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Review article

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The testosterone paradox of advanced prostate cancer: mechanistic insights and clinical implications

Rajendra Kumar[1,2], Laura A. Sena[1,2], Samuel R. Denmeade[1] & Sushant Kachhap[1]

Abstract

The discovery of the benefits of castration for prostate cancer treatment in 1941 led to androgen deprivation therapy, which remains a mainstay of the treatment of men with advanced prostate cancer. However, as early as this original publication, the inevitable development of castration-resistant prostate cancer was recognized. Resistance first manifests as a sustained rise in the androgen-responsive gene, *PSA*, consistent with reactivation of the androgen receptor axis. Evaluation of clinical specimens demonstrates that castration-resistant prostate cancer cells remain addicted to androgen signalling and adapt to chronic low-testosterone states. Paradoxically, results of several studies have suggested that treatment with supraphysiological levels of testosterone can retard prostate cancer growth. Insights from these studies have been used to investigate administration of supraphysiological testosterone to patients with prostate cancer for clinical benefits, a strategy that is termed bipolar androgen therapy (BAT). BAT involves rapid cycling from supraphysiological back to near-castration testosterone levels over a 4-week cycle. Understanding how BAT works at the molecular and cellular levels might help to rationalize combining BAT with other agents to achieve increased efficacy and tumour responses.

Sections

[1]The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, USA.
[2]These authors contributed equally: Rajendra Kumar, Laura A. Sena. e-mail: kachhsu@jhmi.edu

Key points

- Androgens can drive prostate cancer growth providing the rationale for using deprivation of androgens as a first line of treatment for prostate cancer. Unfortunately, prostate cancer cells adapt to low androgen levels and eventually progress to a castration-resistant state.

- Results of several studies have indicated a paradoxical decrease in tumour growth in prostate cancer models upon treatment with supraphysiological levels of testosterone. Evidence indicates several complementary mechanisms, including cell death and cytostasis, which might be responsible for paradoxical growth inhibition by supraphysiological testosterone.

- Adaptive reliance on androgen signalling by castration-resistant prostate cancer cells becomes a therapeutic liability that can be exploited clinically through the administration of supraphysiological testosterone, an approach termed 'bipolar androgen therapy' (BAT). The term bipolar is used to emphasize that, with this strategy, rapid cycling occurs between two extremes: from supraphysiological back to near-castration testosterone levels over a 4-week cycle.

- Understanding how BAT works at the molecular and cellular levels might help to develop biomarkers for patient stratification and to rationally combine BAT with other agents to achieve increased efficacy.

Introduction

Adenocarcinoma of the prostate gland is the second most common cancer in men, with ~2.2 million new instances and ~375,000 deaths estimated to occur during 2022 (ref. 1).

Androgen signalling has an important role in prostate cancer progression, Androsterone was the first androgen to be isolated from men's urine[2,3]. Subsequently, a more potent androgen than androsterone was discovered in the testes, which are a rich source of androgenic hormones, and was termed testosterone from the words testes, sterol and ketone[4]. Testosterone is primarily produced by Leydig cells in the testes in response to luteinizing hormone secreted by the anterior pituitary, and mostly circulates bound to serum hormone-binding globulin[5,6] with only the free form gaining entry into cells owing to its lipophilic nature[7,8]. Upon entry into prostate cells, testosterone is converted to 5 α-dihydrotestosterone (DHT), a highly potent androgen, by the enzyme 5-α reductase[9]. Testosterone is sufficient for the development of embryonic Wolffian ducts but insufficient for the complete development of prostate and external genitalia, which requires 5-α reductase activity and formation of DHT[10,11]. Results of early studies showed that radiolabelled DHT or testosterone was selectively retained by the prostate nucleus[9,12]. These initial observations led to the subsequent identification and cloning of the androgen receptor gene (*AR*)[8,13,14]. *AR* encodes a 100-kDa protein that shares structural similarities with other steroid hormone nuclear receptors, including glucocorticoid receptor, progesterone receptor, mineral corticoid receptor and the oestrogen receptor[15]. AR protein can be functionally divided into four domains: the N-terminal activation domain, the central DNA binding domain, the hinge domain and the C-terminal ligand-binding domain (Fig. 1a). Ligand binding results in dimerization and translocation of AR to the nucleus and subsequent activation or repression of its target genes, such as *KLK3, TMPRSS2,* and *NKX3.1* (ref. 16) (Fig. 1b). Specificity of AR binding to androgen binding sites (ARBS) is determined by chromatin-binding proteins and co-regulators[17–20]. Androgen signalling is important in the development and progression of all stages of prostate cancer[21,22].

The role of androgen signalling in prostate cancer progression forms the basis for using androgen deprivation therapy (ADT) as a standard of care for metastatic or recurrent disease[23–25]. Androgen deprivation is known to provide initial therapeutic benefits, but eventually all men with prostate cancer develop castration-resistant disease[26,27]. Intriguingly, high-dose androgens at supraphysiological levels lead to a paradoxical decrease in the growth of some models of prostate cancer through poorly understood mechanisms. Understanding how androgens promote or inhibit the growth of prostate cancer will help to develop effective clinical strategies to inhibit prostate cancer growth and progression. Bipolar androgen therapy (BAT) is an innovative therapeutic strategy in which high doses of testosterone are periodically administered to achieve supraphysiological serum testosterone levels to inhibit prostate tumour growth[28].

In this Review, the role of androgens in prostate homeostasis and prostate cancer and mechanistic findings of growth inhibition by supraphysiological androgens are described, and insights from the results of prostate cancer clinical trials using supraphysiological testosterone (supraphysiological T) are provided. Finally, the future clinical development of BAT as a therapeutic option against prostate cancer is discussed.

The role of androgens and the AR in the prostate

Accumulated evidence from cellular, molecular and developmental studies indicates that androgens are necessary for the development of the prostate gland and dysregulated AR signalling aids prostate cancer growth and survival.

Androgens and the AR in prostate homeostasis

The prostate gland consists of branched epithelial ducts made up of a pseudostratified epithelium comprising luminal and basal epithelial cells[29,30]. The underlying stroma contains fibroblast cells, smooth muscle cells, nerve cells, endothelial cells, immune cells and rare neuroendocrine cells (Fig. 2). Results of studies conducted with seminal tissue recombination using urogenital sinus mesenchyme showed that paracrine AR signalling in the stromal compartment, but not the epithelial compartment, is essential for prostate development[31,32]. Results of studies using rats further indicated that the adult prostate has a profound regenerative capacity following repeated cycles of androgen withdrawal and replacement[33]. These pivotal studies suggested the presence of castration-resistant stem cells that survive androgen deprivation can regenerate the prostate gland. Prostate regeneration was initially attributed to stem cells in the basal cell compartment, which were largely unaffected by androgen deprivation[34–37]. However, lineage-tracing studies indicated that regeneration after androgen replacement might be mediated by rare luminal cells called castration-resistant Nkx3-1-expressing (CARN) luminal cells that survive androgen deprivation to have a vital role as stem cells in prostate regeneration, with rare basal cells also contributing to proliferation[38,39]. A number of subsequent studies indicated that the adult prostate in mice has self-sustaining basal and luminal compartments[40–42]. The adult prostate is mainly quiescent, but these self-sustaining epithelial cellular compartments might have a role during tissue homeostasis, injury and disease (Fig. 2). However, many of these mechanistic studies to elucidate the role of AR signalling in prostate regeneration involve

