of mCRPC we employed theoretical evolutionary dynamics. This approach let us to investigate the probability if a two-drug

TABLE 1 Baseline model parameters, values, and sources

Symbol	Parameter	Values	Sources
b	Birth rate before treatment	0.129870	Rew and Wilson[17]
b'	Birth rate during treatment of sensitive cells	= b	Assumption
d	Death rate before treatment	0.128909	Calculated
d'	Death rate during treatment of sensitive cells	0.1320778	Calculated
M	Tumor population size (cells)	6.7×10^9	Holmes[10]
n_1	Mutations conferring resistance to AR inhibitor	50	Wadosky18
n_2	Mutations conferring resistance to another targeted drug	50	Bozic[9]
n_{12}	Mutations conferring cross-resistance to both drugs	0 or 1	assumption
s	Net growth rate before treatment	0.0074	Wilkerson[a12]
s'	Net growth rate during treatment of sensitive cells	−0.017	Wilkerson[b12]
u	Mutation rate	1×10^{-9}	Nowak[13]

[a]Median of prednisone treatment group.
[b]Median of docetaxel + prednisone group.

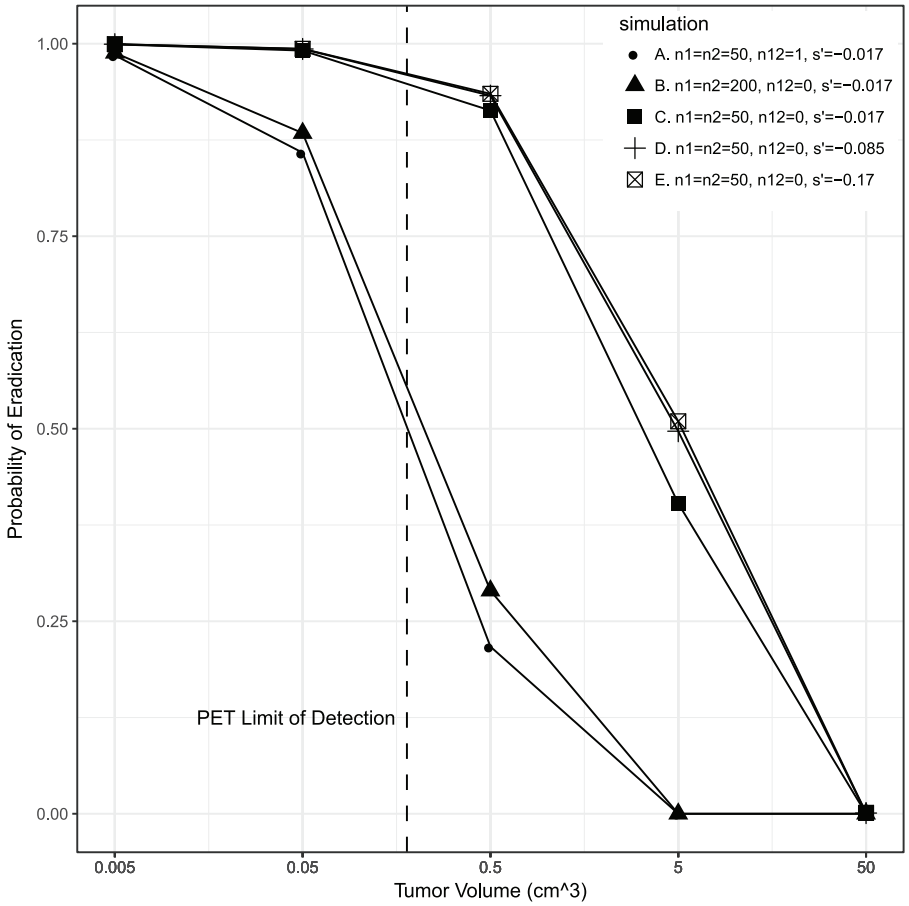

FIGURE 1 Probability of tumor eradication by two-drug combination therapy. On the x-axis the tumor size is plotted on a $\log_{10}$ scale in cm^3 while on the y-axis the probability of eradiation is plotted. The two-drug simulation tested growth rate during drug treatment (s'), as well as mutations conferring resistance (n_1 and n_2) and cross-resistance (n_{12}). Typical metastatic tumors are 2-4 cm^3 in size and genetic background is critical for probability of eradication using two-drug combinations. [Color figure can be viewed at wileyonlinelibrary.com]

combination eradicates mCRPC tumors. We analyzed the literature to parameterize the evolutionary dynamics model in Equations 1-4 with typical values given in Table 1. We investigated five different parameter combinations representing plausible best case, intermediate, and worst case scenarios.

