The unidirectional hypoxia-activated prodrug OCT1002 inhibits growth and vascular development in castrate-resistant prostate tumors

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Funding information
Prostate Cancer UK, Grant number: PG12-02; Department of Employment and Learning, Northern Ireland

Background: OCT1002 is a unidirectional hypoxia-activated prodrug (uHAP) OCT1002 that can target hypoxic tumor cells. Hypoxia is a common feature in prostate tumors and is known to drive disease progression and metastasis. It is, therefore, a rational therapeutic strategy to directly target hypoxic tumor cells in an attempt to improve treatment for this disease. Here we tested OCT1002 alone and in combination with standard-of-care agents in hypoxic models of castrate-resistant prostate cancer (CRPC).

Methods: The effect of OCT1002 on tumor growth and vasculature was measured using murine PC3 xenograft and dorsal skin fold (DSF) window chamber models. The effects of abiraterone, docetaxel, and cabazitaxel, both singly and in combination with OCT1002, were also compared.

Results: The hypoxia-targeting ability of OCT1002 effectively controls PC3 tumor growth. The effect was evident for at least 42 days after exposure to a single dose (30 mg/kg) and was comparable to, or better than, drugs currently used in the clinic. In DSF experiments OCT1002 caused vascular collapse in the PC3 tumors and inhibited the revascularization seen in controls. In this model OCT1002 also enhanced the anti-tumor effects of abiraterone, cabazitaxel, and docetaxel; an effect which was accompanied by a more prolonged reduction in tumor vasculature density.

Conclusions: These studies provide the first evidence that OCT1002 can be an effective agent in treating hypoxic, castrate-resistant prostate tumors, either singly or in combination with established chemotherapeutics for prostate cancer.

Keywords: hypoxia, hypoxia-activated pro-drug, OCT1002, PC3, prostate

1 INTRODUCTION

Hypoxia occurs in most solid tumors and it is known to have a major influence on treatment response to radiotherapy, chemotherapy, and immunotherapy. Untreated prostate tumors are known to be very hypoxic (~0.3% oxygen), which is >12 times lower than oxygen levels found in the normal prostate (~4% oxygen). High levels of hypoxia significantly correlate with increasing clinical stage and can predict biochemical failure following radiotherapy. Furthermore, hypoxia has also been implicated as a causative factor in malignant progression.
genetic instability,\textsuperscript{10} gene amplification,\textsuperscript{11} endothelial-to-mesenchymal transition (EMT)\textsuperscript{12,13} and selection of cells with diminished apoptotic potential and a greater invasive potential.\textsuperscript{14,15} It is, therefore, a viable therapeutic strategy to directly target hypoxic tumor cells in an attempt to improve treatment, meaning hypoxic targeting is likely to be a key part of precision medicine for prostate cancer.\textsuperscript{16,17}

One approach to blocking the influence of hypoxic tumor cells is to directly target this sub-population with a hypoxia-activated prodrug (HAP). HAPs are compounds that are designed to be reduced to an active, toxic form in cells when oxygen levels are very low.\textsuperscript{18,19} However, conventional HAPs (eg, tirapazamine, TH302, PR-104) are reduced in single-electron reduction steps, a reversible process that can redox cycle producing reactive oxygen in normoxic tissue.\textsuperscript{16} In contrast, alkylaminoanthraquinone-di-N-oxides are prodrugs that have a different mechanism of activation. These aliphatic N-oxides are reduced through an obligate two-electron reduction. This results in oxygen atom transfer which under hypoxic conditions results in irreversible formation of a stable anti-proliferative agent and water. For this reason, our previous work has focused on AQ4N\textsuperscript{20} and its more recently described deuterated analogue OCT1002 (OncoTherics Ltd).\textsuperscript{21,22} These unidirectional HAPs (uHAPs) are reduced in hypoxia to the metabolically stable reduction products (AQ4 and OCT1001, respectively). Studies with AQ4N and OCT1002 show that hypoxia-mediated reduction results in a product with high affinity for DNA and targeting of topoisomerase II,\textsuperscript{23,24} which can effect a long-term inhibition of both DNA replication and G2/M cell transition.\textsuperscript{25,26}