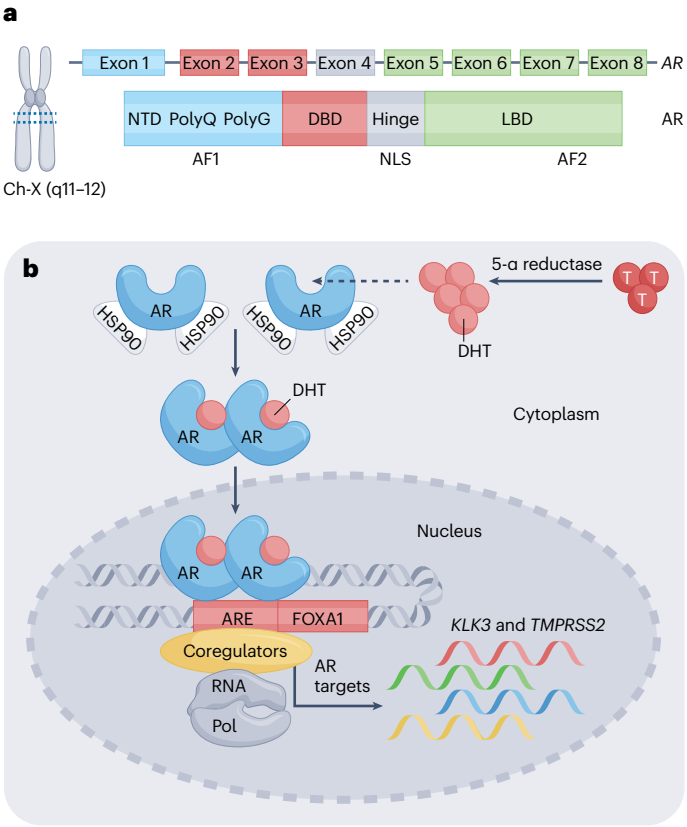

Fig. 1 | AR structure and signalling. a, Structure of the androgen receptor (AR). Location of *AR* on the q arm of the X chromosomes (Xq_{12}). *AR* contains eight exons coding for a 110-kDa protein that has 919 amino acids. The N-terminal domain (NTD) is encoded by exon 1 and has an intrinsically disordered structure. The DNA binding domain (DBD) is encoded by exons 2–3, which contain two zinc finger motifs. The DBD is linked to the ligand-binding domain by the hinge region, which is encoded by exon 4. The ligand-binding domain is encoded by exons 5–8. Both the N terminus and C terminus consist of activation functions called AF1 and AF2, respectively. **b**, Nuclear AR signalling. Testosterone is converted into its highly active metabolite dihydrotestosterone (DHT) by 5-α reductase, which binds to AR sequestered in the cytoplasm by chaperone proteins that include HSP90. Upon binding of DHT, AR dissociates from HSP90, dimerizes, and translocates to the nucleus to bind to androgen response elements (AREs) present in its target genes such as *KLK3* and *TMPRSS2*. Specificity of binding is regulated by co-regulators and pioneer factors such as FOXA1.

AR-knockout models using *cre* recombinase driven by the probasin promoter, which is activated during early postnatal development[43]. Results from these studies leave an open question of whether the observed effects seen are developmental or homeostatic in nature. To address this question, experiments in which basal-specific and luminal-specific AR ablation using inducible *cre* were performed in adult mouse prostates[44]. The results of these studies revealed that cell-autonomous AR signalling is dispensable for basal cell maintenance and required for luminal cell morphology and the bipotentiality of rare basal stem cells. Intriguingly, AR signalling was necessary to maintain daughter cells produced by CARN cells upon androgen replacement, indicating that, unlike average luminal cells of the regressed prostate, CARNs selectively require cell-autonomous AR signalling to produce viable luminal cells during prostate regeneration. Results of a single-cell transcriptomic study suggest that prostate regeneration is driven by all persisting luminal cells that acquire stem cell transcriptional features, not just by rare stem cells[45]. Cumulative evidence from early tissue recombination studies and subsequent knockout and single-cell transcriptomic studies suggests that paracrine AR activity occurs in the mesenchyme rather than in the epithelial compartment, which might be responsible for androgen-driven regeneration of the normal prostate. Understanding the androgen response by the healthy and regenerating prostate could help to delineate the type of prostate cells that are likely to initiate cancer.

Androgen signalling in prostate cancer

Unlike non-malignant prostate epithelial cells in which AR is dispensable, cell-autonomous AR signalling fuels prostate cancer growth[31,32,46]. The modulation of AR signalling through *AR* amplification[21,47], splice variants[48,49], *AR* mutation[50–52], co-activator and co-repressor alteration[19,53] in human prostate cancer underscores the importance of AR signalling in prostate cancer. In the absence of a ligand, the AR receptor is bound to chaperone proteins that keep it in a ligand-binding poised state. Once bound to a ligand, AR dimerizes and enters the nucleus to bind to thousands of ARBS scattered throughout the genome[20,54]. The majority (~90%) of AR binding sites are located hundreds of kilobases away from promoters of target genes in distal enhancer regions, which require chromatin looping to promote or repress AR-target genes[55,56]. In co-operation with its co-regulators and pioneering transcription factors such as FOXO1, AR can influence a number of cancer-relevant cellular processes, such as cell cycle, cell death, metabolism, chromatin remodelling, invasion and DNA repair[46,57–59] (Table 1). Besides its nuclear or genomic role, evidence suggests that AR might also have a non-genomic role[60] in cancer metabolism, proliferation, survival and invasion[61,62] (Table 1).

Clinical utility of reducing AR signalling

Inhibition of AR signalling is the mainstay of the systemic treatment of prostate cancer. Inhibition of AR signalling in patients with prostate cancer can be achieved in three ways: reduction of serum testosterone; inhibition of AR; and degradation of AR. Reduction of serum testosterone can be achieved by blocking its production from the testes and/or adrenal glands[63]. Huggins and Scott first showed the efficiency of this therapeutic strategy by surgical removal of the testes and adrenal glands[64]. Currently, use of medical castration is more common than surgical castration, using luteinizing hormone-releasing hormone (LHRH) agonists (such as leuprolide and goserelin) and antagonists (such as degarelix and relugolix) to block testosterone production from the testes and the CYP17A1 inhibitor abiraterone acetate to block testosterone production by the adrenal glands. Abiraterone acetate in combination with an LHRH agonist has been shown to prolong the survival of patients with prostate cancer when used as a treatment for metastatic castration-sensitive and castration-resistant disease[65–67]. Direct inhibition of AR can be achieved by using antiandrogens that bind to the ligand-binding domain of AR and prevent its nuclear localization and transcriptional activity[68]. First-generation antiandrogens, including flutamide, bicalutamide and nilutamide, have now been replaced by second-generation antiandrogens enzalutamide, darolutamide and apalutamide, which bind AR with higher affinity[69]. These second-generation antiandrogens combined with an LHRH agonist can prolong the survival of patients with prostate cancer when used as a treatment for non-metastatic castration-resistant, metastatic

