

Review

Novel Target Opportunities in Non-Metastatic Castrate Resistant Prostate Cancer

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Simple Summary: Following local treatment by surgical removal or radiation, prostate cancer progression involves non-metastatic state, designated nmCRPC, which is characterized by rise in PSA and lack of detectable metastases. This disease state is eventually progressing to metastatic castrate resistant prostate cancer that invariably leads to death. Here, we review potential therapeutic strategies to interfere with the transition before the cancer becomes deadly.

Abstract: Nearly one third of men will incur biochemical recurrence after treatment for localized prostate cancer. Androgen deprivation therapy (ADT) is the therapeutic mainstay, however almost all patients will eventually transition to a castrate resistant state (castrate resistant prostate cancer, CRPC). Subjects with CRPC generally develop symptomatic metastatic disease (mCRPC) and incur mortality several years later. Prior to metastatic disease, men acquire non-metastatic CRPC (nmCRPC) which lends the unique opportunity for intervention to delay disease progression and symptoms. This review addresses current therapies for nmCRPC, as well as novel therapeutics and pathway strategies targeting men with nmCRPC.

Keywords: prostate cancer; castrate resistance, non-metastatic CRPC, clinical trial, epithelial mesenchymal transition, STAT3

1. Introduction

In the United States, prostate cancer is the most common cancer among men with approximately 175,000 new diagnoses per year [1]. Among men who undergo therapy for localized disease, nearly one third will develop biochemical recurrence as assessed by a rise in prostate specific antigen (PSA) [2]. Given the androgen sensitive nature of prostate cancer, men are generally started on androgen deprivation therapy (ADT) after they recur [3]. ADT includes luteinizing hormone releasing hormone (LHRH) agonists (i.e., leuprolide, goserelin, histrelin), LHRH antagonists (i.e. degarelix) [4], or the very recently approved by US Food and Drug Administration (FDA), oral LHRH antagonist, relugolix [5]. Despite suppression of androgen signals, almost all hormone sensitive prostate cancer patients will eventually transform into hormone refractory, or castrate resistant prostate cancer (CRPC), which carries a poor prognosis and has high rates of metastatic disease (mCRPC). mCRPC is ultimately what causes symptoms and death among prostate cancer subjects with a median survival time around 3-4 years [6, 7]. Studies have shown that nearly 50% of men with nonmetastatic CRPC (nmCRPC) will develop metastases after two years [8]. It is important to note that new imaging modalities, including PSMA-PET, NaF PET, and 11C-choline PET/CT will likely change the landscape of nmCRPC with earlier identification of measurable metastatic disease [9]. However, current disease progres-

sion from nmCRPC to mCRPC typically occurs after 48 months. Given this knowledge, treatment of nmCRPC subjects offers a unique opportunity to delay progression to mCRPC. There has been a surge in research in this domain with three new agents achieving FDA approval in 2018 and 2019 for combination therapy with ADT in the setting of nmCRPC. All of these therapeutics targets were second generation androgen receptor inhibitors. The goal of this review is to discuss current anti-androgen treatment options for nmCRPC, as well as innovative therapeutic targets that have been explored. Lastly, we will discuss a novel pathway, epithelial mesenchymal transition (EMT) process that may have utility in subjects with nmCRPC to delay disease progression.

2. Current Treatment Paradigm for nmCRPC

In 2018 and 2019, three second generation anti-androgen therapeutics were approved by the FDA for combination therapy with ADT in the setting of nmCRPC with PSA doubling time (PSADT) < 10 months: apalutamide, enzalutamide, and darolutamide [4, 10-12]. These small molecule compounds act by three different mechanisms: inhibiting androgen binding to the androgen receptor, inhibiting androgen receptors from entering the nucleus, and inhibiting androgen receptor binding to DNA [10-12]. They also bind to the androgen receptor with a higher affinity than the first generation anti-androgens (i.e., flutamide, bicalutamide, nilutamide) which solely prevent androgen receptor translocation to the nucleus [13, 14].