Previously we have shown that OCT1002 kills hypoxic prostate cancer cells in vitro and in vivo.\textsuperscript{21} We provided the first evidence that OCT1002 has a hypoxia-dependent anti-tumor effect in androgen-sensitive LNCaP prostate tumor xenografts and the effect can be markedly enhanced when combined with daily bicalutamide administration, a drug which targets the androgen receptor (AR). This is consistent with previous studies in the same model, which showed that AQ4N can block re-oxygenation that occurs during prolonged (>14 days) treatment with daily bicalutamide.\textsuperscript{27} We have also shown that single-modality hormone treatment drives development of more malignant tumors, a consequence of reduced vascularization and oxygenation (days 1-14) followed by a revascularization and re-oxygenation over the next 14 days. This was accompanied by a cascade of molecular and phenotypic changes that included evidence of EMT and increased metastasis to the lungs within 4 weeks.\textsuperscript{12,27} The novel analogue OCT1002 caused similar effects in LNCaP xenografts and we also showed that it could block significantly the molecular changes caused by bicalutamide alone,\textsuperscript{21} thereby demonstrating that detrimental hypoxia-induced cellular responses can be effectively blocked.

To further demonstrate the potential of OCT1002, it is important to test its effect in other xenograft models to ensure this is not a cell-line specific result. In particular, it would be instructive to see its impact on androgen-independent prostate tumor cells, since there is a clear clinical need for strategies that can improve the treatment of castrate-resistant prostate cancer (CRPC). Therefore, the current study has been designed to evaluate the effect of OCT1002 on CRPC tumor growth using murine PC3 xenograft and dorsal skin fold (DSF) models. The DSF model allows the extra opportunity to analyze changes in tumor vasculature within the tumor microenvironment in response to drug interaction. For comparison, the effects of three drugs currently used in the clinic to treat CRPC (abiraterone, docetaxel, and cabazitaxel) are similarly investigated, singly and in combination with OCT1002.

## 2 MATERIALS AND METHODS

### 2.1 Cell culture

All cell-lines were obtained from American Type Culture Collection (ATCC, Rockville, MD). Cells were frozen at low passage number and used within 3-6 passages. Cells were authenticated by in-house genotyping service and routinely confirmed as mycoplasma-free (InvivoGen, Toulouse, France). LNCaP cells were cultured in RPMI 1640 culture medium (Life Technologies, Paisley, UK) supplemented with 10% foetal bovine serum (FBS), D-glucose (10 mM; Sigma, Poole, UK) and HEPEs (10 mM; Sigma). PC3 and 22Rv1 were cultured in RPMI-1640 supplemented with 10% FBS. For treatment in hypoxic conditions, 5000 cells were seeded in a 96 well plate and allowed to adhere overnight. After dosing with vehicle (phosphate buffered saline, PBS) or OCT1002 (1 μM), cells were grown at 37°C in 0.1% oxygen in a InvivO2 hypoxia work station (Ruskinn Technology, Bridgend, UK) for 0, 4, 24, 48, or 72 h, before being placed in normoxia (20% oxygen) for 72, 68, 48, 24, and 0 h. The cells were then harvested and cell viability was measured using an XTT assay (Roche, Sussex, UK).

### 2.2 In vivo methods

#### 2.2.1 Animal maintenance

In vivo experiments were conducted in accordance with the Animal (Scientific Procedures) Act 1986 and the UKCCCR guidelines for the welfare of animals in experimental neoplasia.\textsuperscript{28} Male athymic nude mice (8-10 weeks) weighing 30-35 g (Envigo, Cambridgeshire, UK) were housed under standard laboratory conditions in a temperature-controlled (22°C; 50-55% humidity) pathogen-free environment with a 12 h light-dark cycle. Food and water was supplied ad libitum.

#### 2.2.2 Xenograft establishment

Tumor oxygenation and growth delay

LNCaP, 22Rv1, and PC3 xenografts were established on the rear dorsum of nude mice by subcutaneous injection of 2 x 10\textsuperscript{6} cells suspended in 100 μL of matrigel with a 21 g needle (Becton Dickinson, Oxford, UK). When tumors reached approximately 200 mm\textsuperscript{3} intratumor pO\textsubscript{2} was measured using an OxyLiteTM 2000E system (Oxford Optronix, UK) as previously described.\textsuperscript{13,27} For growth delay studies, tumors were measured every 2 days using Vernier callipers. When the tumor volume reached between 150-200 mm\textsuperscript{3}, mice were randomly assigned to treatment groups and dosing initiated (day 0). Mice were
sacrificed on day 28 in the 4-week dosing experiment and on day 42 in the 2-week dosing experiment except in the cabazitaxel group, which required humane sacrifice on day 35 due to toxicity.