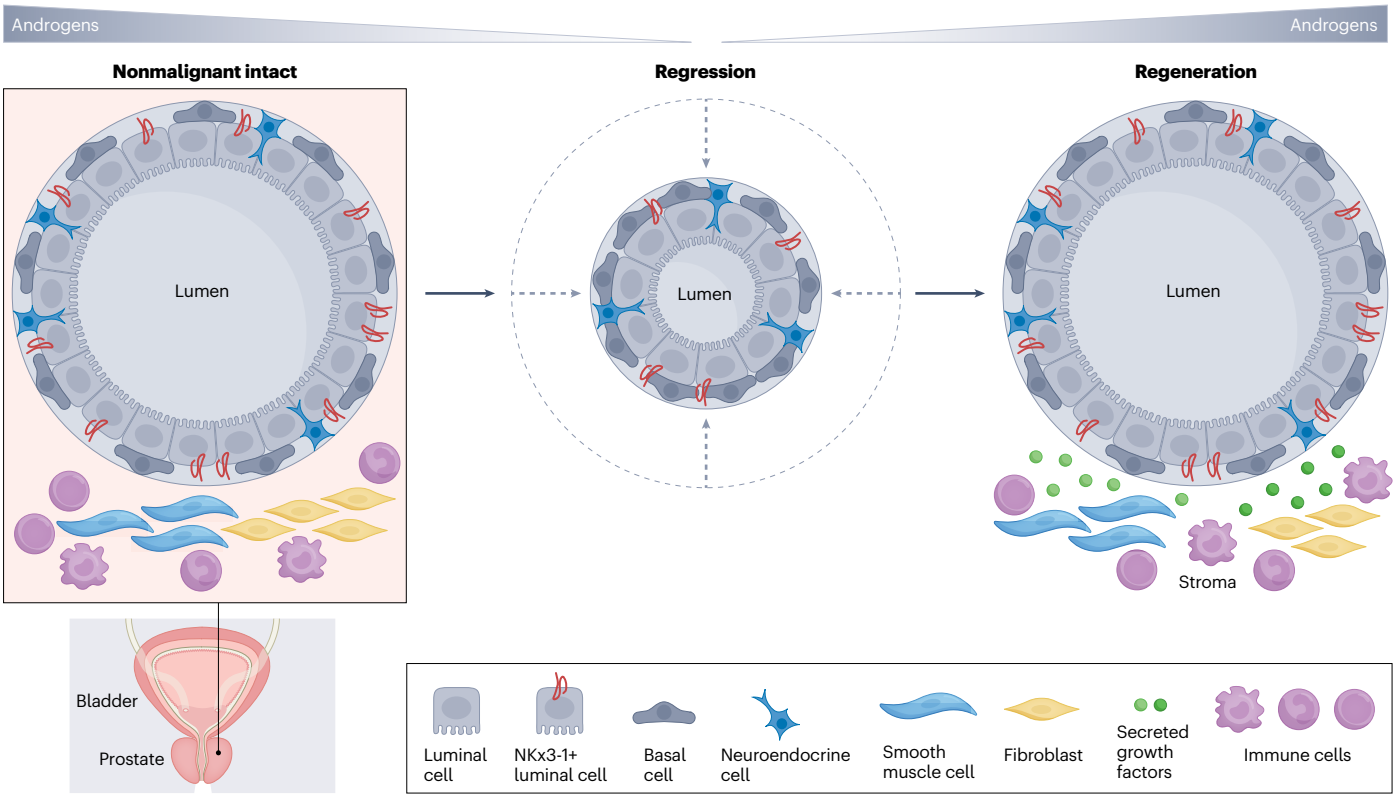

Fig. 2 | Androgens in prostate homeostasis and regeneration. Lineage-tracing studies indicate that the regenerative capacity of the prostate gland following withdrawal and re-administration of androgens can be attributed to luminal cells that acquire stem-like transcriptional features and survive castration. Luminal cell survival and regrowth might be determined by microenvironmental niche factors such as fibroblast growth factor (FGF), insulin-like growth factor (IGF), epidermal growth factor (EGF) and hepatocyte growth factor (HGF).

castration-sensitive and castration-resistant disease[70–74]. The use of AR degraders to inhibit AR signalling is in clinical development. For example, ARV-110 is a proteolysis-targeting chimaera (PROTAC) protein degrader that creates a complex of AR with E3 ubiquitin ligase to result in ubiquitination of AR and degradation by the proteasome[75]. A phase II expansion study testing the efficacy of ARV-110 as a treatment for patients with metastatic castration-resistant prostate cancer (CRPC) with enrichment of T878 and H875 mutations in AR is currently underway (NCT03888612)[76].

The clear clinical benefit of using agents that inhibit AR signalling with increased potency despite previous failure of alternative AR-axis inhibitors reflects the biology of prostate cancer to develop mechanisms to persistently signal through AR despite varied therapeutic approaches to obstruct this pathway. Indeed, the major mechanisms of resistance to AR signalling inhibition include AR overexpression, amplification and mutation, the production of ligand-independent variants and reprogramming of the AR cistrome[21,77–81], all of which can enable ongoing AR signalling in the face of therapeutic inhibition. Reduced dependency on AR signalling, such as trans-differentiation to neuroendocrine, small-cell, or double-negative prostate cancer, presently only seems to occur in a minority of patients. This observation indicates that ongoing efforts to develop agents that target AR signalling are warranted, despite our current relatively large armamentarium of such agents.

The testosterone paradox

Huggins was the first to note that an excess of hormones can cause paradoxical regression of tumours[82]. His observation was based on regression of breast tumours upon treatment with a combination of supraphysiological levels of oestrogens and progesterone. Huggins called this phenomenon 'hormone interference' and noted it as a novel therapeutic approach to treating cancer. To understand the mechanism of this paradoxical effect, the effect of supraphysiological T on prostate cancer cells was tested. Initial studies mainly focused on the effect of supraphysiological T on cell-cycle and cell-death pathways; results of subsequent investigations showed a number of possible mechanisms using both in vitro and in vivo preclinical models; however, the supraphysiological T paradox is not clearly understood.

Initial characterization of lymph node metastasis-derived, AR-positive LNCaP prostate cancer cell line demonstrated a biphasic response to testosterone[83,84], that is, LNCaP cells respond to treatment with low (0.01 nM R1881, synthetic testosterone) testosterone doses by rapidly proliferating, but proliferation is inhibited at supraphysiological T (≥1 nM R1881) concentrations[83,84]. When transfected with AR, AR-negative cell lines such as PC3 cells responded to the synthetic androgen R1881 with growth inhibition[85], suggesting the importance of AR expression in the observed effect. Castration-resistant sublines of LNCaP cells were found to have an adaptive increase in AR expression and their growth was acutely inhibited upon R1881 (0.1 nM and above)

treatment[86,87]. The growth repression by R1881 in these sublines was attributed to a decrease in MYC at the mRNA and protein levels. Furthermore, ectopic expression of MYC reversed the observed growth inhibition, suggesting its importance in supraphysiological T-induced growth inhibition[86]. Results of subsequent investigations indicated that growth inhibition was accompanied by an increase in expression of p21 and p27 and their association with CDK2, which results in G1 cell-cycle arrest[86]. p21 harbours an ARBS in its promoter and is a direct AR target gene, but p27 expression was found to be regulated indirectly by supraphysiological T through AR-mediated downregulation of its degrader SKP2, a subunit of SCF E3 ubiquitin ligase complex[88]. Results of a number of studies in which primary and immortalized normal prostate epithelial cells were used also suggest that ligand-bound AR signalling causes downregulation of MYC, leading to growth arrest and terminal differentiation[89–91]. Another mechanism by which AR can cause a G1 arrest was shown by investigating the role of AR as a DNA replication licensing factor[92–94]. Licensing factors ensure that genomic DNA is replicated once per cell cycle and they are assembled on replication origins