Apalutamide, a nonsteroidal antiandrogen, binds directly to the ligand-binding domain of the androgen receptor with a 7- to 10- fold higher affinity versus first generation agents [10, 13]. It is a selective and competitive androgen receptor inhibitor [14]. Selective Prostate Androgen Receptor Targeting with ARN-509 (SPARTAN) trial was a randomized controlled trial comparing apalutamide with placebo in patients who were at high risk of developing metastasis as defined by a PSA doubling times of less than 10 months. This trial showed that when combined with ADT, addition of Apalutamide (Erleada™) resulted in a metastasis free survival (MFS) of 40.5 months compared to 16.2 months with the combination of ADT and placebo [10]. Of note, the apalutamide group did have a higher incidence of rash (23.8% versus 5.5%), hypothyroidism (8.1% versus 2%) and fracture (11.7% versus 6.5%) [10]. This trial was the basis for FDA approval of apalutamide as a treatment in nmCRPC.

The PROSPER trial was a large, international, randomized controlled trial comparing the addition of enzalutamide or placebo to ADT. Enzalutamide (Xtandi®) is an androgen receptor antagonist that also binds with higher affinity than first generation anti-androgens [14]. Enzalutamide is also approved for use in CRPC per the results from the PREVAIL and AFFIRM trials [6, 15]. Eligible patients for the PROSPER trial had a PSA doubling time less than or equal to 10 months and PSA ≥ 2 ng/ml at screening [11]. Enzalutamide was found to have a MFS of 36.6 months compared to 14.7 months in the placebo group [11]. Enzalutamide also prolonged time to use of antineoplastic therapy. Of note, 31% of subjects in the enzalutamide arm had grade 3 or higher adverse events versus 23% in the placebo group [11]. As a result of the PROSPER trial, the FDA approved enzalutamide in the nmCRPC setting.

The ARAMIS multinational, randomized controlled trial compared Darolutamide with ADT to placebo with ADT [12]. Darolutamide is also an androgen receptor antagonist that as been found to be more efficacious than apalutamide and enzalutamide. Interestingly, Darolutamide is able to bind to the androgen receptor despite various mutations that impact the efficacy of other second generation anti-androgens (typically converting them from antagonist to agonist) [14]. The results of the ARAMIS trial showed that Darolutamide improved MFS (40.4 months versus 18.4 months) with no significant difference in side effects [12]. In 2019, Darolutamide (Nubeqa™) was also granted FDA approval for treatment of patients with nmCRPC [12].

3. Targets Beyond Androgen Receptor for the nmCRPC Therapy

Therapeutics that do not target the androgen receptor have been studied for subjects with nmCRPC and have had mixed results. These therapeutics strive to bypass the proposed mechanisms for biologic conversion to CRPC, which include gain of function mutations to the AR, upregulated intra-tumoral androgen synthesis, overexpression of alternative steroid receptors, and AR protein overexpression [16].

Abiraterone acetate is an irreversible CYP17 inhibitor targeting androgen biosynthesis in the testicles, adrenal glands, and prostate cancer tumor cells. The IMAAGEN trial was a phase II, multicenter study that evaluated PSA responses to abiraterone acetate in 131 nmCRPC patients with a PSA higher than 10 ng/mL or a PSADT lower than 10 months (NCT01314118) [17]. The primary endpoint of the study was PSA response at 6 months. The results demonstrated that 87% of patients exhibited a PSA decline of more than 50%. A decline in PSA of over 90% was noted in 60% of subjects [17]. The median time to PSA progression and to radiographic progression was 28.7 months and not reached, respectively [17]. The toxicity profile of abiraterone was similar to that reported in phase III trials assessing its role in mCRPC patients.

Another small molecule inhibitor of androgen production, orteronel (TAK-700), targeting CYP17A1, was tested in a Phase 2 trial [18]. Data revealed median time to PSA progression and metastases to be 14 months and 25 months, respectively (NCT01046916) [18]. Unfortunately, phase 3 trials exploring orteronel in mCRPC and hormone sensitive metastatic prostate cancer have not shown survival benefit (NCT01193244). There are ongoing trials investigating the role of orteronel in high risk localized prostate cancer (NCT01546987) [19, 20].

Integrins, a family of transmembrane receptors, have been shown to mediate invasion and angiogenesis in prostate cancer bone metastases. A Phase 2 study investigating the effects of cilengitide, a selective antagonist of $\alpha_v \beta_3$ and $\alpha_v \beta_5$ integrins, in nmCRPC was completed in 2015 [21]. While cilengitide was well tolerated, it had no detectable clinical activity [21].

