

Novel Biological Agents for the Treatment of Hormone-Refractory Prostate Cancer (HRPC)

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Abstract: Hormone-refractory prostate cancer (HRPC) is an inevitable evolution of prostate carcinogenesis, through which the normal dependence on hormones for growth and survival is bypassed. Although advances in terms of symptoms palliation and quality of life improvement have been addressed with current treatment options, innovative approaches are needed to improve survival rates. A thorough understanding of HRPC-associated molecular pathways and mechanisms of resistance are a prerequisite for novel potential therapeutic interventions. Preclinical and early clinical studies are ongoing to evaluate new therapies that target specific molecular entities. Agents under development include growth factor receptor inhibitors, small molecules targeting signal transduction pathways, apoptosis and cell-cycle regulators, angiogenesis and metastasis inhibitors, differentiation agents, telomerase inactivators, and epigenetic therapeutics. Incorporation of these agents into existing treatment regimens will guide us in the development of a multidisciplinary treatment strategy of HRPC. This article critically reviews published data on new biological agents that are being tested in HRPC clinical trials, highlights ongoing research and considers the future perspectives of this new class of agents.

Keywords: Biological agents, Gene therapy, Hormone-refractory prostate cancer, Immunotherapy.

INTRODUCTION

Prostate cancer remains the most common non-cutaneous malignancy in the Western world and is the second leading cause of cancer death in males, after lung cancer [1]. In 2002, nearly 189,000 men received a diagnosis of prostate cancer in the United States and there were an estimated 30,200 prostate cancer-related deaths [2]. Autopsy series have revealed small prostatic carcinomas in up to 29% of men 30 to 40 years-old and 64% of men 60 to 70 years-old [3]. Moreover, prostate cancer risk is 1 in 6 and death risk from metastatic disease is 1 in 30 [2]. Unfortunately, localised prostate cancer rarely causes symptoms, thus 38 to 51% of patients present with locally advanced or metastatic disease, while 10% to 50% of these cases will rapidly progress to a hormone-refractory state [4].

Despite these grim statistics, surprisingly little progress has been achieved in extending patients' survival with current treatment modalities. Noteworthy is that since the first observation concerning the beneficial effects of castration, by Huggins and Hodges in 1941, androgen ablation still remains the cornerstone of advanced prostate cancer treatment. Although tumour regression is initially achieved in the majority of patients, progression to hormone-refractory prostate cancer (HRPC) usually occurs within 2 to 5 years [5]. HRPC current therapy is mainly directed at palliation of symptoms and improving the quality of life, offering 7 to 16

months median survival [6,7]. Ongoing research explores in depth the molecular mechanisms implicated in the emergence of hormone independence in prostate cancer [8]. Based on experimental and preclinical findings, novel anti-prostate cancer strategies have been developed. The present review focuses on the rationale of novel biological agents and strategies, which are evaluated for the treatment of HRPC and considers their future perspectives.

1. HRPC DEFINITION

Prostate cancer represents a heterogeneous entity, with both hormone-sensitive and hormone-insensitive cells present since initial diagnosis [9]. HRPC refers to progressive disease despite castration levels of testosterone. Androgen ablation can usually inhibit the progression of endocrine-sensitive prostate cancer cells. However, some cells continue to proliferate despite castration levels of testosterone and remain sensitive to alternative endocrine treatments such as adrenal-androgen ablation, corticosteroids and anti-androgen withdrawal. Noteworthy is that there is not a widely accepted definition of HRPC [10]. Recently, established criteria for patients with HRPC recruited in clinical trials require the presence of at least one new lesion on bone scan or biochemical progression, in the presence of castration levels of testosterone (< 50 ng/mL) [11]. Biochemical progression is considered when two consecutive increases in prostate-specific antigen (PSA) are registered, with a minimal value of 5 ng/mL. Finally, progression occurs following cessation of treatment with androgen receptor blockers for 4 to 6 weeks.

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2. ANDROGEN RECEPTOR (AR) AND HRPC

Prostate tissue development, growth, differentiation and homeostasis depend on androgen activity, mediated by the AR which is a member of the steroid hormone receptors' superfamily and represents a "zinc-finger" transcription factor [12]. The AR is androgen activated upon ligand bonding, resulting in dimerisation and recognition of androgen response elements, located in the promoter or enhancer regions of AR-target genes in the nucleus. Although experimental data suggest that before ligand bonding the receptor is located in the cytoplasm bound with heat-shock proteins, there are also reports supporting that AR largely resides in the nucleus [13].

Table 1. Proposed Mechanisms of HRPC Development(ref.: 15-18)

1.	Bypassing AR signalling pathway
•	Ligand-independent enhancement of AR action from GFs and cytokines
•	Mutations and/or deletions of AR
•	Aberrant methylation of the AR gene promoter with consequent inhibition of its expression
2.	Adapting AR signalling pathway
•	AR amplification
•	AR mutations that change ligand specificity
•	AR ligand-independent activation through cross-talk with other signal-transducing pathways
•	Transcriptional co-factors participation (co-activator amplification or co-repressor down-regulation)

Transcription of the AR gene is cell-type specific and in some tissues also age-specific. Moreover, AR messenger RNA (mRNA) levels are regulated by androgen and other steroid hormones. It is noteworthy that, except for the spleen, there are no other tissues that do not express AR [14]. Therefore, AR expression control through post-translational modifications (e.g. phosphorylations), presence of specific transcriptional co-factors, genetic (e.g. mutations) and epigenetic (e.g. methylation, acetylation) events is of paramount importance for tissue-selectivity determination.

Over 80% of patients with advanced prostate cancer will show some kind of response to androgen blockade [15]. Unfortunately, there are not currently available predictive factors to identify these patients, as well as the duration of this response. However, it seems that clinical responses are not correlated with the levels of AR in cancer tissue. Consistent with this, it has been demonstrated that AR expression is sustained even with androgen blockade [16].

With few exceptions, the AR gene is normally expressed in prostate cancer. However, after hormone treatment is administered, significant changes are noted through which prostate cancer is converted from hormone-sensitive to HRPC [17]. This crucial point of prostate cancer natural history is still not well elucidated, although various molecular mechanisms have been suggested (Table 1). All these mechanisms finally result in the growth of prostate cancer cells in a low-androgen environment and enhanced AR activity with a broad list of ligands [18].

3. NOVEL THERAPEUTIC STRATEGIES FOR HRPC

The current available treatment options for HRPC are supportive care, salvage endocrine manipulations, radiotherapy, radioactive isotopes, biphosphonates and chemotherapy [19]. As all these alternatives have not offered a significant improvement in terms of survival, new strategies are being developed and evaluated (Table 2).

Table 2. Novel Therapeutic Strategies for the Treatment of HRPC(ref.)

