**RADIOBIOLOGY SPECIAL FEATURE: REVIEW ARTICLE**

**An arranged marriage for precision medicine: hypoxia and genomic assays in localized prostate cancer radiotherapy**

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**ABSTRACT**

Prostate cancer (CaP) is the most commonly diagnosed malignancy in males in the Western world, and the lifetime risk of developing CaP exceeds 1 in 6 males. Accurate and consistent clinical staging is essential for optimal treatment planning. Prostate specific antigen (PSA) based staging and histology based staging are the current clinical staging tools. 

**BACKGROUND: THE NEED FOR NOVEL BIOLOGICAL END POINTS FOR PROSTATE CANCER PROGNOSIS**

Prostate cancer (CaP) is the most commonly diagnosed malignancy in males in the Western world. The current clinical staging tools use pathologic Gleason score, pre-treatment prostatic-specific antigen and Union for International Cancer Control-TNM staging to place patients with localized CaP into low-, intermediate- and high-risk categories. These categories are used to represent an increasing risk of biochemical failure and CaP-specific mortality rates. These categories also reflect the need for increasing treatment intensity and justification for increased side effects. In this article, we point out that 30–50% of patients will still fail image-guided radiotherapy or surgery despite the judicious use of clinical risk categories owing to interpatient heterogeneity in treatment response. To improve treatment individualization, better predictors of prognosis and radiotherapy treatment response are needed to triage patients to bespoke and intensified CaP treatment protocols. These should include the use of pre-treatment genomic tests based on DNA or RNA indices and/or assays that reflect cancer metabolism, such as hypoxia assays, to define patient-specific CaP progression and aggression. More importantly, it is argued that these novel prognostic assays could be even more useful if combined together to drive forward precision cancer medicine for localized CaP.
Despite a multitude of treatment options, there are no individualized clinical tests that absolutely tell which patients are unlikely to fail local treatment from those patients who are most likely to fail local treatment within a given clinical risk category. This problem is illustrated by the fact that despite the use of stringent clinical criteria to place patients into clinical prognostic groups, 30–50% of males can still fail precision RT or surgery owing to local resistance and/or systemic spread.1–3 Despite the publication of Phase III dose-escalated EBRT clinical trials in CaP designed to counteract failure due to CaP radioresistance, none of these trials have shown benefit in decreasing PCSM.8 The lack of an effect on survival with EBRT dose escalation can be explained by the fact that in a significant proportion of patients, treatment failure is due to the presence of occult systemic disease rather than local resistance, and that these patients need to be treated with intensification of systemic therapy not EBRT dose intensification, to decrease CaP mortality.1,4 Personalized CaP medicine therefore requires genomic- or biology-based biomarkers, in addition to existing clinical biomarkers, to explain patient heterogeneity in outcomes. Furthermore, even if an increased probability of occult metastases can be predicted, even more biomarkers will be required to favour the use of one systemic agent vs another, let alone the scheduling of these agents relative to each other (Figure 1).10

An additional complication to personalized medicine is the knowledge that many low-risk CaPs are indolent and that their overtreatment results in significant morbidity.4,5,11 For example, two-thirds of low-risk CaPs have an indolent course that can be followed without radical treatment when appropriately placed into AS protocols, thereby preventing the side effects and costs of RT or surgery. The corollary is that one-third of these low-risk patients are being inaccurately classified as having indolent cancers and require treatment.5 On an individual basis, there are no assays that can predict with confidence the need for therapy in low-risk CaP.

So, how do we move forward in precision medicine for CaP using precision RT when faced with such clinical conundrums? One approach is to take advantage of technological advances in genomic medicine to determine patient-specific CaP genomics that reflect tumour progression and metastatic disease in addition to novel biology.12,13 State-of-the-art whole-genome sequencing technologies have the capacity for generating a breathtaking amount of genomic data (in excess of 10 billion bases per day) at a fraction of the cost than a decade ago. DNA- and RNA-based prognostic tests to predict CaP recurrence are being actively developed within the industry and academia for clinical use. Finally, there is also a rich history in radiation oncology for characterizing the tumour microenvironment, including assays for subregions of hypoxia within localized CaPs, which have a prognostic impact. This article will now discuss the potential of genomic and hypoxia assays to help attain the goal of implementing precision cancer medicine for patients undergoing curative RT for CaP.
TUMOUR HYPOXIA MEASUREMENTS ARE PROGNOSTIC IN LOCALIZED PROSTATE CANCER