Endothelin-1 (ET-1) and the ET_A receptor have been implicated in prostate cancer progression. Atrasentan is a selective endothelin-A receptor antagonist. The atrasentan Phase 3 Study Group explored the use of atrasentan in nmCRPC in a randomized placebo-controlled trial. While atrasentan lengthened PSADT and slowed increase in bone alkaline phosphatase levels, this study did not show a significant delay in time to disease progression. However, geographical differences in median time to progression (TTP) were noted: atrasentan did show a prolongation of TTP among patients outside the US whereas it did not delay TTP among US patients [22]. In another study, the ET_A receptor antagonist zibotentan was compared to placebo in patient with nmCRPC [23]. At interim analysis, the zibotentan and placebo groups did not differ in overall survival or progression free survival resulting in trial termination. The authors concluded that zibotentan was no longer under investigation as a potential treatment for prostate cancer [23].

Insulin-like growth factor (IGF) is an endocrine hormone that promotes anabolic activity after signals from growth hormone and has been implicated in the growth of prostate cancer. A Phase 2 trial evaluated the effect of octreotide, a somatostatin analogue that inhibits growth hormone release from the pituitary, in men with nmCRPC [24]. The trial was stopped early after a pre-planned interim analysis showed no decline in PSA levels despite three cycles of treatment and a decline in IGF levels [24].

Bevacizumab (Avastin), a humanized monoclonal antibody against vascular endothelial growth factor A (VEGF-A), a potent proangiogenic and immunosuppressive mediator, was also trialed in nmCRPC patients (NCT01656304) [25]. Fifteen subjects received treatment every 14 days until PSA progression. Median time to PSA progression and new metastases was noted to be 2.8 months and 7.9 months, respectively. This treatment was deemed ineffective [25].

Denosumab is a human RANKL-specific monoclonal antibody that is approved for the prevention of skeletal-related events. A randomized Phase 3 trial was conducted in men with nmCRPC evaluating the effects of denosumab on bone metastasis-free survival [26]. Compared to placebo, denosumab significantly increased bone-metastasis free survival by a median of 4.2 months [26]. However, denosumab was associated with a higher incidence of osteonecrosis of the jaw and hypocalcemia [26]. The FDA denied an expanded indication for denosumab for the prevention of bone metastasis.