1.	Immunotherapy
•	Vaccines ²¹
•	Activated autologous dendritic cells ^{22,23}
•	Monoclonal antibodies ²⁴⁻²⁶
2.	Gene Therapy ^{27,28}
3.	Biological Agents
•	Growth factors inhibitors ³¹⁻⁴⁶
•	Signal transduction inhibitors ⁴⁷⁻⁸⁹
•	Apoptosis regulators ¹⁰⁻¹¹⁰
•	Cell-cycle regulators ¹¹²⁻¹¹⁴
•	Proteasome inhibitors ¹¹⁶⁻¹²⁰
•	Neo-angiogenesis inhibitors ¹²²⁻¹⁵³
•	Anti-metastatic agents ¹⁵⁸⁻¹⁶⁶
•	Differentiation agents ¹⁷⁰⁻²⁰³
•	Epigenetic therapeutics ²⁰⁷⁻²¹⁴
•	Telomerase inactivators ²¹⁵⁻²¹⁷

3.1. Immunotherapy

Until recently, prostate cancer was considered as a non-immunogenic tumour. This assumption has changed and the role of immunotherapy is being extensively explored. Active and passive immune approaches directed against prostate-specific antigens, oncogenic proteins, altered tumour suppressor gene products, and differentiation antigens, are ongoing [20]. A variety of prostate cancer cell-surface glycoproteins and carbohydrates serve as potential targets of synthetic vaccines, which are evaluated in phase I/II trials with encouraging, so far, results [21]. Immunomodulatory cytokines and dendritic cell therapy also represent attractive immunological strategies with encouraging results in HRPC [22,23]. Monoclonal antibody (MA)-based therapeutics is also being applied in HRPC. After the recent clinical success of trastuzumab, a humanised MA against HER-2/neu receptor in the treatment of patients with breast cancer with HER-2 over-expression, its use has also been suggested for the treatment of patients with HRPC. However, a recently published clinical trial revealed that immunohistochemical over-expression of HER-2 is present in only a small percentage of patients with HRPC, 6% have 2+ and only 1% have 3+ immunopositivity for HER-2) [24]. Therefore, further clinical evaluation of trastuzumab is considered rational only

Table 3. Important Published Clinical Trials Evaluating Novel Biological Agents Alone or in Combination with other Therapeutic Strategies in HRPC Treatment

<i>Agent</i>	<i>Target</i>	<i>Primary conclusion</i>	<i>Phase</i>	<i>Ref</i>
1. Growth Factor Inhibitors				
Trastuzumab+ Docetaxel	ErbB-2/HER-2	HER2 overexpression in prostate cancer is infrequent.	II	24
SU101	PDGFR ^a	Modest activity regarding PSA and objective clinical responses as single-agent activity in heavily pretreated patients.	II	43
2. Signal Transduction Inhibitors				
Gefitinib	EGFR-TK ^b	No objective or PSA responses were reported.	II	50
Gefitinib	EGFR-TK	Initial results reporting infrequent PSA responses or early progression as single-agent treatment.	II	51
Gefitinib + Mitoxantrone/Prednisone	EGFR-TK	Preliminary results show promising PSA responses with tolerable toxicity.	I/II	52
Gefitinib + Docetaxel/Estramustine	EGFR-TK	Preliminary results show promising PSA responses with acceptable toxicity.	I/II	53
ISIS 5132	Raf-1 kinase	No objective or PSA responses were observed.	II	68
ISIS 3521	PKC ^c	No objective or PSA responses were observed.	II	68
3. Apoptosis Regulators				
Genasense + Mitoxantrone	bcl-2	Well-tolerated combination without additive toxicity.	I	94
Genasense + Docetaxel	bcl-2	Well-tolerated combination with PSA and clinical responses.	II	95
Atrasentan	ET _A ^d	Well-tolerated agent with mild vasodilatory adverse events.	I	101
Atrasentan	ET _A	Favorable responses in a variety of clinical measures, including time to progression.	II	102
4. Cell-cycle Regulators				
Flavopiridol	CDKs ^e	Significant toxicity without objective responses as single-agent treatment.	II	114
5. Proteasome Inhibitors				
PS-341 + Docetaxel	proteasome	Preliminary results show encouraging efficacy and tolerable toxicity.	I/II	120
6. Neo-Angiogenesis Inhibitors				
Suramin (fixed high dose) + Hydrocortisone		High, but of short duration, efficacy with acceptable toxicity profile.	II	125
Suramin (monthly)		Reported PSA and objective responses in heavily pretreated patients.	II	126
Suramin (three different doses)		No dose-response relationship was reported regarding progression-free and overall survival, whilst toxicity was enhanced with higher doses. II		127
Thalidomide		PSA responses reported in heavily pretreated patients.	II	129
Thalidomide		PSA responses reported in heavily pretreated patients.	II	130
Thalidomide + Docetaxel		The combination achieved better PSA responses than docetaxel alone.	II	131

(Table 3). contd.....

<i>Agent</i>	<i>Target</i>	<i>Primary conclusion</i>	<i>Phase</i>	<i>Ref</i>
Thalidomide + Mitoxantrone/Prednisone		The combination did not achieve response benefit but caused additive toxicity.	II	133
Thalidomide + Dexamethasone (p.o.)		Preliminary encouraging results concerning efficacy.	II	134
Thalidomide + Paclitaxel/Doxorubicin		Preliminary encouraging results concerning efficacy.	I/II	135
Carboxyamido-triazole (CAT)		No clinical activity in HRPC patients with soft tissue metastasis.	II	140
Bevacizumab	VEGFR ^f	No significant objective responses as single-agent treatment.	II	146
Bevacizumab + Docetaxel/Estramustine	VEGFR	Initial results showing remarkable efficacy with acceptable toxicity profile.	II	147
TNP-470		Reversible neuropsychiatric dose-limiting side effects and transient PSA increases. with subsequent decline.	I	150
7. Anti-Metastatic Agents				
Prinomastat Versus Prinomastat/Mitoxantrone/Prednisone	MMPs ^g	No differences were found in the two treatment regimens in terms of PSA responses, progression-free survival, 1-year and overall survival.	III	164
8. Differentiation Agents				
Calcitriol + Docetaxel		Well-tolerated combination regimen with promising results regarding PSA and measurable disease responses, time to progression and survival.	II	172
All- <i>trans</i> -retinoic acid (ATRA)		ATRA is not active against HRPC.	II	180
All- <i>trans</i> -retinoic acid (ATRA)		ATRA has minimal activity against HRPC.	II	181
13- <i>cis</i> -retinoic acid (Isotretinoin) + Androgen Blockade		Isotretinoin does not impair PSA response or cause significant toxicity.	II	182
13- <i>cis</i> -retinoic acid (Isotretinoin) + Interferon alpha/Paclitaxel		First study evaluating the efficacy and safety of this combination, which was well tolerated with encouraging results.	I	183
Troglitazone	PPAR γ ^h	Preliminary encouraging results concerning PSA responses.	II	189
9. Epigenetic zherapeutics				
5-aza-2'-deoxycytidine (azacitidine)	methylation	Well tolerated with modest clinical activity.	II	209

Abbreviations: ^a PDGFR, Platelet-Derived Growth Factor Receptor; ^b EGFR-TK, Epidermal Growth Factor Receptor Tyrosine Kinase; ^c PKC, Protein Kinase C; ^d ET_A, Endothelin-A receptor; ^e CDKs, Cyclin-Dependent Kinases; ^f VEGFR, Vascular Endothelial Growth Factor Receptor;

^g MMPs, Matrix Metalloproteinases; ^h PPAR γ , Peroxisome Proliferator-Activated Receptor γ .

for the subgroup of HRPC patients exhibiting HER-2 over-expression, either as single-agent therapy or in combination

regimens. Finally, several anti-prostate-specific membrane MAs (APSMAs) have been developed against both intra-

and extracellular antigenic epitopes and are under clinical evaluation for radioimmunotherapy of HRPC [25,26].