2.2.3 | Drug administration

OCT1002 (OncoTherics, Shepshed, UK) was prepared in sterile PBS and administered by intraperitoneal (i.p.) injection (30 mg/kg; once per experiment unless otherwise indicated). Cabazitaxel (5 mg/kg; once per week) (Hangzhou Dayang Chemical Industry Limited Company, Zhejiang Sheng, China), docetaxel (10 mg/kg; twice per week) (Sigma) were administered by i.p. injection. Abiraterone (98 mg/kg; daily) (Hangzhou Dayang), was administered orally (p.o.) by gavage. Vehicle was 0.1% DMSO in corn oil (Sigma).

2.3 | Dorsal skin fold model

2.3.1 | Dorsal skin fold (DSF) preparation

A bespoke transparent DSF "window chamber" (APJ Trading Co. Ltd) was attached to the dorsum of anaesthetized nude mice. A tumor fragment (roughly 0.5 mm in diameter, obtained from a mouse PC3 xenograft, was implanted onto surgically exposed panniculus carnosus muscle within the window, washed with saline and covered with a plastic cover slip. After surgery mice were given a topical prophylactic antibiotic (Chloramphenicol; Martindale Pharmaceuticals, UK). Tumors were allowed to vascularize for 7 days following surgery and then randomly assigned to treatment groups, this was designated as day 0, and assigned to treatment groups with drug concentrations as described above. Tumor vasculature was then imaged on days 0, 7, 14, 21 using a stereomicroscope. Image analysis was carried out using Touptek software (Touptek Photonics, China). All surgical procedures were performed under aseptic conditions and the body temperature of animals was kept constant using heated pads.

2.4 | Statistical analysis

Data from in vitro and in vivo studies were analyzed using a two-tailed student's t-test unless otherwise indicated. All statistical analysis was carried out using the Prism 5.0 software (GraphPad). Differences between points were deemed statistically significant with a $P < 0.05$ (95% confidence interval).

3 | RESULTS

3.1 | Effect of OCT1002 on prostate cancer cell growth in hypoxia

Initially, in vitro studies were carried out in three prostate cancer cell lines (LNCaP, 22RV1, and PC3) to confirm their potential for differential cytotoxicity of OCT1002 in normoxia (20% oxygen) and hypoxia (0.1% oxygen). After as little as 4 h exposure to hypoxia, OCT1002 (1 μM) decreased the viability of cells as compared to normoxia in all cell lines (Supplementary Figure S1). At most time points (4, 24, 48, and 72 h) OCT1002 caused more cell death than hypoxia alone.

3.2 | OCT1002 slows tumor growth control in hypoxic PC3 xenograft model

When prostate tumor xenografts were grown in vivo in nude mice, they showed a reproducible and consistently low level of oxygenation that was characteristic of each cell line; LNCaP: $14.5 \pm 2.02$ mmHg (1.9% oxygen), 22RV1: $11 \pm 0.74$ mmHg (1.4% oxygen) and PC3: $3.7 \pm 0.71$ mmHg (0.49% oxygen) (Figure 1A). The toxicity of OCT1002 in nude mice was assessed by measuring their weight for 21 days after administration of a single dose of OCT1002 (10 or 30 mg/kg). No significant difference in weight was observed in either treatment group compared to control. All groups showed a small but steady increase in weight throughout the observation period confirming the lack of toxicity of OCT1002 in nude mice up to at least 30 mg/kg (Figure 1B).

Subsequently, nude mice bearing PC3 tumors were treated with OCT1002 (30 mg/kg; day 1 or days 1 and 7) over 28 days (Figure 1C). For comparison three drugs widely used in late stage CRPC, at dosing schedules reported in preclinical studies, that is, cabazitaxel (5 mg/kg 1× week), abiraterone (98 mg/kg daily), and docetaxel (10 mg/kg 2× week). The effects of all drug treatments on tumor growth were compared to vehicle. OCT1002 as a single low dose treatment showed a marked effect on this severely hypoxic tumor; adding a second dose at day 7 did not have any additional effect. Of the three other chemotherapy drugs, abiraterone showed the highest anti-tumor effect whereas docetaxel and cabazitaxel displayed a growth control very similar to OCT1002. It is interesting that abiraterone was markedly effective against PC3 tumors, which are AR-negative, since its primary action is known to be blockade of androgen synthesis through inhibition of CYP17A1. However, several in vitro studies have demonstrated that the anti-tumor effect of abiraterone in PC3 cells is not solely associated with blockade of androgen synthesis.