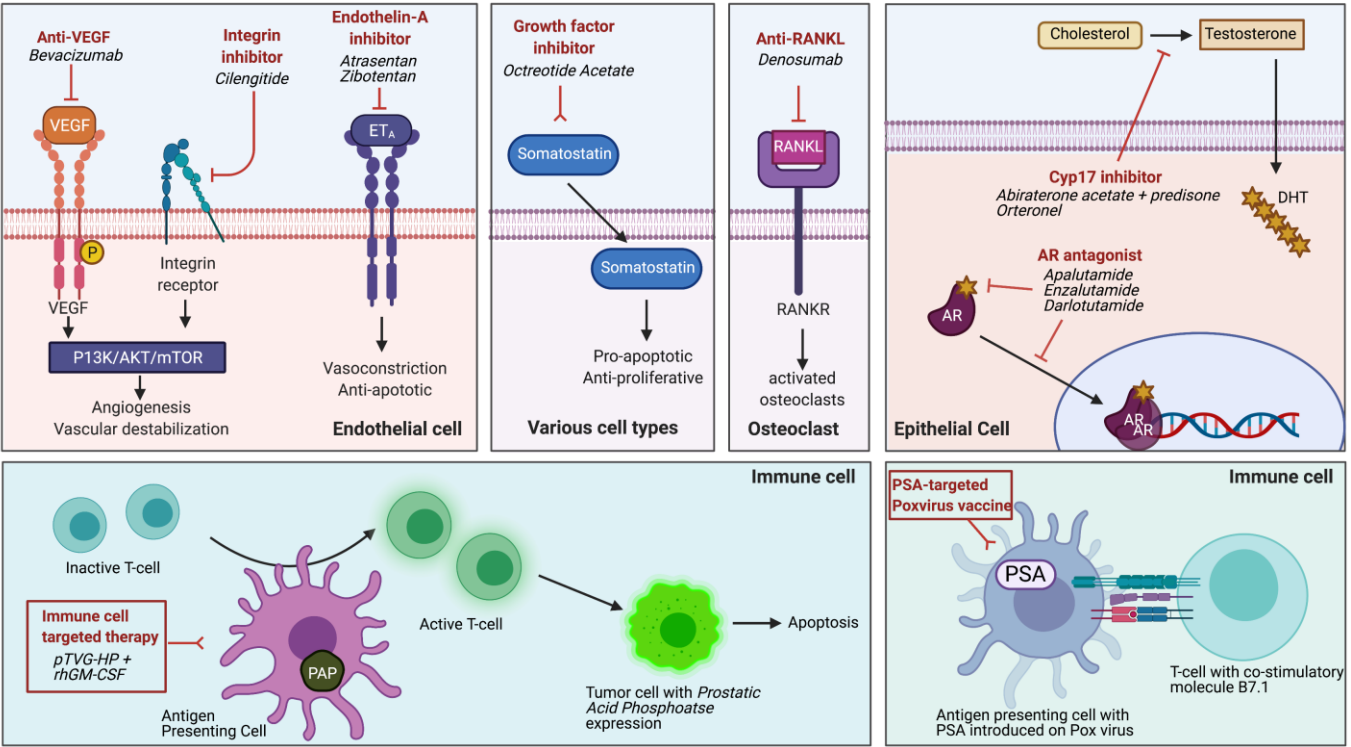


Figure 1. Major pathways targeted in clinical trials of nmCRPC. These include, panels from left to right: angiogenesis and vascular destabilization (Anti-VEGF and Endothelin inhibitor); cell matrix adhesion/EMT (Integrin inhibitor); growth hormone pathway (Somatostatin); androgen pathway (AR inhibitors); bone metastases inhibitor (anti-RANKL); immune cell therapy (rhGM-CSF); and PSA based vaccines (PSA-Pox virus). See Table 1 for clinical trials information related to these targets.

Table 1. Current trials targeting nmCRPC clinical space

Trial Identifier	Trial Name/Details	Name	Drug Target	Conclusions/Metastasis Free Survival (MFS)
NCT01946204	Selective Prostate Androgen Receptor Targeting with ARN-509 (SPARTAN), Phase 3	Apalutamide (Erleada™)	AR antagonist	40.5 months versus 16.2 months with placebo
NCT02003924	PROSPER, Phase 3	Enzalutamide (Xtandi®)	AR antagonist	36.6 months versus 14.7 months with placebo
NCT02200614	ARAMIS multinational, Phase 2	Darolutamide (Nubeqa™)	AR antagonist	40.4 months versus 18.4 months with placebo
NCT01314118	IMAAGEN trial, Phase 2	Abiraterone acetate + prednisone	Cytochrome C17 enzyme (CYP17), androgen synthesis	Median PSA progression at 28.7 months, radiographic progression not reached
NCT00121238	Phase 2	Cilengitide	selective antagonist of $\alpha_v \beta_3$ and $\alpha_v \beta_5$ integrins	No detectable clinical activity
NCT00036556	Atrasentan, Phase 3	Atrasentan	selective endothelin -A receptor antagonist	No significant delay in time to disease progression, but did show a prolongation of TTP among patients outside the US only
NCT00626548	ENTHUSE M0, Phase 3	Zibotentan	ET _A receptor antagonist	No difference in overall survival or progression free survival resulting in trial termination
NCT00510224	Sandostatin, Phase 2 Terminated due to lack of efficiency	Octreotide Acetate (Sandostatin)	Insulin-like growth factor (IGF) signaling pathway inhibition, somatostatin analogue that inhibits growth hormone release from the pituitary	No decline in PSA levels despite three cycles of treatment and a decline in IGF levels
NCT00286091	Phase 3	Denosumab	anti-RANKL monoclonal antibody	20 months versus 15.8 months with placebo
NCT01046916	TAK-700, Phase 2	Orteronel (TAK-700)	CYP17A selective inhibitor	Median time to PSA progression and metastases to be 14 months and 25 months
NCT00849121	DNA vaccine, Phase 2	DNA Vaccine: pTVG-HP with rhGM-CSF	Vaccine has DNA that encodes for a protein which is produced by the prostate gland, prostatic acid phosphatase (PAP) and given with an adjuvant granulocyte-macrophage colony-stimulating factor (GM-CSF)	Repetitive immunization with the DNA vaccine maintained antigen-specific T-cells that target prostate cells
N/A	Randomized control trial	PSA-based Poxvirus vaccine	Vaccine with transgenes for PSA and human t-cell costimulatory molecule B7.1; priming vaccine followed by monthly boosts with GM-CSF	Noted trend toward survival benefit for patients randomized to vaccine arm
NCT01656304	Pilot phase 2 trial	Bevacizumab (Avastin)	Humanized monoclonal antibody against vascular endothelial growth factor (VEGF)	No benefit noted