3.2. Gene Therapy

Prostate cancer gene therapy represents a promising distant future treatment strategy. A number of different approaches are being explored, such as correcting aberrant gene expression, exploiting apoptotic cell pathways, introducing toxic or lytic "suicide genes", targeting crucial cell functions, enhancing host anti-tumour immunologic response and various combinations [27]. Preclinical, *in vitro* and *in vivo*, results are encouraging, although most research is currently focused on the development of more effective vector delivery and selective targeting [28].

3.3. Novel Biological Agents

A great number of new therapeutic strategies are under development (Table 2) and early clinical evaluation for the treatment of HRPC (Table 3), based on the rapidly increasing knowledge pertaining to the molecular biology of the prostate carcinogenesis process.

4. GROWTH FACTOR INHIBITORS

Growth factors are necessary for cell proliferation. Many human solid tumours, such as prostate cancer are associated with over-expression of growth factors and their receptors, and the hypothesis is that dysregulated stimulation of growth factor receptors contributes to carcinogenesis, and *vice versa*. Experimental data have shown that among the mechanisms associated with the development of HRPC, is the bypassing of the AR-signalling cascade through activation of growth factor receptors and enhanced intracellular signalling activity with subsequent increase of cancer cell proliferation, inhibition of apoptosis and increased expression of markers of drug resistance. Therefore, targeting of growth factor signalling represents a possibly promising new therapeutic approach in treating HRPC [29] (Fig. 1A).

4.1. Inhibitors of Epidermal Growth Factor Receptor (EGFR)

EGFR, ErbB1/HER1, is one of the four known members of the HER-family of growth factor receptors including: ErbB1, ErbB2/HER2, ErbB3 and ErbB4, which are mediators of cell growth, differentiation and survival [30]. Enhanced expression of EGFR has been associated with tumour progression in various tumours, including HRPC [31]. EGFRs are normally expressed in normal prostatic tissue, while their expression increases with androgen-independence [32]. Anti-EGFR therapeutic approaches in prostate cancer include MAs directed against the extracellular bonding domain, small-molecule tyrosine kinase inhibitors, ligand conjugates, immuno-conjugates and antisense oligonucleotides [6]. Agents in clinical development include IMC-C225 (cetuximab), EMD 55900, ICR 62, ABX-EGF and others that directly block EGFR [33,34]. *In vitro* studies have suggested that cetuximab is capable of inhibiting tumour growth and metastasis, while paclitaxel and doxorubicin enhanced these results in HRPC cells [35,36]. A novel use of anti-EGFR MAs includes their combination

with MAs targeting other tumour antigens, such as HER-2. GW572016 (lapatinib) is a reversible small-molecule selective dual inhibitor of both EGFR and ErbB2 tyrosine kinases, which has recently entered clinical trials as an oral agent [37].

4.2. Inhibitors of Platelet-Derived Growth Factor Receptor (PDGFR)

The PDGF proteins are suggested to be potent stimulators of cell proliferation and play a major role in intracellular communication. Experimental data have shown that PDGFRs are expressed in prostatic intraepithelial neoplasia and carcinoma, but not in benign prostate hypertrophy or normal prostatic epithelium [38], suggesting that PDGF signalling might significantly contribute to the development of primary and metastatic prostate cancers [39].

Imatinib mesylate (ST1571-Gleevec[®]) has been found to exert a direct inhibiting action towards the bcr-abl kinase activity with significant clinical effects, thus its use in patients with chronic myeloid leukemia and Ph+ acute lymphoblastic leukemia represents a valid therapeutic option [40]. It has also been found that imatinib mesylate is a potent inhibitor of PDGFR kinase and clinical trials are underway to evaluate its efficacy in the treatment of patients with HRPC [41,42].

SU101 (leflunomide) is also a novel potent and highly selective inhibitor of PDGFR. After the encouraging results observed in phase I studies, a large scale phase II study of SU101 as monotherapy of HRPC patients resulted in a modest objective clinical benefit with the most frequent adverse events being nausea, anorexia and anemia [43]. Despite these results, further studies are warranted to assess the efficacy of SU101 either as a single treatment agent or in combination regimens for the treatment of HRPC.

4.3. Inhibitors of Insulin-like Growth Factor Receptor (IGFR)

IGFR is suggested to be involved in tumour cell proliferation, invasion and survival. Several studies have indicated that IGF axis contributes to prostate cancer progression [44]. Recently, it was suggested that a direct correlation exists between IGFR inhibition and down-regulation of zinc-dependent matrix metalloproteinase-2 (MMP-2), as well as with increased rate of apoptosis in androgen-independent cancer cells [45]. However, differential expression of certain IGF family members has been recently reported in various histological entities during prostatic carcinogenesis [46]. Therefore, thorough understanding of the role of these growth factors and their associated ligands and receptors will elucidate their potential therapeutic application in HRPC.

5. SIGNAL TRANSDUCTION INHIBITORS

Cancer cells receive external signals through surface receptors that stimulate their growth and proliferation. The transduction of the membrane-bound receptor activation signal to the nucleus is achieved and enhanced through various intracellular biochemical reactions. All these signal transduction molecular pathways are often dysregulated

during carcinogenesis. The deeper understanding of these molecules and their downstream and cross-talk relationships has generated intense research efforts in designing specific inhibitors of key proteins that are gradually entering into the clinical evaluation phase in the treatment of patients with solid tumours, including HRPC (Fig. 1B).

5.1. Tyrosine Kinase Inhibitors

Inhibition of growth factor receptor kinase-dependent signalling pathways is one of the most promising novel treatment strategies for prostate cancer treatment [47]. EGFR-tyrosine kinase (EGFR-TK) activity leads to activation of downstream pathways such as ras/MAP kinase and STATs (signal transducers and activators of transcription) transcription factors [30]. These signal transduction events are critical for the growth of many human tumours. EGFR has been found to be over-expressed in a wide range of human cancers, including prostate cancer [31], while this over-expression seems to be associated with poor outcome [32]. Several small-molecule inhibitors of EGFR-TK have been developed, such as ZD1839 (Iressa®), OSI-774 (Tarceva®), PD182905, PKI-166 and CI-1033 [48]. Iressa, given either intermittently or continuously, has resulted in remarkable efficacy with low-toxicity profile in patients with prostate cancer in recent phase I/II studies [49-51]. In addition to their single-agent activity, EGFR-TK inhibitors have also shown synergy with chemotherapy and radiation therapy. Phase I/II clinical trial evaluations of the combination of ZD1839 with mitoxantrone/prednisone [52] and docetaxel/estramustine [53], resulted in 23% and 33% decreases in PSA responses respectively, with no additive toxicity. An oral pan-ErbB TK irreversible inhibitor, CI-1033 [54], and PKI-166, a dual ErbB1/ErbB2 TK inhibitor [55], have recently reported to have *in vitro* antitumour activity against prostate cancer cells either alone or in combination with radiation therapy and are now entering the clinical testing phase. Oncogenic TKs seem to represent an attractive anti-tumour target. However, combination of TK small-molecule inhibitors and conventional chemotherapy and/or radiation therapy might result in better clinical results.