Table 1 | AR-influenced genomic and non-genomic cellular processes

Biological process	Biomolecules involved	Mechanism	Refs.
Cell cycle and proliferation	Cyclin-dependent kinases 2 and 4 and cyclins D1 and E	Increase in cyclin-dependent kinase activity and stimulation of the cell to enter the S phase	170
Genetic fusion	*TMPRSS2* and *ETS* oncogene families (*ERG*, *ETV1*) and other non-random fusion events	ERG overexpression induced MMPs and plasminogen activation and cell invasion	171–174
Cistrome modification	AR cistrome reprogramming	Loss of canonical AR and enrichment of non-canonical AR cistrome Enrichment of *HOXB13* and *FOXA1* motifs near AR binding sites	55,175–177
Growth inhibition	p21, p27	G1 cycle arrest, inhibition of CDK2 activity	178,179
Apoptosis	G1 cell-cycle arrest	G1 cycle arrest, fragmentation of DNA	87,119
Cell survival and anti-apoptosis	HSP27, FLIP, and FOXO3a	AR-mediated upregulation of anti-apoptotic FLIP	180
	Phosphatidylinositol 3-kinase and AKT and PTEN loss	MTORC2-mediated AKT activation and increased AR activity PTEN loss causes increased FLIP expression, constitutive PI3K activity-mediated AKT phosphorylation	181
Invasion, migration and metastasis development	MMP-2 upregulation	AR-mediated increase in pro-MMP-2 levels	182
	Ezrin expression and phosphorylation	Androgen-mediated direct increase in ezrin followed by androgen-activated PKC-α-induced ezrin phosphorylation (Thr567)	183,184
	Interaction of AR with filamin A and regulation of FAK, paxillin and RAC	AR interaction with filamin A and control integrin beta 1 and FAK, paxillin and RAC	185
Metabolism	Glucose-6-phosphate dehydrogenase expression Lactate dehydrogenase A and MCT4	Pentose phosphate pathway for generation of NADPH and nucleotide precursors Pyruvate to lactate metabolism	186,187
	Combined targets of AR and SREBP: ELOV6, SCD1, FASN, and A-CoA carboxylase	Increased fatty acid synthesis (monounsaturated and saturated FA)	188,189
	Amino acid transporters (LATs and ASCTs)	ASCT2-mediated glutamine uptake	190,191
	Folate cycle pathway and methionine cycle	Trans-sulfuration and polyamine synthesis	192,193
	Poly (ADP-Ribose) polymerase1	PARylation of XRCC1	194,195
DNA repair	DNAPKcs	*PRKDC* (encoding the protein product DNAPKcs) *XRCC2* and *XRCC3* (*RAD51*)	196
Non-genomic ligand-independent crosstalk with growth factors, cytokines and non-receptor tyrosine kinase pathway	EGF-induced AR tyrosine phosphorylation IL6 IL8 IGF1–insulin signalling HER2neu	AR tyrosine phosphorylation at positions 267 and 534 Jak–STAT3 signalling, MAPK and PI3K signalling Androgen receptor expression and activation Removal of FOXO1-mediated co-repression of AR AR stabilization, increased binding of AR to AREs	197–201
	MAPK and effectors SRC, ERF1 and ERF2, and PI3K and AKT signalling	Increased ERK1 and ERK2 phosphorylation RAF and ERK2 activation	202
	Calcium signalling	Increased intracellular calcium by *GPCR* and/or EGFR	203,204

A-CoA carboxylase, acetyl-CoA carboxylase; AR, androgen receptor; ASCTs, alanine/serine/cysteine/threonine transporter; CDK2, cyclin-dependent kinase; DNAPKcs, DNA-dependent protein kinase catalytic subunit; EGF, epidermal growth factor; *EGFR*, epidermal growth factor receptor; *ERG*, ETS-related gene; ERK, extracellular signal-related kinase; *ETS*, E-26 transformation specific; *ETV1*, ETS translocation variant 1; FAK, focal adhesion kinase; FASN, fatty acid synthase; FLIP, FLICE-like inhibitory protein; *FOXA1*, Forkhead Box A1; FOXO1, Forkhead box O1; FOXO3a, Forkhead box protein O3a; *GPCR*, G protein-coupled receptor; HER2neu, human epidermal growth factor receptor 2; *HOXB13*, Homeobox protein B13; HSP27, heat shock protein 27; IGF1, insulin-like growth factor 1; IL6, interleukin 6; IL8, interleukin 8; JAK–STAT3, Janus kinase-signal transducer and activator of transcription; LATs, linker for activation of T cells; MAPK, mitogen-activated protein kinase; MCT4, monocarboxylate transporter 4; MMP-2, matrix metalloproteinase-2; MTORC2, mechanistic target of rapamycin kinase; NADPH, nicotinamide adenine dinucleotide phosphate; PARylation, poly(ADP-ribose)-ylation; PI3K, phosphatidylinositol-4,5-bispohosphate 3-kinase; PTEN, phosphatase and tensin homologue; *RAD51*, RAD51 recombinase; SREBP, sterol regulatory element binding protein; *TMPRSS2*, transmembrane protease serine 2; XRCC, X-ray repair cross complementing.

in G1 phase, an obligatory event for activation of replication origins in the S-phase[95]. These factors are tightly regulated in the G1 phase either through inactivating phosphorylation or proteasomal degradation[96,97]. AR was found to interact with many licensing factors, namely ORC2, CDC6, CDT1 and MCM7 (ref. 98). Moreover, AR, like other licensing factors, undergoes proteasomal degradation in mitosis[93,94] before the next cell cycle. Ligand-bound AR under supraphysiological T conditions was proposed to prevent AR from degradation during mitosis. This inhibition of degradation would result in origins of replication with bound AR, preventing relicensing and causing a G1 arrest.

Another mechanism for growth suppression by supraphysiological T could be through self-regulation of AR transcription. A decrease in both mRNA and protein levels of AR in castration-resistant LNCAP cell sublines treated with R1881 had been observed[86]. A reduction in AR transcript upon androgen stimulation was also noted in other studies[99,100]. In a subsequent investigation, a highly conserved ARBS site was identified in the second intron of *AR*[101]. Ligand-bound AR was shown to decrease *AR* expression by recruiting the lysine-specific histone demethylase LSD1 (ref. 101), a known transcriptional repressor[102]. Recruitment of LSD1 leads to demethylation of H3K4 and repression of AR transcription. This phenomenon is intriguing as LSD1 has been shown to primarily act as an AR co-activator, which it achieves by demethylating the K270 residue of the pioneering factor FOXA1 to enhance its chromatin binding, maintaining the AR enhancer accessibility that is needed to transcribe AR target genes[18]. These observations also highlight how the AR transcript increases under castration conditions to enhance prostate cancer growth and survival.

A decrease in tumour growth can also be brought about by senescence, quiescence or cell death[103]. All of these mechanisms have been investigated in the context of supraphysiological T treatment. Re-expression of AR in AR-negative prostate cancer cells was shown to induce apoptosis[104]. However, apoptosis in AR-negative DU145 cells was contingent upon co-expression of retinoblastoma (RB) protein[104]. AR-negative PC3 cells, when transfected with full-length *AR* (PC3-AR), exhibited effects ranging from a decrease in proliferation without apoptosis to a G1 arrest that culminated in apoptosis with an increase in time of treatment[87]. Castration-resistant LNCaP sublines have also been reported to induce BAX-mediated apoptosis upon androgen treatment[105,106]. Results of other studies also indicate that supraphysiological T can induce senescence in LNCaP cells[107,108]. Treatment of LNCaP cells with 1 nM R1881 for 72 h was sufficient to induce the formation of senescence-associated heterochromatic foci and senescence-associated β-galactosidase activity[107]. Supraphysiological T treatment increased p16, a known senescence marker that mediates the hypophosphorylation of RB, which resulted in downregulation of its target cyclin D1 and E2F1. These results indicated that supraphysiological T might regulate the p16–RB–E2F1 pathway to mediate cellular senescence. In line with these observations, results of another study demonstrated that supraphysiological T could be combined with a CDK4 and CDK6 inhibitor, strengthening the chromatin binding of the RB–E2F repressor complex by blocking the hyperphosphorylation of RB proteins[109]. Results of a previous study using PC3-AR cells had shown that androgen-mediated senescence proceeds after a G1 arrest[108]. Senescence was brought about by AR-dependent expression of p21 and depletion of p63. In this study, RB hypophosphorylation was mediated through AR-induced reactive oxygen species (ROS)[108]. Intriguingly, MTORC1 activity remained high in PC3-AR cells after supraphysiological T treatment, which was also shown to be active in LNCaP cells treated with supraphysiological T: MTOR activity promotes cellular senescence, but the mechanism is not well understood[110,111]. Transient exposure to androgens in AR-positive LNCaP and VCaP cells plated at low density in hypotonic growth media has been shown to induce quiescence or dormancy through redox imbalance and TGFβ–BMP signalling[112]. Some of the responses to supraphysiological T might seem to be varied and depend upon the cellular models, passage number, supraphysiological T treatment concentration and duration, but many of these effects might be true and not mutually exclusive (Fig. 3a).