Despite the limited efficacy of immune checkpoint blocking antibodies in prostate cancer, several studies suggested potential of immunization-based strategies in CRPC patients. A Phase 2 trial explored a DNA-based vaccine, sipuleucel-T, that targets prostatic acid phosphatase (PAP), a protein specifically produced by the prostate gland [27, 28]. When given in conjunction with granulocyte-macrophage colony-stimulating factor (rhGM-CSF), PAP-specific T cells are generated which leads to an immunologic response to prostate specific cancer cells [27]. This pilot study did show that repetitive immunization with the DNA vaccine-maintained antigen-specific T-cells that target prostate cells in a safe manner (NCT00849121) [27].

Use of a PSA-based poxvirus vaccine (PSA-TRICOM) in conjunction with ADT (nilutamide) in nmCRPC was explored previously [29, 30]. The study included 21 subjects who were randomized to receive either the vaccine or ADT. Upon PSA progression without evidence of metastatic disease on imaging, patients could cross-over to receive both therapies. Time to PSA progression was 7.6 months with nilutamide versus 9.9 months with vaccine first [29]. At 6 years, a trend was noted toward survival benefit for patients randomized to the vaccine arm [30].

Finally, the recent phase 1 study by Kyriakopoulos et al. explored the potential of targeting AR not as an oncogene but as a tumor-specific antigen using a DNA vaccine [31]. The study demonstrated safety of this approach in patients with metastatic CSPC with early evidence of the activation of tumor-specific cytotoxic T cells. Whether the approach will be effective in patients with more advanced prostate cancer is currently being tested in combination with PD1 blockade (NCT04090528 and NCT03600350).

4. Novel Epithelial Mesenchymal Transition (EMT) process to delay nmCRPC

Novel targeting of the dysregulated epithelial mesenchymal transition (EMT) process may provide opportunity to delay nmCRPC disease progression [32, 33]. EMT describes the physiologic and pathologic process by which epithelial cells de-differentiate into mesenchymal cells. Epithelial cells, which are normally polarized with intact cell-to-cell junctions, de-differentiate into mesenchymal cells which allows for wound healing in normal cells, but also migration and metastatic spread in tumor cells [34]. Changes in morphology and signaling lead to conversion to a poorly differentiated cell [32, 34]. Epithelial cells require the structural stability of adherens junctions which are comprised of cadherin proteins [35]. E-cadherin is a calcium dependent transmembrane glycoprotein that facilitates extracellular interactions with other epithelial cells [35]. The downregulation of E-cadherin has been noted to be a hallmark of early stages of EMT [36-38]. The emergence of EMT related transcription factors (EMT-TF), such as TWIST1 and SNAIL, silence E-cadherin expression through direct binding to the E-cadherin gene, which disrupts cell junctions and allows for tumor migration [39].

Research has shown that anti-androgen treatments such as enzalutamide have resulted in the upregulation of EMT-TF, such as TWIST1 and SNAIL, via the Twist1/Androgen Receptor (AR) axis [40]. Patients on ADT with high TWIST1 expression may benefit from TWIST1 inhibition to prevent EMT [41]. TWIST1 inhibitors have been studied in lung cancer, and results have shown cell growth inhibition and apoptosis [42, 43]. Martin et al, proposed EMT as a mechanism of resistance to Cabazitaxel and antiandrogen therapy in advanced prostate cancer, thus justifying more research inquiry into the pathway [44].