The tumour microenvironment is characterized by subregions of nutrient deprivation, low extracellular pH, high interstitial fluid pressure and hypoxia. Hypoxic areas arise in tumours when oxygen consumption rate exceeds that of supply. The blood vessels within a tumour microenvironment are usually irregularly organized and have abnormal architecture such that tumours will contain regions where the partial oxygen concentration (pO₂) is significantly <5 mmHg (i.e. normal tissues range from 10 to 80 mmHg). Intratumoural hypoxia limits the effectiveness of RT and chemotherapy. Cells that are hypoxic or anoxic are usually two to three times more resistant to ionizing radiation when compared with oxic cells unless they are DNA repair deficient (see hypoxia section below). Chemotherapy-related tumour cell kill in this scenario is also limited owing to poor drug distribution and decreased tumour cell proliferation that limits the effectiveness of S-phase-specific chemotherapeutics. Therapy-resistant cells can adapt to hypoxic regions and result in cycles of selection for aggressive mutator phenotypes with faulty DNA repair and increased genetic instability. Pre-clinical data have also linked tumour cell hypoxia to increased experimental and spontaneous metastasis. Metastasis is a multistep process that involves intravasation through the basement membrane and extracellular matrix into the host vasculature, extravasation through vessel walls and then forming a new nidus with the organ of metastatic spread, and then tumour angiogenesis during secondary tumour growth. Indeed, hypoxia alters metastasis gene expression including that of E-cadherin (cell–cell contact), urokinase-type plasminogen activator receptor (degradation of extracellular matrix proteins), hepatocyte growth factor (cellular motility) and vascular endothelial growth factor (VEGF; angiogenesis and vascular permeability). Therefore, pre-clinical data support two general aspects relating to the resistance of hypoxic tumours during RT: increased local tumour cell radioresistance and/or an increased capacity for systemic metastases.

Clinical studies that have attempted to directly measure the level of hypoxia in CaP have used pO₂ electrodes, hypoxia imaging (positron emission tomography (PET)) and immunohistochemistry (IHC) (Table 1). These studies support the concept that hypoxic subregions exist within localized CaPs and are associated with higher rates of biochemical failure following surgery or RT. For example, a prospective clinical trial showed that localized CaPs have uptake of the hypoxia biomarker, pimonidazole, and that this uptake correlated with GS but not vascularity. Turaka et al showed that decreased prostate-to-muscle oxygen ratio was an important predictor of early biochemical recurrence following brachytherapy secondary to occult metastases at the time of treatment. In the largest clinical study of CaP hypoxia using direct pO₂ measurements, Milosevic et al showed that hypoxia is associated with both early biochemical relapse and local recurrence in the prostate gland. Despite these data, hypoxia-targeted therapy is still not a standard current cancer treatment. Agents that may be useful in this context might include the use of ADT (given the ability of androgen suppression to improve CaP oxygenation), molecular-targeted agents or hypoxia-targeted systemic agents. Importantly, as we will see below, hypoxia can also drive genetic instability by inhibiting DNA repair. Understanding these additional consequences of the hypoxic microenvironment on the development of genetic instability may give novel treatment approaches to combat hypoxia-associated resistance.

GENETIC INSTABILITY AND GENOMIC ASSAYS IN LOCALIZED PROSTATE CANCER

Chromosomal instability and aneuploidy are associated with cancer progression and adverse prognosis in CaP. Patients with tetraploid or aneuploid CaP tumours have increased mortality following radical prostatectomy when compared with patients whose CaP tumours are diploid. The specific genomic events that might link to this aspect of aggression are now being understood in the context of abnormal gene copy number loss, gene mutation and abnormal gene expression relating to oncogenes and tumour suppressor genes. Hypoxia-targeted studies have used array comparative genomic hybridization (using DNA from diagnostic CaP biopsies prior to therapy) to associate specific gene copy number alterations with prognosis following EBRT or radical prostatectomy. Copy number loss of the tumour suppressor genes novel human prostate-specific, androgen-related homeobox gene (NX3.1) or Phosphatase and tensin homologue (PTEN) or the androgen synthesis genes steroidogenic acute regulatory protein (StAR) and hydroxysteroid (17-beta) dehydrogenase 2 (HSD17B2) are novel and independent genomic prognostic factors (hazard ratio ranges from 2 to 4 for failing local therapy). When associated with copy number gain of the proto-oncogene cMYC, NXX3.1 loss was also associated with local radioresistance. Furthermore, males who
carry germ-line mutations in the breast cancer 2, early onset (BRCA2) tumour suppressor gene develop aggressive CaPs (higher GSs and increased nodal metastases) that have very poor prognosis following surgery or RT.36,37 Surprisingly, the presence or absence of a fusion gene between transmembrane protease serine 2 and the ETS-related gene (TMPRSS2–ERG) fusion (found in >50% of all CaP patients) is not prognostic.38,39