An interesting aspect of ligand-bound steroid receptors, including AR, is their ability to cause DNA damage[113–115]. Response of cells to DNA damage can range from apoptosis to growth arrest and senescence, an effect that is observed in supraphysiological T treatment. The exact mechanism of how androgens cause DNA damage is unknown; evidence suggests a role for ligand-bound AR in recruiting enzymes that actively induce DNA double-strand breaks (DSBs). Insights into this mechanism came from the observation that in prostate cancer, translocations of AR-driven *TMPRSS2*, which is located on chromosome 21, were common with *ERG* or *ETV1* located on chromosomes 21 and 7, respectively[116]. Ligand-bound AR was observed to rapidly locate to these translocation sites to recruit cytidine deaminase (AID) and LINE-1 repeat-encoded ORF2 endonuclease, which induce DNA DSBs and proximity-mediated gene rearrangements leading to *TMPRSS2–ERG* fusions[114]. Recruitment of TOP2B to these sites was shown to generate DSBs, leading to *TMPRSS2–ERG* rearrangements[115]. The effects of TOPO2B are probably not restricted to rearrangement of this genomic region but are likely to occur at other AR binding sites as well. Furthermore, transcription induced by AR would be expected to lead to DNA opening, making it susceptible to ROS-induced DNA damage[117,118]. Cells with defects in the DNA repair pathway might be particularly susceptible to androgen-induced DNA damage under supraphysiological T conditions. In agreement with this notion, prostate cancer cell lines and patient-derived xenografts that harbour DNA repair mutations have been shown to have inhibited growth on supraphysiological T treatment[119,120]. Moreover, patients with prostate cancer whose disease responds well to treatment with supraphysiological T had mutations in DNA repair genes, suggesting mutations in DNA repair genes could be positively associated with response to therapy[121–123]. In AR-positive LNCaP cells that harbour mutations in DNA repair genes, two parallel autophagy-mediated pathways could be triggered: ferritinophagy and nucleophagy[124]. Ferritinophagy involves selective degradation of the iron-storage molecule ferritin, increasing the labile pool of intracellular iron, leading to non-apoptotic death by ferroptosis upon supraphysiological T treatment. Supraphysiological T-treated cells shuttled their damaged DNA to autophagosomes for degradation through nucleophagy. Activation of nucleophagy in this context might be a cytoprotective phenomenon, enabling cells to get rid of their damaged DNA; however, it can also trigger cytosolic nucleic acid sensors, and NF-κB-mediated innate immune signalling, which includes secretion of cytokines and chemokines that attract innate and adaptive immune cells[124]. This mechanism might occur in vivo to cause immune clearance of the tumour. Supraphysiological T considerably increased immune cell infiltration in preclinical animal xenograft models of prostate cancer and an increase in cytotoxic CD8 T cells was observed in biopsy samples from patients with prostate cancer after supraphysiological T treatment[124] (Fig. 3b).

The above observations show that perturbation of transcription proteins such as AR, which affect many cellular processes, is likely to have a pleiotropic effect. One aspect of supraphysiological T biology that remains to be studied is how supraphysiological T might regulate immune cells and the tumour microenvironment. Androgens are also known to affect the development of lymphocytes in both the thymus

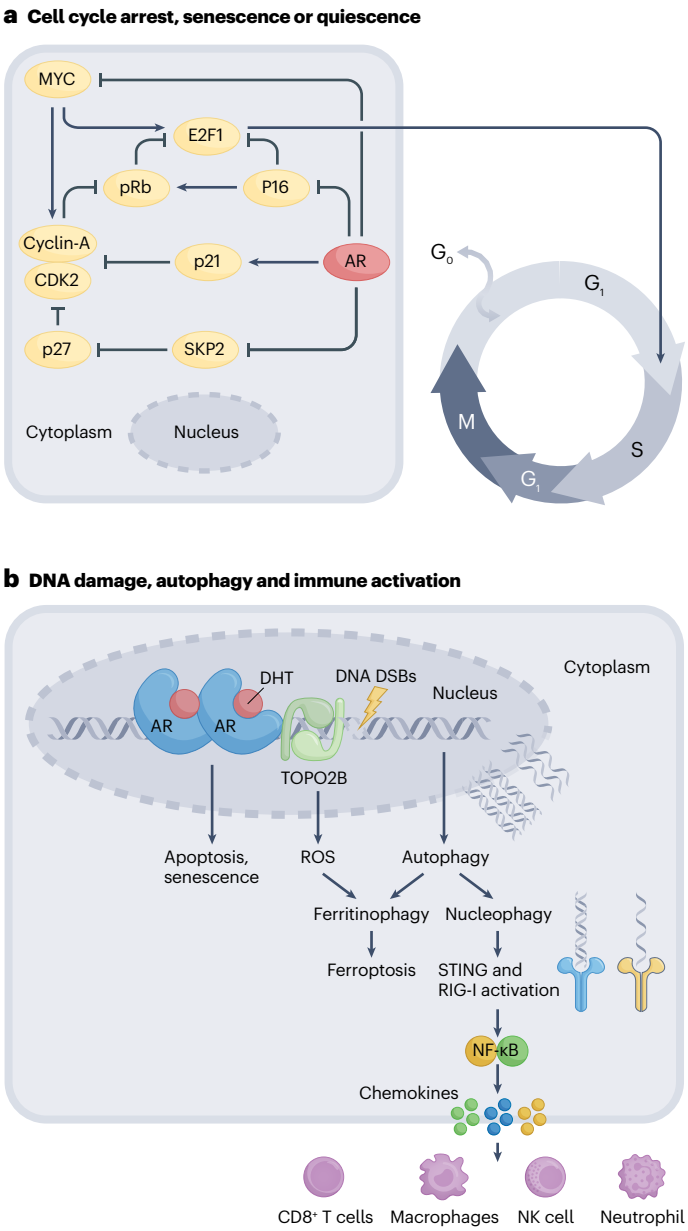

Fig. 3 | Mechanisms of action of supraphysiological testosterone. a, Cell-cycle regulation. Supraphysiological testosterone (supraphysiological T) inhibits the transcription of MYC, which is required for cyclin and cyclin-dependent kinase-mediated passage of cells from the G1 to the S phase. Downregulation of MYC suppresses CDK2 and CyclinA activity, which prevents phosphorylation-mediated degradation of RB leading to cell-cycle arrest. Supraphysiological T also increases p21 levels through transcriptional upregulation by the androgen receptor (AR) and inhibits the expression of S-phase kinase-associated protein (SKP2), a subunit of SCF-type cullin ubiquitin ligase. Downregulation of SKP2 by supraphysiological T increases p27, which, in conjunction with p21 and p16 upregulation, causes a G1 phase arrest leading to cell death and quiescence and/or senescence. **b**, Autophagy and immune activation. Supraphysiological T mediates DNA double-stranded breaks (DSBs) by recruiting TOPO2B to DNA binding sites. Unrepaired DNA lesions cause apoptosis, cell-cycle arrest or senescence. Supraphysiological T also causes an induction of two parallel autophagy-mediated pathways: ferritinophagy and nucleophagy. Ferritinophagy, which involves autophagy-mediated degradation of ferritin, results in increased lipid reactive oxygen species (ROS) and ferroptotic cell death. Supraphysiological T-damaged DNA can be degraded in the autophagosomes by the process of nucleophagy. Cytoplasmic autophagosomal DNA activates a nucleic acid-sensing mechanism through STING and RIG-I. Activated STING and RIG-I signal through NF-κB and cause the release of pro-inflammatory chemokines, including CXCL10, attracting natural killer (NK) cells, T cells, macrophages and neutrophils. DHT, dihydrotestosterone.