5.2. Farnesyl-Protein Transferase (FPTase) Inhibitors

Ras family proteins regulate important growth factor receptor-induced signalling pathways contributing to cellular differentiation and proliferation. Key to the functionality of Ras proteins is the post-translational farnesylation of the amino-terminus of Ras by a cytosolic enzyme, the so-called farnesyl-protein transferase (FPTase) [56]. Many solid tumours, such as prostate cancer, have been reported to exhibit Ras dysregulation [57]. Although agents that either down-regulate Ras expression or reverse Ras activation have not been developed yet, several FPTase inhibitors (FPTIs) are currently under clinical evaluation [58].

A number of FPTIs are undergoing phase I/II trials both as monotherapy and in combination with chemotherapeutic agents in HRPC [59]. Combined efficacy was demonstrated with FPTIs SCH66336 and SCH58500 in androgen-insensitive DU145 prostate cancer cells [60]. Tipifarnib (R115777) has been broadly studied in a wide variety of

tumour types with myelosuppression being the major toxicity [61]. Moreover, FPTIs can antagonise the growth enhancing properties of Ras-related small GTP-binding proteins such as Rho and Rac. Notably, the Rho kinase inhibitor Y-27632 has shown to inhibit tumour growth and angiogenesis in androgen-insensitive prostate cancer cells [62]. All these findings support the further clinical evaluation of FPTIs alone or in combination treatments in HRPC.

5.3. Raf Kinase Inhibitors

Raf-1 is a downstream kinase of the activated Ras signalling pathway [63]. The first orally active inhibitor of Raf kinase, BAY 43-9006 (sorafenib), has been assessed in phase I studies in patients with various solid tumours, among them prostate cancer [64-67]. Antisense technology has also been used for the development of Raf kinase selective inhibitors. The Raf kinase inhibitor ISIS 5132 has been evaluated in phase I/II trials in HRPC patients and it was found to lack significant effects on the PSA response and to have a non-tolerable toxicity profile [68].

5.4. Mitogen-Activated Protein Kinase (MAPK) Inhibitors

MAPK pathways have been associated with the progression of prostate cancer to its androgen-independent stage [69]. At least four distinct groups of MAPKs have been identified: (a) ERKs, extracellular signal-related kinases, 1/2; (b) JNK, c-Jun amino-terminal kinases, 1/2/3; (c) p38, α - δ proteins; and (d) ERK5 [8]. It has been suggested that in HRPC the ERK cascade is relatively activated whereas the stress-activated protein kinase (SAPK) pathway is suppressed [70]. Components of MAPK pathways represent candidate targets for therapeutic intervention [71]. Differential regulation of the interaction between MAPKs can be accomplished with selective inhibition, i.e. by phosphatases such as Cdc25A phosphatase [72]. The MAPK inhibitors PD098059 and PD184352 are among novel MAPK small-molecule inhibitors that have been developed and evaluated in preclinical prostate tumour models [73,74]. Recently, it was also demonstrated that angiotensin II induces the phosphorylation of MAPK in androgen-independent prostate cancer cells. Oral administration of angiotensin II receptor blocker was found to inhibit the growth of prostate cancer xenografts in a dose-dependent manner [75]. Therefore, these agents are currently being evaluated as a therapeutic option for HRPC.

5.5. Mammalian Target of Rapamycin (mTOR) Inhibitors

The tumour suppressor gene PTEN (phosphatase and tensin homologue) on chromosome 10q23 is the most frequently mutated gene in prostate cancer [76]. Although its expression remains in normal prostate. Prostatic carcinomas usually exhibit down-regulation of this protein supporting a possible key role for gradual PTEN functional loss in the progression of prostate cancer to its hormone-independent state [77]. PTEN functions as a negative modulator of the phosphoinositide-3 kinase (PI3K)/Akt signal transduction pathway [78]. Akt protein is a tyrosine/threonine kinase with various modulatory effects regarding cellular proliferation,

apoptosis and translational control, while a possible relationship of activated Akt and AR expression has also been postulated [79]. In addition, mammalian Target of Rapamycin (mTOR) is a downstream kinase implicated in Akt-mediated translational control [80]. It has also been reported that mTOR inhibition results in cyclin-dependent kinases (CDKs) inhibition, Rb phosphorylation inhibition and enhancement of cyclin D1 degradation, culminating in cell-cycle arrest in G1 phase [81]. Therefore, blockage of mTOR function should lead to inhibition of PI3K/Akt-mediated proliferation signals and cell arrest [82].

Rapamycin, which is a macrolide fungicide, and its analogs, CCI-779, RAD001 and AP23573 have been found to share mTOR inhibitory properties, thus suppressing its carcinogenic potential [83]. The CCI-779 analogue has shown a significant anti-proliferative effect and favourable toxicology profile in phase I/II trials in various human cancers, including HRPC [84,85]. Major toxicities observed with CCI-779 included anemia, hyperglycemia, hypertriglyceridemia, hypophosphatemia, stomatitis, mucositis and bowel perforation. Several studies evaluating the combination of mTOR inhibitors with chemotherapeutic agents in HRPC are being developed.

5.6. Protein Kinase C (PKC) Inhibitors

PKC is a negative growth regulator of human prostate cancer cells due to its involvement in triggering apoptosis [86]. Antisense oligonucleotides targeting PKC have shown promising results, either as a monotherapy or in combination with chemotherapeutics in various solid tumours, such as lung and prostate cancers [87]. Preclinical

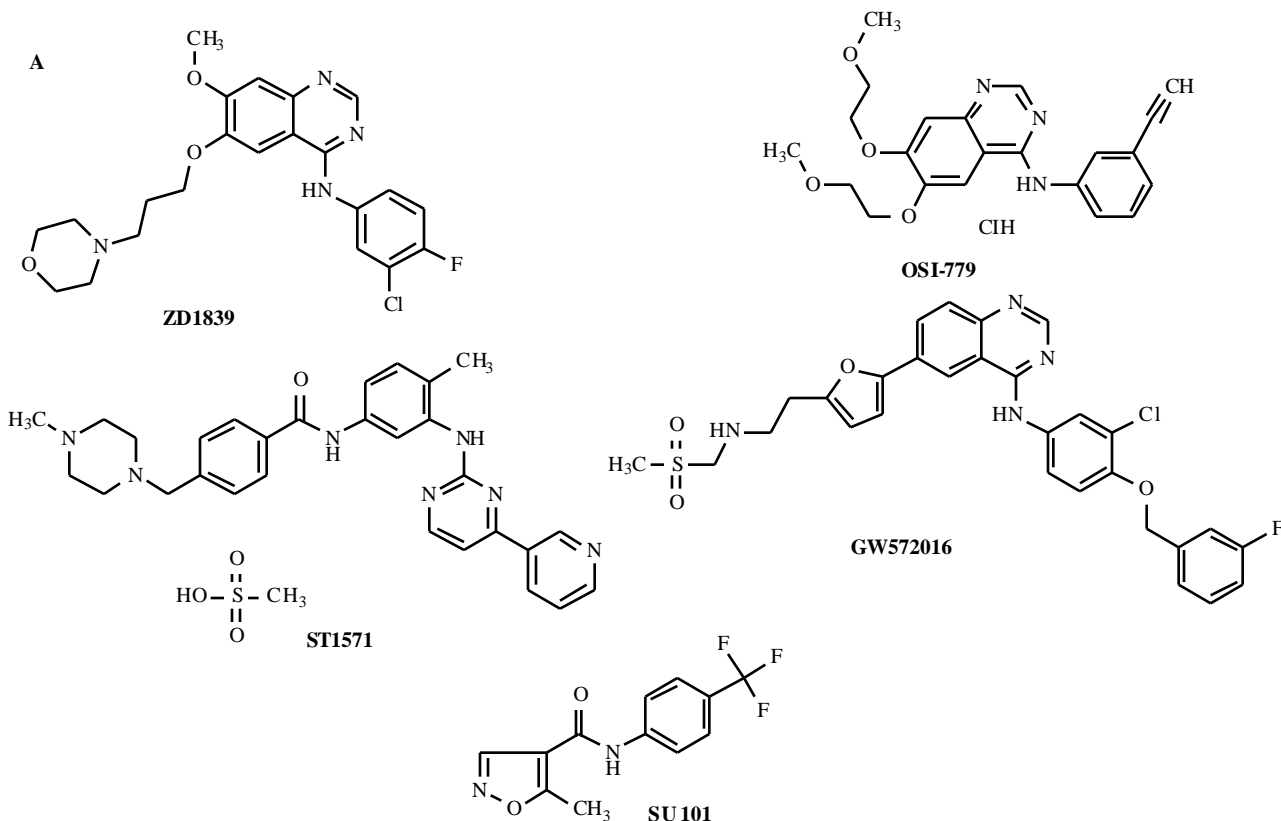
evidence suggested that ISIS 3521 causes specific inhibition of PKC mRNA with accompanying anti-tumour activity. Phase I/II clinical trials have been completed or are ongoing, evaluating this agent in HRPC [68].