Unbiased genome-wide signatures based on DNA or RNA indices have recently been developed to predict PSA recurrence in the post-operative setting. A set of DNA-based biomarkers (genomic evaluators of metastatic CaP) has improved utility over the sole use of clinical recurrence nomograms (e.g. Kattan nomogram) in the prediction of recurrence following surgery.40 Recent evidence also suggests that miRNAs may also have prognostic value, although only a limited number of studies have correlated genome-wide analysis of miRNA species with differential prognosis.41–43 High levels of miR-96 were prognostic of biochemical recurrence in a series of 155 radical prostatectomies.41 Similarly, others have noted an independent prognostic value (e.g. independent from currently utilized clinical parameters of PSA, T-category and GS) for miR-191, miR-145, miR-100 and miR-122, many of which are arranged in genomic clusters.42,43

Table 1. Clinical studies of hypoxia assays and prognosis in localized prostate cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>T-category</th>
<th>Assay</th>
<th>Prognostic value and details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turaka et al23</td>
<td>57</td>
<td>cT1–3</td>
<td>pO2 probe</td>
<td>Prognostic: lower prostate/muscle pO2 ratio predicted early biochemical failure after brachytherapy</td>
</tr>
<tr>
<td>Milosevic et al21</td>
<td>247</td>
<td>cT1–2</td>
<td>pO2 probe</td>
<td>Prognostic: largest study showing that hypoxia predicted early biochemical relapse after radiotherapy and local recurrence</td>
</tr>
<tr>
<td>Vergis et al24</td>
<td>201 (RT); 289 (surgery)</td>
<td>cT1–3</td>
<td>IHC-VEGF, HIF-1α, OPN</td>
<td>Prognostic: increased expression of VEGF, HIF-1α and, for patients treated with surgery, OPN identified patients at high risk of biochemical failure</td>
</tr>
<tr>
<td>Carnell et al18</td>
<td>43</td>
<td>cT1–3</td>
<td>IHC-PIMO</td>
<td>Not tested, but a positive correlation of PIMO +3 binding with Gleason score was demonstrated</td>
</tr>
<tr>
<td>Boddy et al17</td>
<td>149</td>
<td>cT1–3</td>
<td>IHC-VEGF, HIF-1α</td>
<td>Not prognostic: there was a significant correlation between HIF-1α and HIF-2α expression, and with AR and VEGF expression. VEGF was also significantly related to the androgen receptor, whereas PHD2 was inversely related to HIF-2α expression. No significant association was shown between HIF-1α or HIF-2α and time to PSA recurrence</td>
</tr>
<tr>
<td>Green et al20</td>
<td>50</td>
<td>cT3</td>
<td>IHC</td>
<td>Prognostic: high VEGF expression was associated with lower disease-specific survival</td>
</tr>
<tr>
<td>Thoms et al22</td>
<td>199 (T1–3); 37 (M1)</td>
<td>cT1–T3</td>
<td>ELISA-OPN</td>
<td>Not prognostic: within localized prostate cancers plasma OPN was not predictive of more aggressive disease or response to radiotherapy or hormone therapy</td>
</tr>
<tr>
<td>Weber et al25</td>
<td>103</td>
<td>cT1–3</td>
<td>IHC</td>
<td>Prognostic: high nuclear expression of HIF-1α and low EGFR expression was associated with a good prognosis in patients treated with RT ± ADT</td>
</tr>
<tr>
<td>Garcia-Parra et al19</td>
<td>14</td>
<td>pT2b–T3a</td>
<td>PET-FAZA + IHC</td>
<td>Not prognostic: negative 18F-FAZA accumulation and CAIX staining in primary prostate cancer despite documented large lesions (up to 4 cm). HIF-1 staining was positive and independent of Gleason score</td>
</tr>
</tbody>
</table>