For the worst case scenario, we assumed a single alteration conferring resistance to two-drug combination therapy, for example, increased drug efflux (scenario A). The probability of eradication is below 25% for tumors that are 10^8 cells (~0.5 cm^3 volume or ~0.5 cm radius). Another scenario concerns a very high mutation rate on the genomic level. In scenario B, there are no cross-resistance mutations, but a fourfold higher number of mutations capable of conferring resistance to each drug alone, that is, from 50 to 200. Similar to A, scenario B also predicts very poor probability for eradication (see Figure 1). Both results show that at the limit of detection for PET scan, the probability of eradicating the tumor is 50%. In the case of typical tumors (2-4 cm, 33 cm^3), there is very little chance of eradication using only two drugs. Hence, genomic background and instability are two main factors contributing to drug resistance in mCRPC.

A best case scenario does not have a single mutation that can confer cross-resistance to both drugs, has a moderate number of resistance conferring mutations of 50 and the two-drug combination reduces tumor size based on median values achieved by docetaxel reported by Wikerson et al[13] (scenario C). For an initial tumor burden of 10^8 cells (~0.5 cm^3 volume or ~0.5 cm radius) the probability of tumor eradication is ~90% (see Figure 1). However, for tumors with 10^9 cells (~5 cm^3 or ~1 cm radius) at the initiation of treatment, the probability of eradication is less than 50%. Even under this best case scenario constant monitoring and early detection of small tumors is key to eradicating tumors using a two-drug combination.

To investigate whether potency of the two-drug combination is critical to eradication, we changed the drug efficacy by an order of magnitude (scenarios D and E) leaving all other parameters as best case scenario C. Surprisingly, the 10-fold increase in drug potency had little effect on the probability of eradication given a certain tumor size (see Figure 1).

4 | DISCUSSION

Overall, this study finds that two-drug combinations are unlikely to be effective at overcoming the emergence of resistance in mCRPC. This is

due to large initial tumor burden associated with the limit of detection for current Positron Emission Tomography (PET) scan technology. At the time a tumor is detectable by PET, tumors with unfavorable genetic background only have a 50% chance of tumor eradication. Advances in PET imaging technology, for example, digital PET, will undoubtedly shift the boundaries to earlier detection of tumors increasing the chance of tumor eradication using a two-drug combination.

Another contributing factor is tumor heterogeneity, where clones are present in the tumor at the start of therapy that are resistant to both drugs. Through selective pressure during pharmacological treatment these resistant clones will be selected due to their fitness. Additionally, by chance new mutations will arise in clones and evolve during treatment. In an examination of patient data, Hieronymus et al showed that the higher the total level of copy number variation (CNV) change the worse the patient's outcome.[15] This is in agreement with our calculations that indicate greater genomic instability increases the probability of disease progression. A potential cause for inaccuracies of our evolutionary dynamics model is the estimation of mutation rate. Prostate cancer typically shows more CNV than mutations. For the purpose of our evolutionary dynamics model we rationalized that the number of DNA repair events is critical, but not the nature of the events. Hence, we set a typical value for human cancers to reflect CNV and mutations. If the mutation rate increases due to genomic stability or is much greater than is typical for cancer used here, the simulations would conclude an even lower probability of eradication.

Additionally the number of single mutations that can confer cross-resistance to two drugs are another confounding factor. In that worst case scenario there is an even lower probability of eradication. For example, if there are multiple drug transporters capable of conferring cross-resistance, then a copy number amplification of a single gene will confer cross-resistance and the parameter n_{12} is greater than 1, perhaps as high as 10. This greatly diminishes the probability of eradication.

Despite significant experimental challenges, higher-order drug combinations consisting of more than two drugs should be pursued in preclinical models. Recent theoretical advances suggest that higher-order drug combinations may be predictable from a dose series of drug pairs.[16] This method potentially provides an efficient means of developing drug combinations from a large drug library. In a complementary approach, knowledge from sequencing prostate cancer and mechanistic preclinical studies will help assemble effective drug combinations drug-by-drug.[17]

5 | CONCLUSION

Our results show that genomic background and tumor heterogeneity have a greater influence on expected eradication of mCRPC than 10-fold increase in efficacy of a two-drug combination therapy. Most clinical studies investigating combination therapy contain only two drugs targeting mCRPC tumors and our theoretical evolutionary dynamics simulations indicate a low probability of success. Therefore, two research priorities are improving detection limits of mCRPC because smaller tumors have a higher eradication probability, and development of drug combinations with 3 or more drugs to increase the probability of targeting resistant clones.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTION

AR conceived and performed simulations. AR and HAE wrote the manuscript. Both authors critically revised the manuscript.

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