Dysregulated ABI1, a protein involved in cellular cytoskeleton stabilization and signaling, may also contribute to the EMT process [45]. Downregulation of ABI1 is associated with loss of E-cadherin, the key protein involved in maintaining the adherens junction [45]. This may propel disease progression and metastatic spread of tumor through activation of EMT. ABI1 loss has also been associated with upregulated STAT3 activity [45]. STAT3 is a master regulator of EMT transcriptional programming that promotes cellular adhesion, migration, proliferation and differentiation

[34, 46, 47]. Gujral et al, identified the critical mechanism of STAT3-mediated invasion through activation of non-canonical WNT pathway [48]. WNT pathway is one of the key pathways associated with prostate tumor progression and invasion [49, 50].

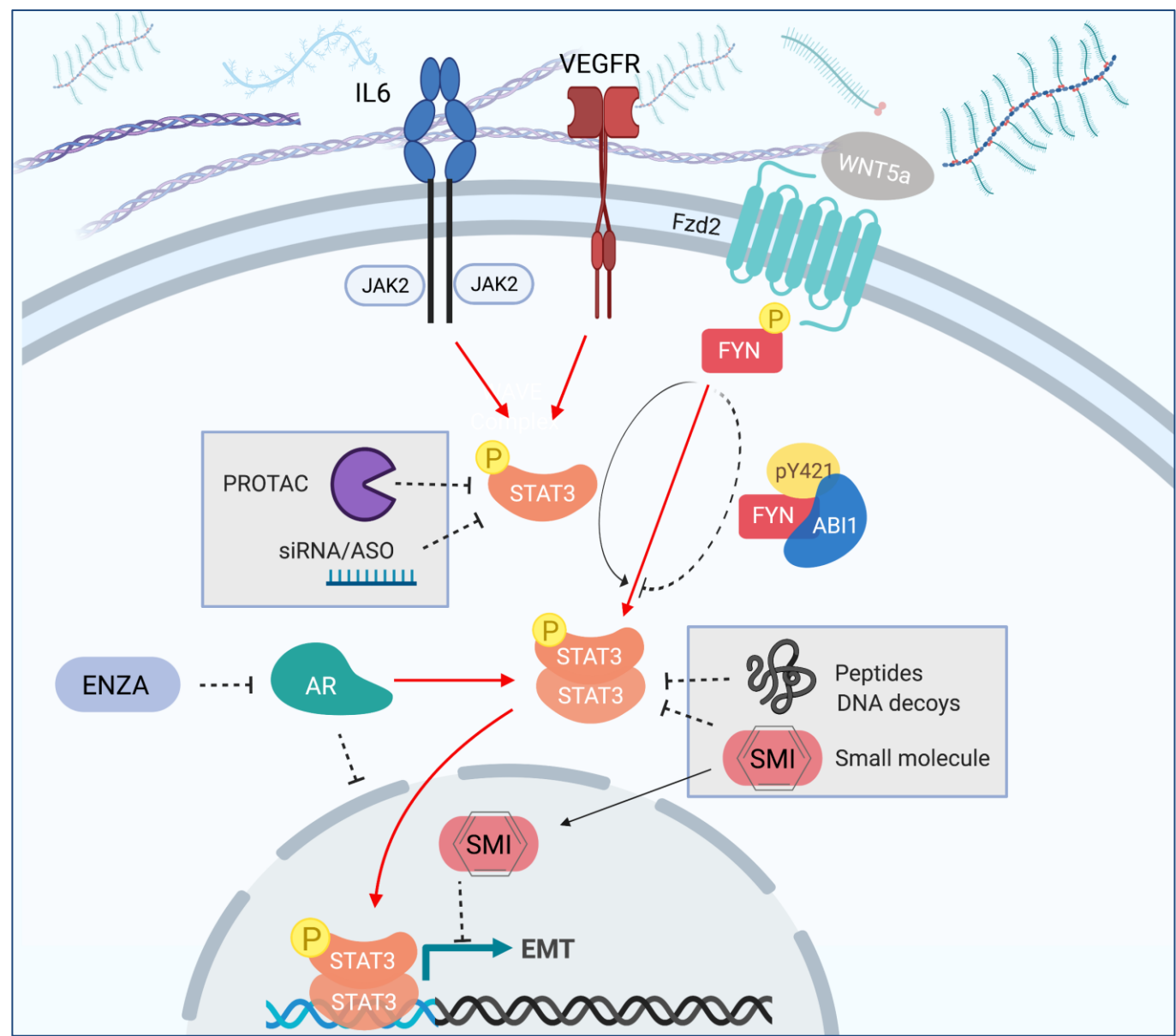


Figure 2. STAT3 as potential target pathway in nmCRPC. Major types and mechanisms of current STAT3 inhibitors: PROTAC, siRNA/ASO, polypeptides, DNA decoys, and small molecule inhibitors. The goal of inhibitors is to degrade STAT3 before it acts in the nucleus (PROTAC, siRNA/ASO); or inhibit its nuclear translocation (DNA decoys), or transcriptional activity by interfering with DNA binding (polypeptides, small molecule inhibitors). Major regulatory pathways for STAT3 in prostate epithelial cells are depicted.

5. Targeting STAT3 as Master Regulator in nmCRPC

Much has been said about inhibiting STAT3 in cancer. STAT3 is an established target in the majority of advanced human tumors including prostate cancers [51, 52] [53]. STAT3 is elevated prostate cancer cells as well as in many types of tumor-infiltrating immune cells, therefore pharmacological approaches aim to downregulate STAT3 function. Another justification for potentially targeting STAT3 in nonmetastatic CRPC is the fact that inhibition of STAT3 might prevent treatment-induced neuroendocrine-like prostate tumor phenotype, also termed t-NEPC. Incidence of this type of tumors is expected to rise upon increased use of novel anti-AR agents [54-56]. These tumors alike classical NEPC tumors [57] are likely to be challenging to treat as they are resistant to anti-AR agents and usually treated with platinum therapy subsequent to exhausting taxane therapy [55, 57-59].