5.7. Ansamycins

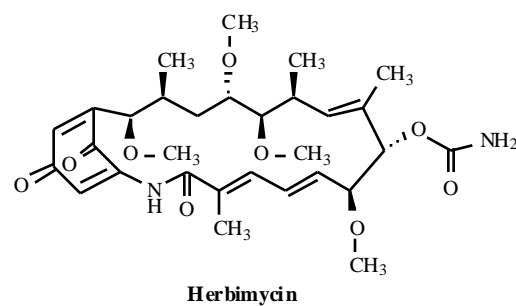
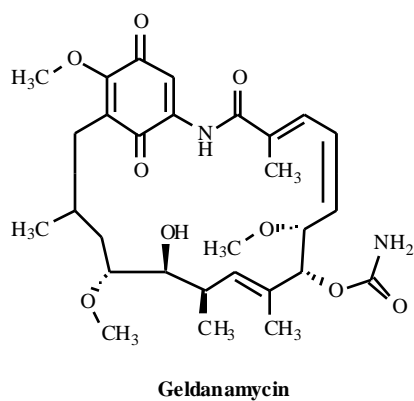
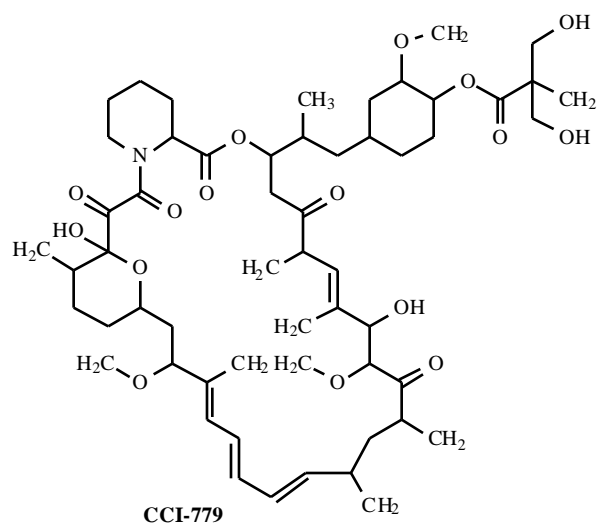
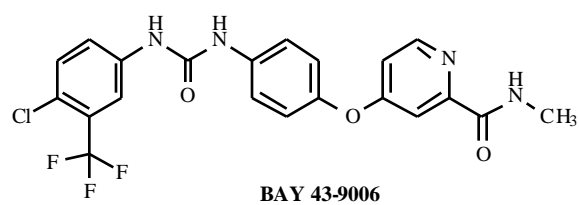
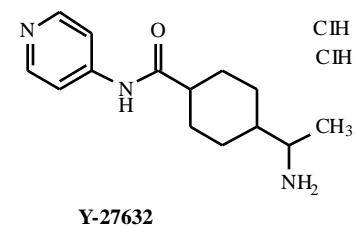
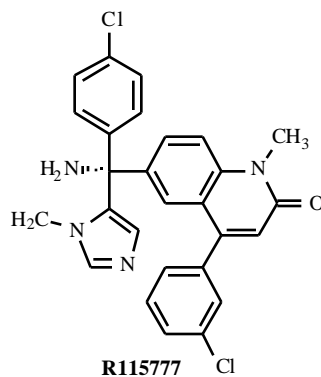
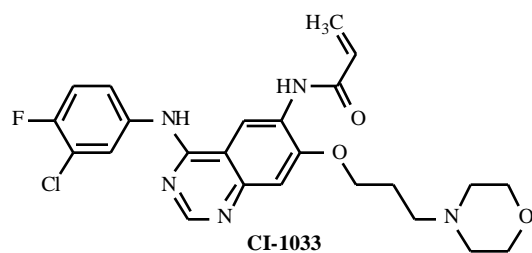
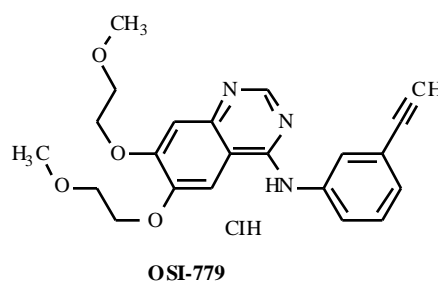
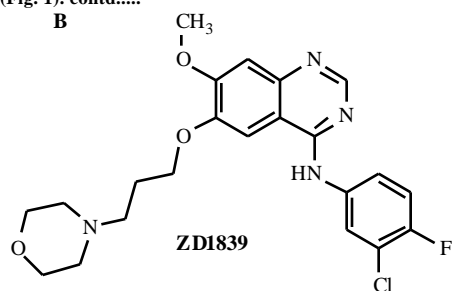
The benzoquinoid ansamycin antibiotics are derived from *Streptomyces hygroscopicus* and include geldanamycin and herbimycin. Ansamycins appear to have an antitumour effect based on their action against chaperone proteins (i.e., Hsp90), which are responsible for maintaining the active conformation of selected protein kinases, steroid receptors, Raf-1 kinase, cyclin D1 and EGFR [88]. One agent can thereby target multiple protein kinases, including the HER-2 axis, as well as the AR, a particularly promising strategy for the treatment of prostate cancer [89]. Phase I clinical trials in patients with HRPC are underway.