Our in vivo studies would confirm these observations. In a second experiment, tumors were treated only with the selected regimens for 14 days and the effect of the drugs on tumor growth was followed for a further 28 days (Figure 1D). Regrowth of the tumor was considerably more marked for cabazitaxel and this drug caused considerably more toxicity, to the extent that by day 35 all of the animals in this group had been sacrificed. The other treatment regimens were well tolerated until day 42 when the experiment was ended. The single dose of OCT1002 (30 mg/kg on day 1) was as effective as the 14-day scheduled dosing of abiraterone and docetaxel in controlling tumor regrowth. Representative images of mice and the excised tumors are provided in Figure 1E.

3.3 | OCT1002 inhibits tumor growth and reduces PC3 tumor vasculature in DSF model

PC3 tumor fragments were grown in DSF “window chambers” and these were used to measure the effect of OCT1002 on tumor growth...
and vasculature. Abiraterone, cabazitaxel, and docetaxel were also tested for comparison. All drugs had some effect on tumor growth in comparison to vehicle treatment, with abiraterone being the most effective, showing a particularly large anti-tumor effect between days 14 and 21 (Figures 2A and 2B). Vasculature was assessed by calculating the mean density of tumor blood vessels, which markedly increased for control tumors from day 0 to day 21 (Figures 2C and 2D). OCT1002 treatment resulted in a significant reduction in the mean vessel area covered on days 14 and 21, while abiraterone resulted in a significant vessel reduction at days 7, 14, and 21. Docetaxel significantly reduced vasculature compared to vehicle on day 7 and 14, but by day 21 tumor vasculature was restored to levels similar to that displayed in control tumors. Cabazitaxel treatment showed a trend toward reduced vasculature compared to control but these measurements did not reach significance.

3.4 | Effect of drug combinations on PC3 tumor growth and vasculature

Each of the clinically approved chemotherapy drugs was then given in combination with OCT1002 on day 1. In the PC3 xenograft model, no marked advantage was observed in the combinations, although it was noted that cabazitaxel was better tolerated when the mice were treated with cabazitaxel in combination with OCT1002 (Figures 3A-3C). However, when tumors were analyzed in the DSF model, OCT1002 caused a significant growth reduction when used in combination with the other drugs, compared to single treatment (Figures 4A-4C). This effect was most marked when OCT1002 was used in combination with cabazitaxel and docetaxel (Figures 4B and 4C).

When the mean vessel density was measured in the PC3 DSF model it was clear that the drug combinations also had an effect on tumor vasculature. When OCT1002 was combined with abiraterone the vascular density was further reduced on day 14 and 21, compared to OCT1002 alone (Figure 5A). OCT1002 appeared to cause some further reduction on the vasculature in combination with cabazitaxel, though it only reached significance on day 14 (Figure 5B). Combining OCT1002 with docetaxel resulted in significantly reduced vasculature at day 14 and 21 compared to either drug used singly (Figure 5C).

4 | DISCUSSION

Improving treatment of CRPC remains a major challenge for clinicians. Current chemotherapy includes cytotoxic agents such as docetaxel and cabazitaxel, or drugs targeting AR axis signalling, such as enzalutamide and abiraterone. However, drug resistance to these
agents is common, meaning novel strategies and/or combinations are required to combat this.\textsuperscript{32,33} Since hypoxia is a common feature of prostate tumors and a known driver of prostate cancer progression, the use of HAPs/uHAPs to target hypoxic cells represents a novel approach which can address developing drug resistance. This study presents further pre-clinical evidence to demonstrate the potential of the uHAP OCT1002 as a novel drug for treating prostate cancer.