and the bone marrow. AR expression has been found on endothelial cells, thymic epithelial cells and innate and adaptive immune systems, including T cells, B cells, innate lymphoid cells and many cell populations present in the bone marrow[125–128]. Neutrophils also have considerable levels of AR protein expression[126]. AR is universally expressed on all neutrophil lineages starting from proliferative to terminally differentiated matured phenotype. Upon activation, neutrophils give rise to pro-inflammatory cytokine expression (IL-6, IL-1β and TNF) and chemokines (CCL2, CCL3, CCL4, CXCL1, CXCL4 and CXCL7), and the expression of these were reduced upon AR knockout. Similarly, the expression of AR on monocytes and macrophages suppresses cutaneous wound healing by increased TNF production. A mouse model of myeloid-specific AR-knockout showed rescued wound healing by inhibiting the TNF-mediated inflammatory response[129]. Supraphysiological T is likely to directly influence the function of these cells, which might contribute to the observed tumour growth inhibition.

Testosterone as a drug

In the past decade, in spite of its reputation as a growth factor for prostate cancer, testosterone has been tested as a therapeutic agent for treatment of this disease.

Early use of testosterone for patients with prostate cancer

Testosterone was initially given to patients with prostate cancer to confirm that the beneficial effect of castration was a result of the reduction of testosterone[23]. Indeed, many early reports indicated that testosterone administration reversed the benefits of castration, resulting in elevation of tumour markers that were used at that time (including acid phosphatase and alkaline phosphatase) and symptomatic progression[130–132], supporting the role of androgens as growth factors for prostate cancer. Given this observation, androgens were given to patients with the intent of stimulating cancer cell proliferation to sensitize them to subsequent DNA damaging agents, such as radioactive phosphorus (32 P), cyclophosphamide, 5-FU, methotrexate and doxorubicin[133–135]; however, the results of these studies were uniformly negative in improving patient outcomes. Yet, scattered among these initial descriptions of testosterone administration for patients with prostate cancer are anecdotal case reports of patients who paradoxically improved with testosterone monotherapy. In 1957, patient HG, a 68-year-old man with metastatic prostate cancer that had progressed following orchiectomy and hypophysectomy, was described as having a dramatic decrease in serum acid phosphatase from near 200 BU/100 cc to undetectable levels and improvement in cancer symptoms following treatment with testosterone propionate. In 1967, patient CJS, a 76-year-old man with 'preterminal' metastatic CRPC (mCRPC), was described to improve from an "extremely feeble" state, "unable to sit without assistance," to "totally pain-free" and "dancing weekly" following treatment with testosterone propionate 100 mg

three-times weekly[136]. Yet these case reports were anti-dogmatic, and further clinical investigation into whether testosterone could be used as a therapy for prostate cancer was slow. Notably, a substantial body of literature describes the use of androgen replacement in men with hypogonadism and prostate cancer. The results of these studies suggest that androgen replacement does not result in rapid prostate cancer disease progression, contrary to the previously widely held view that androgens would rapidly increase prostate cancer growth[137–142]. They established a precedent that testosterone could be safely administered to patients with prostate cancer, which enabled subsequent studies assessing testosterone as a prostate cancer therapy. Thus, in 2009, two groups reported on the use of transdermal testosterone as a treatment for patients with CRPC[143,144]. Using transdermal testosterone, physiological levels of serum testosterone of 300–850 ng/dl, which were generally well tolerated, were achieved in both studies. However, the efficacy of this approach was quite modest, with 3 of 15 patients with non-metastatic CRPC demonstrating a decrease in PSA (no patient with >50% decrease) in one study, and only 1 of 12 patients with mCRPC demonstrating a reduction in PSA of 50% in the other study[143]. Despite this limited efficacy, these studies supported the growing appreciation that testosterone could be administered safely to patients with advanced prostate cancer.

Bipolar androgen therapy

BAT is the administration of testosterone cypionate 400 mg intramuscularly every 28 days concurrent with an LHRH agonist to result in oscillation of serum testosterone from supraphysiological (>1,500 ng/dl) to near-castration levels[145]. This therapy was first tested when it was given to 16 patients involved in a pilot clinical trial in combination with etoposide as a treatment for asymptomatic mCRPC[145]. Remarkably, this combination therapy resulted in PSA and radiographic responses in about half of the patients involved, with 4 patients treated with BAT for >1 year[145]. The design of this trial was such that patients received BAT and etoposide for the first 3 months, then subsequently received BAT monotherapy if they were experiencing a PSA decline. Notably, most patients who responded to BAT and etoposide continued to respond to BAT monotherapy; thus, etoposide was thought to contribute minimal benefit but considerable toxic effects and was omitted from subsequent trials of BAT.

BAT differs in two important ways from transdermal testosterone administration: first, it achieves supraphysiological levels of serum testosterone; and second, the testosterone level is not clamped but rather is cycled between high and low levels (hence the name 'bipolar' androgen therapy)[28]. This strategy was selected given preclinical data suggesting that CRPC exhibits a biphasic response to re-exposure to androgens, whereby physiological levels of androgens induce growth and proliferation, and supraphysiological levels of androgens are required to induce growth arrest and cell death[146]. Moreover, this cycled approach was hypothesized to target the heterogeneity and adaptability of prostate cancer cells present in metastases, some of which might be inhibited by high testosterone and others by low testosterone.

Following the promising results of the pilot clinical trial, BAT has been tested in five subsequent clinical trials for patients with advanced prostate cancer: a single-arm trial for castration-sensitive prostate cancer (BATMAN)[147]; a single-arm, multicohort trial for CRPC (RESTORE)[148–150]; a randomized trial for mCRPC comparing BAT with enzalutamide (TRANSFORMER)[151]; a single-arm trial of BAT in combination with the anti-PD1 agent nivolumab for patients with mCRPC (COMBAT)[152]; and a single-arm, multicohort trial of BAT in combination with the poly (ADP-ribose) polymerase (PARP) inhibitor olaparib for mCRPC[153]. Overall, nearly 300 patients with prostate cancer have been treated with BAT, and a great deal has been learned regarding safety, efficacy, and novel vulnerabilities and opportunities for synergistic combination therapies with BAT, although much remains to be understood.