6. APOPTOSIS REGULATORS

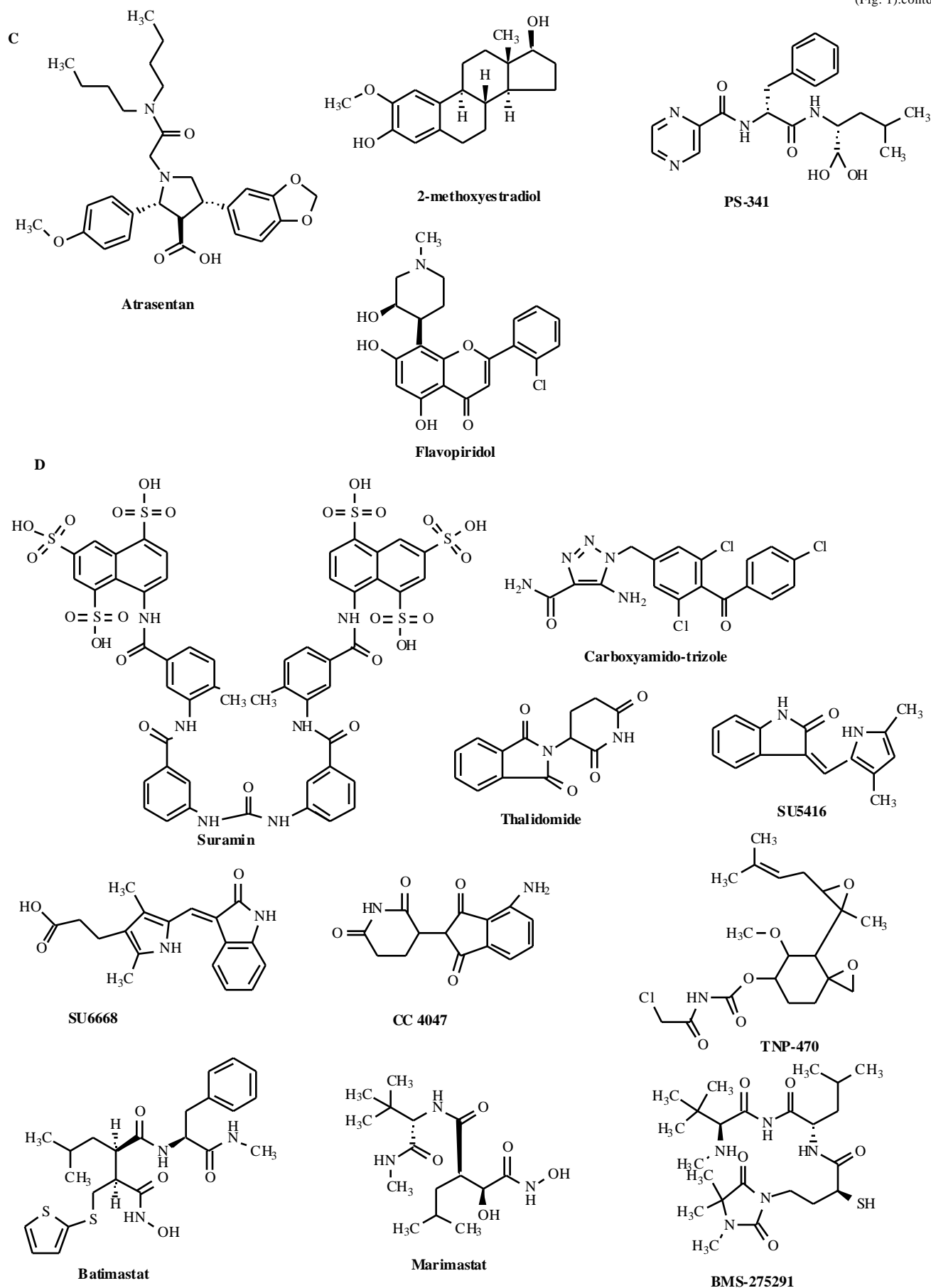
Apoptosis, the programmed cell death mediated by proteases called caspases, is essential for normal tissue functions. Impaired apoptosis is a central step during prostate cancer natural history [8]. Anti-apoptotic bcl-2 family members and pro-apoptotic proteins interplay control the release of cytochrome *c* from the mitochondrial membrane, activation of the caspase cascade and apoptosis execution. Conventional cytotoxic and radiation therapy indirectly induces apoptosis, but outcomes that are more effective should be achieved by direct activation of the apoptotic machinery. Thus, an alternative way to modulate



(Fig. 1). contd.....

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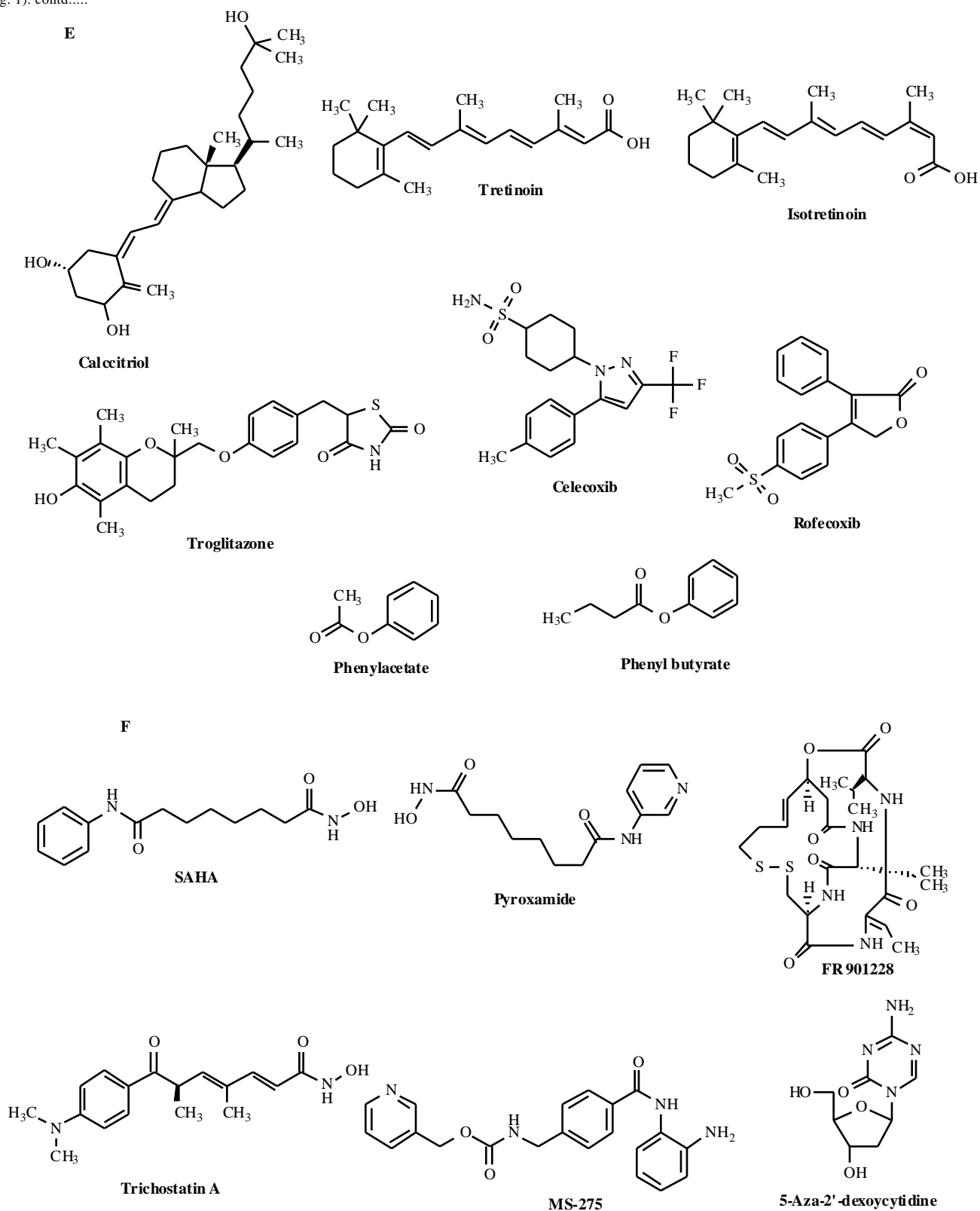