ADT, androgen-deprivation therapy; AR, androgen receptor; CAIX, carbonic anhydrase IX; cT, clinical T-category; EGFR, epidermal growth factor receptor; ELISA, enzyme-linked immunosorbent assay; 18F-FAZA, 18F-labelled fluoroazomycin arabinoside; HIF-1, hypoxia-induced factor 1; IHC, immunohistochemistry; OPN, osteopontin; PET, positron emission tomography; PHD, prolyl hydroxylase enzyme; PIMO, pimonidazole; pO2, partial oxygen concentration; PSA, prostate-specific antigen; pT, pathologic T-category; RT, radiotherapy; VEGF, vascular endothelial growth factor. pO2, measured with pO2 electrode.
Major research efforts have led to the development of RNA-based signatures to better stratify patients between indolent vs aggressive CaPs (Table 2). A 31-gene signature of biochemical recurrence following radical prostatectomy has been reported based on RNA expression of cell cycle progression genes; this prognostic assay was also validated in the setting of recurrence following EBRT. A 22-gene RNA expression panel has been similarly validated across multiple independent cohorts by several groups. Likewise, Wu et al have reported a 32-gene RNA expression signature that is prognostic of biochemical recurrence and metastatic disease following radical prostatectomy. Using DNA-based indices, Taylor et al have shown independent prognostic utility for six genetic clusters that are prognostic for biochemical recurrence, independent of the GS.

Table 2. DNA- and RNA-based prognostic signatures for localized prostate cancer

<table>
<thead>
<tr>
<th>Signature (DNA or RNA)</th>
<th>Signature development cohort</th>
<th>Outcomes predicted</th>
<th>Validation in separate cohorts (yes/no)</th>
<th>Evaluated in other treatment modality cohorts</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA-based CNAs for NKX3.1, PTEN, cMYC, STAR</td>
<td>Pre-radiotherapy</td>
<td>BCR</td>
<td>No</td>
<td>Yes: surgery</td>
<td>Zafarana et al; Locke et al</td>
</tr>
<tr>
<td>DNA-based CNA clusters (6 clusters)</td>
<td>Post-surgery recurrence</td>
<td>BCR</td>
<td>No</td>
<td>No</td>
<td>Taylor et al</td>
</tr>
<tr>
<td>RNA 22-gene expression signature</td>
<td>Post-surgery recurrence</td>
<td>M, PCSS, OS</td>
<td>Yes (two cohorts)</td>
<td>No</td>
<td>Cooperberg et al; Erho et al</td>
</tr>
<tr>
<td>RNA 31-gene expression signature</td>
<td>Post-surgery recurrence post-TURP recurrence</td>
<td>BCR, PCSS</td>
<td>Yes (three cohorts)</td>
<td>Yes (conservatively managed and radiotherapy)</td>
<td>Cuzick et al</td>
</tr>
<tr>
<td>RNA 32-gene expression signature</td>
<td>Post-surgery recurrence</td>
<td>BCR, M</td>
<td>Yes (one cohort)</td>
<td>No</td>
<td>Wu et al</td>
</tr>
</tbody>
</table>

BCR, biochemical recurrence; cMYC, proto-oncogene cMYC; CNA, gene copy number; M, metastases; NKX3.1, novel human prostate-specific, androgen-regulated homeobox gene; OS, overall survival; PCSS, prostate specific-cancer survival; PTEN, phosphatase and tensin homolog; TURP, transurethral resection of the prostate.

Additional DNA epigenetic modifications may drive individualized CaP biology and progression. Using high-throughput genome sequencing and DNA methylation analyses, >147,000 cancer-associated epigenetic alterations were observed in 51 tumour and 53 benign prostate samples; the specific nature of these alterations were dependent on the presence of absence of a TMPRSS2–ERG rearrangement. This observation of differential methylation events in fusion-negative tumours (based on enhancer of zeste homologue 2 (EZH2) gene activation) explains CaP carcinogenesis in the 50% of patients who are TMPRSS2–ERG fusion-negative. Systematic overviews of studies for the prognostic role of specific gene methylation (e.g. GSTP1, APC, RAR-β, RASSF1A, PITX2, CCND2, EDNRB and HOX family of genes) in CaP concluded that their prognostic roles are still unknown and require further validation in large clinical cohorts.