Safety and tolerability of BAT

Given the efficacy of ADT in treating prostate cancer[67], the safety and tolerability of BAT (as the opposite therapy of ADT) have been heavily scrutinized. Evidence from early reports suggested that testosterone can exacerbate pain owing to bone metastases[130,134,154,155], and many have voiced concern that testosterone could induce tumour flare that might result in the dangerous spinal cord or urethral compression. Thus, all clinical trials of BAT have excluded patients with pain caused by prostate cancer requiring opiate medications and those with evidence of disease in sites that might put the patient at risk of complications should tumour flare occur. With these exclusion criteria in place, BAT has seemed to be relatively safe and very well-tolerated among treated patients. Overall, the rate and severity of adverse events seem similar to the standard-of-care agent enzalutamide[151]. Common adverse events tend to be low grade and include musculoskeletal pain, lower extremity oedema and breast tenderness[149,151]. Notably, spinal cord compression, urethral compression causing urinary obstruction or other objective evidence of tumour flare have not been observed with the use of BAT. This observation suggests that BAT does not cause tumour flare, but this possibility will be continuously assessed as increased numbers of patients are treated.

Efficacy of BAT monotherapy

The efficacy of BAT monotherapy has been tested in patients with castration-sensitive prostate cancer (BATMAN)[147], CRPC that has progressed on only ADT (RESTORE cohort C)[149], CRPC that has progressed on abiraterone (RESTORE cohort B and TRANSFORMER)[148,151], and CRPC that has progressed on enzalutamide (RESTORE cohort A)[150]; however, only the TRANSFORMER trial[151] was a randomized controlled trial, which means it included a control arm to assess the benefit of this therapy most accurately. On average, among patients with mCRPC, BAT results in a PSA decline ≥50% (PSA_{50} response) in 20–25% of patients, an objective response in 30–40% of patients, and a median progression-free survival of ~6 months. Efficacy end points studied include the PSA_{50} response rate (the percentage of patients with at least a 50% decline in PSA on therapy), the objective response rate (ORR) per RECIST 1.1 (ref. 156) and Prostate Cancer Working Group 3 (PCWG3) definitions[157], clinical or radiographic progression-free survival PCWG3 definition[157] and overall survival (OS) (Table 2).

Biomarkers for predicting response to BAT

Given that tumour regression seems to occur in a minority of patients treated with BAT, identifying biomarkers that predict sensitivity could enhance the utility of this therapy. Preclinical cell line and mouse xenograft models suggest that high AR expression induced by prolonged castration might improve sensitivity to growth inhibition by supraphysiological androgens[146]. The expression of full-length AR and the splice variant AR-V7 in circulating tumour cells had no correlation with response in patients included in the TRANSFORMER trial[86,151]. However, this approach was limited given that circulating tumour cells were not detectable in most patients, and the assay reported a binary, rather than continuous, measurement of AR expression.

Table 2 | Efficacy of BAT

Trial	Therapy	Patient population	Number of patients	Efficacy	*Clinicaltrials.gov* number	Ref.
Pilot	Single arm: BAT plus etoposide	nmCRPC and low-volume mCRPC	16	PSA_{50} RR: 4 of 14 ORR: 5 of 10	NCT01084759	205
BATMAN	Single arm: alternating ADT plus BAT	nmCSPC	29	PSA <4 ng/ml at 18 months: 17 of 29	NCT01750398	206
RESTORE	Single arm: BAT	Cohort A: mCRPC that has progressed on enzalutamide	30	PSA_{50} RR: 9 of 30 ORR: 6 of 12 Median crPFS: 8.6 months	NCT02090114	159
		Cohort B: mCRPC that has progressed on abiraterone	29	PSA_{50} RR: 5 of 29 ORR: 2 of 7 Median crPFS: 4.3 months		
		Cohort C: De novo CRPC	29	PSA_{50} RR: 4 of 29 ORR: 4 of 13 Median rPFS for mCRPC: 8.5 months		
TRANSFORMER	Randomized: BAT versus enzalutamide	mCRPC that has progressed on abiraterone	94 (BAT) and 101 (enzalutamide)	PSA_{50} RR: 24 of 85 (BAT) and 24 of 94 (enzalutamide) Median rPFS: 5.7 months (BAT) and 5.7 months (enzalutamide) Median OS: 32.9 months (BAT) and 29 months (enzalutamide)	NCT02286921	207
COMBAT	Single arm: BAT followed by BAT plus nivolumab	mCRPC that has progressed on enzalutamide and/or abiraterone, plus or minus taxane therapy	45	PSA_{50} RR: 18 of 45 ORR: 10 of 42 Median rPFS: 5.7 months	NCT03554317	208
BAT plus olaparib	Single arm: BAT plus olaparib	mCRPC that has progressed on enzalutamide and/or abiraterone	36	PSA_{50} RR: 14 of 30 Median PFS: 12.6 months	NCT03516812	209

ADT, androgen deprivation therapy; BAT, bipolar androgen therapy; crPFS, clinical or radiographic progression-free survival; mCRPC, metastatic castration-resistant prostate cancer; nmCRPC, non-metastatic castration-resistant prostate cancer; ORR, objective response rate; OS, overall survival; PSA_{50}, PSA decline ≥50%; PFS, progression-free survival; rPFS, radiographic progression-free survival; RR, response rate.

High AR activity predicts growth inhibition by supraphysiological androgens and BAT in patients[158]. High androgen receptor activity is required for growth inhibition of prostate cancer by supraphysiological androgens by enabling downregulation of MYC[158]. A gene score that estimates AR activity based on a ranking of expression of 10 canonical AR target genes among the top expressed genes in tumours before BAT therapy (ARA_{MW} score) enabled prediction of PSA response and objective response and increased progression-free survival (PFS) and OS on BAT treatment. Notably, BAT results in significant downregulation of AR ($P < 0.0001$), which was found to be a mechanism of resistance to growth inhibition by supraphysiological androgens. Future prospective trials are required for validation of the ARA_{MW} score as a predictive biomarker of response to BAT.

Beyond AR, results of retrospective analyses of patients treated with BAT have suggested that patients with mutations in *TP53* and/or homologous recombination in DNA repair genes might exhibit enhanced responses to BAT[119,121]. These observations support the idea that BAT can induce AR-mediated DNA damage that is enhanced in cancer cells with defective DNA repair mechanisms. Ongoing studies are being conducted to prospectively assess the benefit of BAT in a biomarker-selected group of patients with *TP53*, *PTEN* or *RB1* pathogenic alterations (NCT02090114)[159] and separately in the biomarker-selected group of patients with homologous recombination defect mutations (NCT03522064)[160].

Sequencing of BAT with AR-axis inhibitory therapies

A notable finding of the pilot clinical trial of BAT was that it seemed to re-sensitize CRPC to AR-axis inhibition[145]. Overall, 12 of 13 patients exhibited a PSA decline to subsequent AR-directed therapy administered after progression on BAT, despite previous progression on similar agents before BAT. This idea was further explored in the RESTORE[148–150] and TRANSFORMER[151] trials. In RESTORE, patients who had previously progressed on enzalutamide subsequently exhibited a PSA_{50} response rate of 52% on enzalutamide after BAT, whereas patients who had previously progressed on abiraterone subsequently exhibited a PSA_{50} response rate of 16% on abiraterone after BAT[148]. In TRANSFORMER, the PSA_{50} response rate to enzalutamide without previous BAT was 25.5%, the PSA_{50} response to enzalutamide following BAT was 77.8%[151]. Moreover, the PSA PFS was 3.8 months and OS 28.6 months on enzalutamide without previous BAT, but improved to 10.9 months and 37.1 months, respectively, on enzalutamide following BAT.