Fig. (1). Chemical formulae of novel biological agents being tested for the treatment of hormone-refractory prostate cancer (HRPC). **A:** Growth factor inhibitors; **B:** Signal transduction inhibitors; **C:** Apoptosis regulators, Cell-cycle regulators and Proteasome inhibitors; **D:** Neo-angiogenesis inhibitors and Anti-metastatic agents; **E:** Differentiation agents; **F:** Epigenetic therapeutics.

apoptosis is by interfering with the expression of essential regulatory molecules or critical apoptosis-associated molecular pathways (Fig. 1C).

6.1. Anti-bcl-2 Agents

The anti-apoptotic protein bcl-2 has been shown to be over-expressed with androgen withdrawal and HRPC

emergence. Thus, targeting bcl-2 represents a rational therapeutic approach [90,91]. Genasense (G3139) is a phosphorothioate antisense oligonucleotide complementary to the bcl-2 mRNA open reading frame that has shown significant activity with regard to inhibition of bcl-2 expression, delaying of androgen independence and improving chemo- and radio-sensitivity of prostate cancer cells [92,93]. The bcl-2 antisense treatment has also been evaluated in combination with chemotherapeutic drugs. A phase I study of Genasense with mitoxantrone in patients with HRPC revealed no major toxicities but only 2/26 responses by the PSA criteria, although patients' selection was not based on bcl-2 expression [94]. Genasense has also been combined with docetaxel in phase I/II studies with encouraging so far PSA and clinical responses [95]. This might be explained by experimental data suggesting that taxanes and vinca alkaloids induce bcl-2 phosphorylation with resulting further abrogation of its anti-apoptotic effect [96]. A randomised trial of docetaxel and Genasense versus docetaxel alone in patients with HRPC has been initiated.

6.2. Endothelin-1 (ET-1) Antagonists

ET-1, the main endothelin isoform, is produced by endothelial and epithelial cells and has been shown to represent an important mediator of growth, survival and angiogenesis signalling pathways in both normal and tumour cells [97]. ET-1 mediates its effects through two G protein-coupled receptors, of which the Endothelin-A (ET_A) receptor is most important in prostate cancer. Up-regulation of ET_A receptor levels and down-regulation of ET_B receptor has been found in HRPC cells, while elevated ET-1 concentrations have been reported in HRPC patients [98]. Moreover, ET-1 expression is directly correlated with the stage and grade of human prostate cancer. A number of studies have also shown that, apart from the vascular and mitogenic properties, ET-1 has effects on bone remodelling and pain, rendering it an attractive target for preventing formation and progression of bone metastasis frequently found in HRPC patients [99].

Selective inhibition of ET_A receptors, but not ET_B receptors, has been found to inhibit ET-1-mediated effects in various tumour models. Given the crucial role that ET-1 appears to play in the progression of prostate cancer, much attention has been focused on the design of ET-1 receptor antagonists [99,100]. At least three ET-1 antagonists are currently under development, with atrasentan being the most extensively studied. Atrasentan is an orally effective and highly selective ET_A receptor antagonist, with favourable tolerability in HRPC patients [101]. Adverse events, such as headache, rhinitis, peripheral edema, blood pressure changes and slight hemodilution, are mainly attributed to the vasodilatory effect of the drug. Randomised phase II clinical trials have demonstrated statistically significant improvements in reducing pain, PSA kinetics, biologic markers of bone changes and development of bone metastasis. There have also been consistent improvements in time to progression but not in median survival [102,103]. Phase III MOO-211 study group (includes metastatic HRPC patients) and MOO-244 study group (includes HRPC patients with rising PSA but without metastasis) trials are currently underway to further evaluate the clinical

effectiveness of atrasentan versus placebo [104]. The favourable toxicity profile of atrasentan might also prove beneficial in future clinical trials combining this agent with other cytotoxic drugs or bone-directed treatments, as well as in evaluating atrasentan in earlier stages of prostate cancer.

6.3. 2-methoxyestradiol (Panzem)

Two-methoxyestradiol is an anti-angiogenic and anti-proliferative human endogenous metabolite of estradiol. Various mechanisms of action have been suggested, including up-regulation of the death receptor 5 (DR5) in prostate cancer cell lines, with subsequent activation of caspases 3, 8 and 9 [105,106]. Phase I/II trials in HRPC patients are ongoing, evaluating different dosing schedules of 2-methoxyestradiol alone or in combination with taxanes and mitoxantrone.

6.4. Survivin Inhibitors

Caspases are the central initiators of apoptosis. The apoptosis proteins inhibitors (APIs) are a family of recently described caspase inhibitors [107]. Recent studies have revealed a novel, highly conserved, apoptosis inhibitor, survivin, which is expressed in the majority of prostatic carcinomas, but not in normal prostate [108]. Although its exact role remains to be elucidated, survivin might represent a potential target for apoptosis-based therapy in HRPC.

6.5. Tumour Necrosis Factor (TNF)-Targeted Therapeutics

The TNF family of ligands has multiple biologic functions. Many of them are directly involved in the regulation of cell homeostasis by inducing apoptosis or enhancing cell survival and proliferation. To date, members of this family interact with 26 receptors and include 18 different ligands [109]. Novel cancer treatment strategies are evolving based on the understanding of TNF family members' actions. Selected TNF-targeted therapeutic agents are under development and early clinical evaluation. Among them are agents against CD30, CD40, RANKb (receptor activator of nuclear factor-Kb) and TRAIL (TNF-related apoptosis-inducing ligand) [110]. Results of ongoing clinical studies with these agents will clarify their future potential in solid tumours therapeutics.

7. CELL-CYCLE REGULATORS

Dysregulation of cell-cycle control is a hallmark of human cancers, causing lack of differentiation and aberrant growth. Proper regulation of this process involves external signals ultimately leading to the activation of CDKs, which are a family of serine/threonine kinases playing a pivotal role in controlling cell cycle. The regulatory subunits of the CDKs, known as cyclins along with their cyclin-dependent kinase inhibitors (CDKIs), contribute to the transition between the different phases of the cell cycle, through crucial checkpoints. The thorough understanding of these molecular mechanisms has identified novel targets within cancer cell-cycle regulation processes as a basis of anticancer treatments [111].