We first confirmed OCT1002 was capable of killing prostate cancer cells grown in hypoxic conditions in vitro (Supplementary Figure S1) to corroborate cell-line analysis from our previous study.\textsuperscript{21} Having demonstrated the effect of OCT1002 on PC3 cells in vitro, we proceeded to use PC3 cells in murine models in vivo. In our previous work, we measured the oxygen levels in LNCaP and 22Rv1 tumors in SCID mice and found that each untreated tumor type had an intrinsic level of oxygenation that was specific and consistently reproducible.\textsuperscript{13,21} Here, we demonstrated that PC3 tumors grown in nude mice are considerably more hypoxic than LNCaP and 22Rv1 tumors (Figure 1A). Based on our experience of tumor xenograft models, Figure S1) to corroborate cell-line analysis from our previous study.\textsuperscript{21} Having demonstrated the effect of OCT1002 on PC3 cells in vitro, we proceeded to use PC3 cells in murine models in vivo. In our previous work, we measured the oxygen levels in LNCaP and 22Rv1 tumors in SCID mice and found that each untreated tumor type had an intrinsic level of oxygenation that was specific and consistently reproducible.\textsuperscript{13,21} Here, we demonstrated that PC3 tumors grown in nude mice are considerably more hypoxic than LNCaP and 22Rv1 tumors (Figure 1A). Based on our experience of tumor xenograft models, agents is common, meaning novel strategies and/or combinations are required to combat this.\textsuperscript{32,33} Since hypoxia is a common feature of prostate tumors and a known driver of prostate cancer progression, the use of HAPs/uHAPs to target hypoxic cells represents a novel approach which can address developing drug resistance. This study presents further pre-clinical evidence to demonstrate the potential of the uHAP OCT1002 as a novel drug for treating prostate cancer.

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we propose that the intrinsic hypoxic status of the tumors is determined by several inter-dependent factors. Clearly a major factor will be the extent of the vascular network that develops in vivo, which can subsequently change in response to anti-tumor treatments.13,21 Linked to this, these prostate tumor cell lines have a varying genetic background and exhibit considerable differences in a number of signalling pathways that affect both metabolism and the cells’ ability to develop a functioning vascular network.34 Thus, it is not surprising that they exhibit differing levels of vasculature and oxygenation in the tumors grown in vivo. Furthermore, it is likely that tumor hypoxia will vary depending on the mouse strain used in these models. For example, we get consistent results for each model that we have used, but interestingly LNCaP tumors are more hypoxic when grown in SCIDs than nudes, suggesting the specific characteristics of the growth environment plays a role too. It therefore appears that oxygenation of tumors in vivo is determined by different overlapping physiological and molecular influences, which will be important to our understanding of tumor biology in the treatment responses of preclinical models. Indeed, many of these factors are likely to influence treatment responses for CRPC.

The intrinsically low oxygen levels in PC3 tumors suggested that OCT1002 should certainly be activated in these tumors. Indeed, it should be noted that all three of these xenograft tumors have oxygen levels which are well below that of normal prostate tissue ∼4%. The range demonstrated in the mouse tumor xenografts is consistent with oxygenation levels exhibited by human prostate tumors, making them a clinically relevant model for testing OCT1002.6

We first showed a lack of OCT1002 systemic toxicity in this PC3 xenograft model, as measured by mouse body weight retention (Figure 1B). This is consistent with OCT1002 acting as a prodrug which is inactive in normal tissues. We then proceeded to test its ability to control tumor growth, using three approved chemotherapy drugs for...
comparison purposes. All drugs demonstrated significant tumor growth control at day 28 compared to vehicle treatment. Cabazitaxel (5 mg/kg; 1× week) had some anti-tumor efficacy, but was also the most toxic to the mice. Docetaxel (10 mg/kg; 2× week) showed a similar inhibition of tumor growth but was better tolerated. Abiraterone (98 mg/kg; daily) exhibited the best tumor growth control. Comparing these drugs with OCT1002, we found it particularly noteworthy that a single, low dose of OCT1002 (30 mg/kg; administered on day 1) demonstrated similar tumor growth control to both cabazitaxel and docetaxel (Figure 1C). As observed above, this may be because PC3 tumors are extremely hypoxic and thus particularly susceptible to OCT1002. This also underlines the potential success that OCT1002 may have in the clinic if used in patients identified to have hypoxic tumors, either by molecular or physical biomarkers. Encouragingly, PET-based imaging techniques have been established that can be used to quantify the hypoxic fraction, including an ongoing clinical trial (NCT01567800), which could identify those patients most likely to benefit from OCT1002. For example, CRPC patients predicted to have worse outcome on enzalutamide or abiraterone could be offered a new treatment option based on use of OCT1002.