Mechanistically, given that AR inhibition results in AR overexpression that can confer resistance to AR inhibition[161], BAT might result in AR downregulation that can confer re-sensitization to AR inhibition. Indeed, BAT did cause downregulation of AR in all samples analysed in the COMBAT trial[158]. However, the results of these studies suggest that AR antagonism and AR agonists (BAT) might be repeatedly alternated to pre-empt and/or overcome resistance to either therapeutic modality. This approach is currently being tested in a prospective

clinical trial of BAT alternating with enzalutamide in the STEP-UP trial (NCT04363164)[162].

Opportunities for synergistic combination therapies

BAT is generally well tolerated[151]. Moreover, in contrast to second-generation AR-axis inhibitors, BAT is associated with minimal financial toxicity and requires no commitment of compliance on behalf of the patient, as it is administered by rapid intramuscular injection monthly in the clinic[151]. Thus, BAT is an ideal foundation on which to layer additional therapies that might augment responses. Treatments that have been tested in combination with BAT include the anti-PD1 agent nivolumab (COMBAT[152]) and the PARP inhibitor olaparib[153]. Outcomes of these clinical trials have been reported currently in abstract form only[152,153].

The rationale for combining BAT with nivolumab comes from three anecdotal instances of patients with microsatellite-stable mCRPC exhibiting remarkable responses to anti-PD1 following progression on BAT[122]. These responses were notable given that microsatellite-stable mCRPC is immunologically cold and shows near-uniform resistance to anti-PD1 therapy[163]. The responses were hypothesized to occur through the induced vulnerability of AR-mediated activation of nucleic acid sensors and immune signalling that might recruit and activate cytotoxic immune cells to the tumour bed[124]. The design of the COMBAT trial[152] was a 3-month lead-in of BAT monotherapy followed by combined therapy with BAT and nivolumab. The complete analysis describing the antitumour benefit attributed to nivolumab is currently in preparation; however, the overall PSA_{50} response rate was 40%, and the median radiographic PFS was 5.7 months[152]. The PSA_{50} response rate was slightly higher than in previous trials, but the median rPFS was identical to BAT monotherapy in the TRANSFORMER[152] trial. This observation suggests that further research into the effect of BAT on prostate cancer tumour immunity is needed to understand whether BAT has a role in enhancing durable immune responses to prostate cancer.

The other combination therapy approach that has been tested is BAT in combination with olaparib[153]. The rationale for this approach is that supraphysiological androgens can induce AR-mediated DNA DSBs[115,119] that are hypothesized to be more detrimental in the presence of PARP inhibition than not, similar to the synthetic lethality of BRCA1 and BRCA2 deficiency and PARP inhibition in prostate cancer and other cancer types[164]. The possible sensitivity of prostate cancer with homologous recombination deficiency mutations to BAT[165] further supports the idea that efficient DNA repair is crucial to the persistence of CRPC treated with BAT. Of note, the results of the pilot clinical trial of BAT suggested minimal additional benefit from concurrent treatment with etoposide[145], which exerts antitumour effects through induction of DNA DSBs. Nonetheless, olaparib has a different mechanism of action from etoposide by inhibiting PARP and impairing the repair of DNA DSBs[166], which might provide enhanced synergy with BAT. Some results from this trial were presented at European Society of Medical Oncology 2021, and a PSA_{50} response rate of 47% and a median PFS of 12.6 months were reported[153]. Teasing out whether synergy between BAT and olaparib occurs in this trial will probably be challenging, given that both agents are known to be active agents as treatment for mCRPC when given as monotherapy (unlike anti-PD1).

Future directions

Many questions remain in a quest to define the optimal clinical application of the testosterone paradox in prostate cancer. The optimal schedule and dose of testosterone administration remains to be determined. Results of previous studies indicate that strategies that achieve sustained physiological serum levels of testosterone are not as effective as BAT[143,144], which produces cycling of serum testosterone from supraphysiological to near-castration levels over the course of 28 days[145]; however, whether BAT is more effective simply owing to its ability to expose tumours to increased concentrations of testosterone or whether the cycling of testosterone is important to prevent rapid adaptation of the cancer cells to high levels of testosterone (or both) is currently unknown. One feature of testosterone cypionate is that it has variable pharmacokinetics[145]. Future clinical studies should consider whether other forms of AR agonists, such as novel formulations of oral testosterone including Jatenzo, an oral lipoprotein-coated testosterone undecanoate, or selective AR modulators, small-molecular non-steroidal AR agonists, might be more or less effective than testosterone cypionate.

Patient factors that predict sensitivity to BAT also need to be determined. Clinical studies of BAT have shown that only 20–40% of patients with CRPC are sensitive to BAT[151]. Thus, understanding mechanisms of sensitivity and primary resistance are essential to limiting the use of BAT to only patients who are likely to respond and developing novel strategies to overcome primary resistance to BAT to expand the population of patients who benefit. Promising features that might predict response to BAT include high AR activity[158] and homologous recombination repair mutations[119], although these biomarkers require prospective validation. A related question is the optimal timing of administration of BAT in the sequence of therapy for patients with CRPC. Current evidence suggests that progression on prolonged and potent AR-axis inhibitors might enhance sensitivity to BAT[151]; however, BAT priming can improve sensitivity to AR-axis inhibitors[149]. Thus, future studies should assess the optimal timing of BAT usage for the treatment of patients with advanced prostate cancer.

A challenge is that we have not tested BAT among patients with pain from prostate cancer. An understanding of the molecular mechanisms by which testosterone administration causes or exacerbates pain is needed to broaden the population of patients who might receive and benefit from BAT. Given the usual rapid onset of pain flares, it seems unlikely that this pain is a result of cancer cell proliferation and is more probably a neuromodulatory effect owing to production of cytokines or other pain-inducing chemical substances, but this idea is currently speculation and future research should directly address this question.

The drivers of acquired resistance to BAT also need to be determined. The majority of patients who initially respond to BAT unfortunately go on to develop resistance at around 6 months to 1 year[151]. BAT results in considerable downregulation of AR expression[158] and this reduction is probably a substantial driver of acquired resistance to therapy. Deciphering this mechanism is important given that this adaptive resistance might be reversible. Alternative mechanisms of resistance should also be considered and studied.

The key mechanisms of tumour growth inhibition by BAT occurring in patients are important to discover. Given the diverse maladaptive effects of supraphysiological androgens in models of prostate cancer[158], clinically, several mechanisms probably occur. This knowledge might lead to an understanding of novel vulnerabilities or adaptive responses induced by BAT that could be targeted concurrently with BAT to result in expanded efficacy.

Finally, the cancer cell-extrinsic effects of supraphysiological androgen and BAT that might alter prostate cancer progression need to be understood. Androgens can affect the function of diverse cell types, including immune and stromal cells within the tumour microenvironment[167,168], and those of distant tissues such as bone and muscle, which might indirectly affect cancer progression[169].

Conclusions

Despite the fundamental function of androgens as growth factors for prostate cancer, preclinical and clinical studies have established that supraphysiological androgens can paradoxically suppress the growth of CRPC. Accumulated preclinical evidence suggests that this growth inhibition can result from multiple mechanisms including cell cycle arrest, senescence, apoptosis, non-apoptotic cell death and immune clearance. The scientific community has made substantial progress in defining and elucidating mechanisms of the testosterone paradox of advanced prostate cancer, but considerable knowledge still needs to be gained to maximize opportunities for patient benefit. BAT is an innovative approach based on paradoxical growth inhibition of prostate cancer by supraphysiological testosterone; however, it has not been incorporated into standard-of-care practices, given the uncertainty in the optimal use of such therapy. We hope that ongoing research efforts will soon establish a role for this therapy to expand options and improve outcomes for patients with advanced prostate cancer.

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Competing interests
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Additional information
Correspondence should be addressed to Sushant Kachhap.

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