The direct effect of STAT3 targeting depends on the genetic background of cancer cells. In PTEN-deficient cancer cells, STAT3 may act as tumor suppressor [60] and promote tumor senescence by transcriptional regulation of ARF-p21-P53 axis [61]. The disruption of STAT3 signaling in PTEN null prostate cancer cells can stimulate tumor growth in mice [61]. However, targeting STAT3 in the whole tumor microenvironment, including tumor-associated myeloid cells, was shown to generate potent antitumor effects independently from PTEN status of cancer cells [53]. These preclinical results underscore therapeutic potential and priority in targeting STAT3 activity in tumor-associated immune cells rather than in cancer cells alone [53, 62]. Synergistic activity of anti-STAT3 inhibitors on tumor microenvironment might be just as important as its anti-tumor activity in prostate cancer [63]. STAT3 is activated in myelosuppressive cells [64, 65]. Hence targeting STAT3 in microenvironment allows for tumor shrinkage, due to proper immune system re-activation [53, 62].

Multiple approaches to inhibit STAT3 levels or activity have been tested in preclinical studies [66]. Small molecule Janus kinase inhibitors allow for targeting upstream regulators of STAT3 activity [51, 52]. Peptide-, decoy DNA or small molecule-based approaches aim at targeting the SH2 domain of STAT3 to prevent dimerization, which is involved in DNA binding activity and STAT3 transcriptional activity [51, 52]. Efficient downregulation of STAT3 can be obtained by either oligonucleotide-based inhibitors, such as antisense oligonucleotides [53], or, by small molecules acting as proteolysis targeting chimeras (PROTACs) [67].

ABI1 regulates STAT3 expression through SRC kinase FYN providing a novel, potential strategy for STAT3 targeting [45]. Enhanced STAT3 levels are associated with enzalutamide resistance [68]. Therefore, loss or downregulation of ABI1 with overactive STAT3 signaling may contribute to enzalutamide resistance [45]. This suggests a potential role of ABI1 as a novel biomarker for early EMT events, as well as STAT3-mediated enzalutamide resistance. This also suggests a potential role for STAT3-inhibitors to re-sensitize tumors to enzalutamide as well as other ADTs [68].

6. Conclusion

Given that 50% of men with nmCRPC will undergo progression to mCRPC, novel therapies are needed. Currently, three FDA approved anti-androgen therapies are used in this sphere: apalutamide, enzalutamide, and darolutamide. Numerous targets for non-androgen pathways have been explored previously. This review introduces the novel concept of targeting the EMT process early on in nonmetastatic prostate cancer, as EMT plays a key role in disease progression and may serve as a potential target for future therapeutics and biomarkers. Further exploration of disrupted signaling pathways and cellular architecture may uncover potential opportunities for disease control.

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