The persistent, long-lasting effect of OCT1002 was further demonstrated when we stopped treatment with the three approved drugs at day 14 and compared tumor regrowth over the next 28 days to the OCT1002 treatment group, which had received a single dose on day 1. This single, early dose of OCT1002 showed a similar ability to inhibit tumor regrowth as abiraterone and docetaxel (Figures 1D and 1E). It was markedly better than cabazitaxel treatment, which proved quite toxic to mice in this experiment, to the extent that mice had to be prematurely sacrificed. This demonstrates the unique uHAP properties of OCT1002, in that a long-lasting effect is achieved due to the irreversible activation within hypoxic cells to the toxic reduction product OCT1001. We propose this selective targeting prevents the ability of hypoxia-resistant cells to re-establish the tumor as quickly.

Since our previous work had identified the importance of drug effects on LNCaP tumor vasculature, we wanted to extend this to other tumor models and therefore examined how OCT1002 impacted upon PC3 tumor vasculature in vivo. We compared the effects of OCT1002 and the three clinically approved drugs on tumor growth and vasculature using the DSF window chamber assay (Figure 2). As was observed using the dorsal tumors, a single dose of OCT1002 (30 mg/kg; administered on day 1), demonstrated similar tumor growth control to both cabazitaxel and docetaxel (Figures 2A and 2B). Abiraterone was again the most effective in controlling tumor enlargement in the window chamber.

As for tumor vasculature, all drugs demonstrated a significant effect compared to vehicle-treated tumors at various time intervals following dosing (Figures 2C and 2D). OCT1002 resulted in significantly reduced tumor vasculature density at day 14 and 21, compared to control tumors, an effect we had previously noted in LNCaP xenografted tumors. In contrast, docetaxel showed a marked anti-vascular effect at day 7 and 14, but by day 21 the vasculature had recovered to levels displayed by control tumors, despite continuance of the twice weekly treatment. Cabazitaxel treatment reduced vasculature, but not significantly. Abiraterone proved to have the most consistent effect, resulting in significant reduction of vessel coverage compared to control tumors from day 7 to 21. This corroborates a similar anti-vascular effect in LNCaP tumors treated with bicalutamide in DSF experiments. It is likely that this is a consequence of the blockade of tumor cell growth, which will disrupt its capacity for pro-angiogenic signalling and hence vessel formation. However, our previous work also demonstrated that tumor vasculature could re-establish itself eventually (after 14-21 days), even with continued dosing. We hypothesized that resistant tumor cells which survived the increased hypoxic stress caused by vascular reduction were able to promote re-growth of the tumor vasculature. This new data provides further evidence for this, in the observation that the initial inhibitory effect of docetaxel on blood vessels was abrogated by day 21, which is very similar to the effect we noted using bicalutamide against LNCaP tumors. This ability of tumors to recover quite quickly from chemotherapeutic interventions is a worrying observation as it suggests that resistant cells within tumors adapt to treatment by adopting a more pro-angiogenic phenotype. In LNCaP tumors we have shown that this adaptation was accompanied by changes associated with development of a more malignant genotype. This would help to explain why patients frequently relapse to a more aggressive disease following initially successful therapy. To address this, it seems clear that strategic drug combinations are required to overcome resistance, either concomitantly or sequentially. Indeed, recent results from the CHARITY and STAMPEDE clinical trials have revealed that docetaxel in combination with androgen deprivation therapy improved relapse-free survival in patients with high-risk localized prostate cancer. A more recent report from the STAMPEDE trial also showed that men with locally advanced or metastatic prostate cancer who received ADT plus abiraterone and prednisolone had significantly higher rates of overall and failure-free survival than those who received ADT alone. Hence the right drug combinations can overcome tumor resistance in prostate cancer sufferers. As hypoxia is widely recognized to play a crucial role in developing tumor resistance to therapy, the current study supports the use of for OCT1002 as a strategic drug for use in such a combination.