Integrating genome-, epigenome-, transcriptome- and proteome-wide data sets to iterate a multimodal genetic test will no doubt improve subgroup prognostication. Therefore, similar to hypoxia assays, patient-specific indices of genetic instability may be utilized to further define aggressive subsets of CaP for treatment intensification (Figure 2). But is there a biological link between genetic instability and hypoxia that leads to adverse prognosis? We will now discuss the potential interplay between hypoxia, DNA repair and genetic instability.

HYPOXIA AND GENOMIC INSTABILITY IN PROSTATE CANCER: A NOVEL THERAPEUTIC TARGET?

One model of interaction between the CaP tumour microenvironment and CaP genomics is that hypoxic tumour cells have down-regulated DNA repair function (e.g. decreased capacity for the repair of DNA double strand breaks; DSBs) in addition to any genetic instability due to oncogene activation or tumour suppressor gene inactivation. Such repair-deficient hypoxic tumour cells could adapt to low oxygen levels and acquire an aggressive “mutator” phenotype leading to treatment resistance and metastases. For example, there are two major pathways of DSB repair: non-homologous end-joining (NHEJ; active throughout the cell cycle) and homologous recombination (HR; requiring a homologous chromosome available only during S and G2 phases of the cell cycle). Hypoxia causes decreased transcription and translation of a series of HR and NHEJ genes, including Rad51, BRCA1, BRCA2 and DNA–protein kinase catalytic subunit (DNA-PKcs). Furthermore, functional studies using isogenic cells have reported hypoxia-induced NHEJ and HR defects. As a consequence, despite lower levels of initial DSB formation following ionizing radiation (IR) in hypoxic tumour cells, hypoxia-induced defects in DSBs repair increased the level of unrepaired DSBs and chromosomal aberrations at the first mitosis post-irradiation. If even a fraction of these hypoxic mutant tumour cells survive subsequent cell division, unstable genetic mutants could undergo clonal selection.

Hypoxia induces activation of common fragile sites throughout the genome (i.e. chromosomal regions prone to breakage) and
down-regulation of DSB repair has been implicated as an underlying mechanism to this chromosomal fragility.61 The compromise in DSB resolution in hypoxic cells and the resulting increase in chromosome aberrations is a "perfect storm" towards genomic instability in tumour cells that adapt and continue to proliferate under low oxygen conditions.27 Hypoxia can also lead to a decrease in function of other DNA repair pathways, including that of mismatch repair (MMR), nucleotide excision repair and the Fanconi anaemia (FA) pathway.62–65 The parallel reduction in various DNA damage repair pathways can all potentially contribute to the acquisition of aggressive tumour phenotypes.27

The genetic instability in hypoxic cells would at first seem a complex phenotype to target with standard or molecularly targeted therapies. Any clinical approach would require careful assessment of the tumour microenvironment and genomic status (using assays mentioned in previous sections) to incorporate both hypoxia and genomic assessment as part of a standard of care. However, the repair-defective phenotype might just be the undoing of the hypoxic resistance phenotype, providing an opportunity to specifically target hypoxic tumour cells and improve the therapeutic index.27 Hypoxic tumour cells can be directly targeted using cytotoxic agents that induce DNA damage only under low oxygen; these include the bioreductive drugs, tirapazamine and apaziquone.66,67 A newer drug, TH-302 (a 2-nitroimidazole triggered hypoxia-activated cytotoxin) directly decreases the hypoxic fraction in xenografts of varying histology and is undergoing Phase II–III clinical trials in combination with chemotherapy.68 Additional targeting of hypoxic subregions can be achieved by targeting HIF-1-dependent transcription or targeting the unfolded protein response, which controls gene translation under cellular stress (e.g. targeting the mechanistic target of rapamycin (mTOR) signalling pathway).69–71

One could also target the faulty DNA repair and genetic instability in hypoxic cells using the concept of "contextual" synthetic lethality.72 "Genetic" synthetic lethality is a concept first developed using yeast genetics in which mutations in two genes (e.g. gene A and gene B) result in cell death, while a mutation in only one gene (e.g. either gene A or gene B) results in cell viability.73 This concept has been successfully used to target tumours deficient in HR (e.g. BRCA1- or BRCA2-deficient ovarian cancer, breast cancer and CaPs) by the additional inhibition of the poly (ADP-ribose) polymerase (PARP1) protein.74 PARP1 normally functions in single-strand break and base-excision repair, and its inhibition is synthetically lethal when the HR pathway is also compromised. Similarly, PARP1 inhibition can be toxic to the HR defects associated with hypoxic cells; hence the concept of “contextual” synthetic
lethality. We have shown in principle that PARP1 inhibition can preferentially kill repair-defective hypoxic tumour clonogens, while sparing normal tissues that maintain their DNA repair capability. Other contextual synthetic lethal approaches could be the inhibition of DNA polymerase-β in MMR-deficient hypoxic cells or inhibition of the ataxia telangiectasia mutated (ATM) kinase in hypoxic cells that have defective FA pathway function.