To further investigate this we therefore examined the effect of combining OCT1002 with agents clinically used in prostate cancer on PC3 tumor growth. We could detect no additional benefit of these combinations on xenograft tumor growth inhibition using the particular schedules chosen. However, as we have shown PC3 tumors are very hypoxic so we predicted we might get a maximal effect with OCT1002 as a single agent. Previously in a pilot study of another intrinsically very hypoxic xenografted pancreatic tumor (0.3% oxygenation in control tumors) we found a marked effect equivalent to treatment with the standard-of-care drug gemcitabine (unpublished data). Others have noted a similar finding with AQ4N. This confirms to us that when assessing uHAP effects in a tumor that is intrinsically very hypoxic then significant effects can be achieved even with single agent therapy, meaning the scope for seeing an additive effect is limited. Another important consideration in assessing drug combinations is the potential for negative interactions. However, our findings provide a firm basis for excluding negative interactions between
OCT1002 and other standard-of-care drugs. This will enable combinations to be explored to identify the potential for additivity or even synergy in dose schedule studies.

In the current study we felt that, before progressing to further scheduling combinations, it was important to look for effects in the DSF model since we have found this model to be more sensitive and capable of showing subtler differences. Using this approach, we did observe that OCT1002 can enhance the anti-tumor effects of the three other drugs. Abiraterone was very effective as a single agent at tumor growth inhibition in this model but nevertheless a statistically significant improvement was noted at day 7 and 14 when it was used in combination with OCT1002. The combination was also significantly better at controlling tumor growth than OCT1002 alone at day 7-21. Similarly, combining OCT1002 with cabazitaxel was significantly better at controlling tumor growth than either treatment alone at day 7-21. Finally, combining OCT1002 with docetaxel was significantly better at controlling tumor growth than either treatment alone at day 14 and 21. When we looked at the effect of the combinations on vasculature density, the most interesting effect was for docetaxel (Figure 5C). At day 14 and 21, the combination showed a significant reduction of vasculature compared to either treatment alone. At day 21, the restoration of vascular density observed with docetaxel alone was completely abrogated when combined with OCT1002. This corresponds with the inhibitory effect of the docetaxel/OCT1002 combination on tumor growth observed in this model, showing promise for this treatment combination in the clinic for CRPC. Indeed, the low toxicity of OCT1002 and its mode of action may help address issues of polypharmacy and toxicity associated with treatment of metastatic CRPC patients41 by allowing lower doses of standard chemotherapy drugs to be potentially used.

Overall, our results demonstrate that OCT1002 has potential for treating CRPC. Further work is now needed to probe the specific effects of OCT1002 at a molecular and cellular level, as well as optimizing the dosing and scheduling of OCT1002 as a single agent or in combination with other drugs. Furthermore, it is now clear that a wide molecular diversity exists in human prostate tumors.52 Understanding the complexities of this diversity is important for patient stratification as different sub-types of prostate cancer exist, each with different druggable pathways which may be potentially targeted.42,43 In OCT1002, we have an agent that when activated inhibits topoisomerase II,24 a critical player in DNA replication, DNA repair and AR signalling,24 therefore impacting upon targets that are important in driving prostate cancer. It therefore shows considerable promise as a novel precision therapy for prostate cancer.

5 | CONCLUSIONS

In conclusion, we have shown that OCT1002 can control the growth of CRPC xenografted PC3 tumors, in a similar way to that observed in androgen-sensitive LNCaP tumors. At a single dose of OCT1002 the effects compared favorably to the multiple dosing schedules of abiraterone, cabazitaxel, and docetaxel in these prostate cancer models. Our DSF studies showed the promise of using OCT1002 in combination with other drugs to inhibit tumor growth and significantly diminish tumor vasculature density. Together, our results provide the first evidence for the potential for OCT1002 to improve treatment responses in CRPC, especially where it is shown that patient tumors are hypoxic, a feature which is widely reported for this tumor type.

ACKNOWLEDGMENT

This study was supported by a Prostate Cancer UK research grant (PG12-02). Additional support was provided by Department of Employment and Learning, Northern Ireland.

DISCLOSURE STATEMENT

SR McKeown and PJ Smith are directors of OncoTherics Ltd; RJ Errington, LH Patterson and PJ Smith are directors of BioStatus Ltd. J Worthington and SR McKeown are directors of Axis Bioservices. H Nesbitt and DJ McKenna declare no conflict of interest.

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