Of interest, clinicians may already be combatting genetic instability and hypoxia in CaP with the combined use of ADT and RT. This combination has led to improved overall and CaP-specific survival in high-risk and locally advanced CaP. The use of neoadjuvant ADT (150 mg per day of bicalutamide) was shown to improve CaP oxygenation (based on PO2 measurements) prior to RT. Furthermore, three recent studies have suggested that ADT treatment reduces expression and function of the NHEJ and other DNA repair pathways, supporting the use of DNA-PKcs inhibitors in combination with ADT as a novel treatment for CaP. Any approach that tries to increase cell kill in hypoxic cells using contextual synthetic lethality or alterations in DNA repair will require assays that can measure the function of NHEJ, HR, MMR and FA proteins in situ to ascertain the fraction of hypoxic cells within a tumour that may be repair deficient. These uses of the predictive biomarkers in addition to pharmacodynamic biomarkers that confirm drug activity in vivo will be required for maximal impact of the use of this targeted approach in combination with RT, surgery or chemotherapy.

A CAVEAT: THE PROBLEM OF PROSTATE CANCER MULTIFOCALITY AND STEM CELL SUBPOPULATIONS

CaP is unique in that it is a multifocal cancer with clonal subpopulations that have varied histological and molecular abnormalities that could relate to differential outcome. Heterogeneity exists both within and between patients. The vast majority of prostatectomies have more than one malignant focus within a prostate gland, which can be subcategorized by differential genomics based on PTEN, c-MYC and NKX3.1 gene abnormalities, therefore containing CaP foci with differential prognostic information. It is now well appreciated that tumours with identical GSs may exhibit profound genetic heterogeneity within a single prostate gland. Anatomically distinct tumour metastases can be derived from a single progenitor clone, a concept elegantly proven in renal cancer, whereby single needle biopsies did not predict the genetic heterogeneity within the primary tumour nor distant metastases for an individual patient. Studies using circulating tumour cells and circulating cell-free DNA, RNA and miRNA, after improvements in assay specificity and sensitivity, could be useful as a means to detect the most aggressive features of CaP within so-called, "liquid biopsies", as a function of staging, prognosis and treatment response. These findings must be also placed into the context of CaP tumour initiating cells (TICs), which constitute that subfraction of cells, which must be sterilized by RT to prevent tumour cell repopulation after treatment (i.e. treatment failure). Pre-clinical studies suggest that CaP TICs may have increased biological growth under hypoxia and exist as a radioresistant hypoxic niche. However, rigorous studies are required to delineate the exact TIC markers that will differentiate this subpopulation for specific genomic studies as this relates to individualized prognosis. Finally, sub tumoural heterogeneity in cancer metabolism (e.g. both acute and chronic hypoxia co-exist within a tumour and lead to significant gradients of oxygen consumption) could also confound quantification and summary statistics for assaying the fraction of tumour hypoxia from one patient to another. Intraprostatic heterogeneity must therefore be adequately "sampled" across multiple foci and TIC subpopulations with genomic or hypoxia assays, such that the most aggressive and key features of tumour progression and/or prognosis are not missed.

CONCLUSIONS

A robust understanding of the interplay between hypoxia and genomics in the context of tumour heterogeneity is required to facilitate precision medicine for CaP. We must embrace these complexities if we are to target the most aggressive cases of CaP to improve cure rates (Figure 2). The genome-wide RNA- and DNA-based prognostic signatures developed and validated on post-treatment radical prostatectomy specimens must now be validated using pre-RT biopsies. The enormous reduction in cost and materials required for whole-genome and whole-transcriptome sequencing will further detail the genomics of CaP. Hypoxia-based assays must include functional assessment of DNA repair pathways if the concept of contextual synthetic lethality is to be acted upon within the clinic. Arranging the marriage between these biological end points will highlight key features of aggressive CaP variants that will best explain heterogeneous clinical outcome and provide novel treatments to offset both local and systemic resistance.

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This review is dedicated to the late Professor Donal Hollywood.

CONFLICTS OF INTEREST

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