Flavopiridol is a semisynthetic flavone, which inhibits CDK1, 2, 4 and 7, causing arrest at the transition of G2/M and G1/S phases (Fig. 1C). Flavopiridol has been found to inhibit growth of various tumour cells *in vitro*, including prostate cancer cells, while this inhibitory potential varied from cytostatic to cytotoxic depending on its concentration, type of tumour cells and duration of exposure [112]. It has been evaluated in phase I clinical trials either as a monotherapy or in combination with cytotoxic agents against various solid tumours [113]. Nevertheless, a recent phase II study with HRPC patients, demonstrated that flavopiridol treatment was associated with significant side effects and disappointing single-agent activity [114]. However, the synergistic effect of the combination of flavopiridol with other cytotoxic drugs seems to be a more promising treatment proposition.

8. PROTEASOME INHIBITORS

The ubiquitin-proteasome system plays an important role in cell homeostasis. The proteasome is the primary component of the protein degradation pathway of the cell and its action is crucial in the activation or repression of many cellular processes, including cell-cycle regulation and apoptosis [115]. The proteasome degrades proteins that have been marked for elimination and conjugated to multiple units of the polypeptide ubiquitin. Several proteasome inhibitors have been developed, such as lactacystin, peptide aldehydes, and dipeptide boronate derivatives [116]. Bortezomib (PS-341) consists the first proteasome inhibitor that entered human clinical trials [117] (Fig. 1C). Preclinical evidence revealed the activity of PS-341 in a variety of tumours as single-agent or in combination with other cytotoxic drugs. Interestingly, PS-341 was well-documented to cause infrequent induction of resistance to this agent [116,118]. Following the successful application of PS-341 in the treatment of refractory multiple myeloma [119], many phase I/II clinic trials have been completed or are in progress in patients with solid tumours, such as HRPC [116]. Dose-dependent clinical activity of this agent was found in a phase I clinical study in HRPC [120], and phase II trials are underway to evaluate PS-341 as monotherapy or in combination with other chemotherapeutic drugs, such as docetaxel, with encouraging results.

9. NEO-ANGIOGENESIS INHIBITORS

Neo-angiogenic activity is required by all tissues for normal maintenance and function. However, it is recognised that cancer cells also require neo-angiogenesis for their growth and metastatic spread [121]. Neo-angiogenesis is induced by hypoxia and controlled by a large number of tumour-related pro-angiogenic and anti-angiogenic factors. Stimulating factors that have been identified so far are vascular endothelial growth factor (VEGF), acidic and basic fibroblast growth factors (FGF α and β), tumour growth factor β (TGF- β) and others [122]. Tumour angiogenesis is a complex process, which requires coordinated interactions between numerous proteins, signalling pathways and cell types. Potential molecular targets vary according to steps involved in the angiogenesis process. Prostate cancer may be an attractive candidate for anti-angiogenic therapy. Based on

the great success in preclinical models, several agents are currently under clinical development, such as suramin, carboxyamido-triazole (CAT), thalidomide and its novel analogues, SU5416, SU6668, bevacizumab, TNP-470 and endostatin/angiostatin [122,123] (Fig. 1D). The development of these agents has faced significant problems, such as understanding the exact mechanism of their action and defining accurate surrogate endpoints, while maintaining normal tissue angiogenesis. The anti-angiogenic agents are generally divided based upon those that directly target specific molecules involved in neo-angiogenesis and others that indirectly inhibit endothelial cell function or response. It has been suggested that combining these agents with current treatment options would enable better clinical results, although the initial clinical experience has generated great skepticism.

9.1. Suramin

Suramin is an anti-fungal agent with recently documented *in vitro* anti-neoplastic activity. Many mechanisms of action have been reported, such as modulation of the interaction of growth factors with their receptors, inhibition of DNA polymerase, topoisomerase II, PKC and angiogenesis as well [124]. Phase I/II clinical trials in patients with HRPC using suramin as single-agent or in combination with hydrocortisone have revealed significant objective and PSA response rates, but a high incidence of side effects such as neurotoxicity, rash and grade III fatigue [125,126]. A recent randomised study in patients with HRPC failed to show dose-response relationship for suramin in terms of survival and progression-free survival, while dose increase was accompanied by enhanced toxicity [127]. The role of suramin in the therapeutic armamentarium for HRPC is still under evaluation.

9.2. Thalidomide and Novel Analogues

Thalidomide, infamous for its teratogenic potential is now emerging as a therapeutic option as an anti-neoplastic agent, due to its documented immunomodulatory and anti-angiogenic effects. Clinical studies have shown encouraging results of thalidomide in patients suffering from refractory myeloma, whilst further trials are in progress to evaluate its efficacy in various other malignancies, among them HRPC [128]. Phase I/II trials of thalidomide in patients with HRPC have been associated with considerable decreases regarding PSA levels, with the most prevalent toxicities being constipation, fatigue and neurocortical/neurosensory defects [129,130]. Preclinical data indicate that synergistic activity could occur when combining anti-angiogenic and cytotoxic agents [128]. Consistent with these findings, a recent phase II randomised study of thalidomide plus docetaxel versus docetaxel monotherapy suggests significant decreases in PSA responses, 51% versus 35%, with a tolerable toxicity profile [131], although thrombotic events were seen more frequently in the combination regimen [132]. Co-administration of thalidomide with mitoxantrone/prednisone [133], oral dexamethasone [134] and paclitaxel/doxorubicin [135] are among treatment schedules that have been tested in phase II trials with encouraging, so far, results. Nevertheless, there is also *in vitro* documentation of thalidomide enhanced

secretion of PSA from prostate cancer cells, raising great concerns of the validity of PSA as a surrogate marker in clinical trials evaluating thalidomide in the treatment of HRPC [136].

A number of novel anticancer thalidomide analogues have been developed and are gradually entering clinical evaluation for the treatment of HRPC [137]. Alpha-(3-aminophthalimido) glutarimide, CC5013 (revimid), has multiple actions mediated in part by the ability to down regulate over-production of tumour necrosis factor α (TNF- α). Phase I/II studies to elucidate its clinical efficacy are ongoing [122]. Another analogue of thalidomide, CC4047 (actimid), is also currently undergoing evaluation for the treatment of prostate cancer [122,137].

9.3. Carboxyamido-Triazole (CAT)

Carboxyamido-Triazole (CAT) is an inhibitor of signal transduction *via* non-voltage-gated calcium channels. It has been proposed that CAT exerts its anti-angiogenic, anti-invasive and anti-metastatic effects *via* the down-regulation of key cellular regulatory proteins, including the zinc-dependent MMPs [138]. However, recent phase I/II trials have reported minimal clinical activity along with intolerable toxicity of CAT in patients with HRPC [139,140].

9.4. SU5416 and SU6668

It has been documented that there are two subtypes of VEGF receptors (VEGFRs) with discrete roles in prostate carcinogenesis. Moreover, it has been reported that VEGFR1 expression is associated with early and more differentiated carcinomas, whilst VEGFR2 expression is correlated with advanced and more poorly differentiated prostatic carcinomas [141]. An anti-angiogenic compound, SU5416, acts as a selective inhibitor of Flk-1, a VEGF receptor (VEGFR2) on endothelial cells [142]. In preclinical studies, treatment with SU5416 resulted in decreased tumour vascularisation and growth [143] and is currently being evaluated in early clinical trials for the treatment of patients with HRPC.

A broad spectrum anti-angiogenic signalling compound, SU6668, may act by inhibiting VEGFR Flk-1/KDR, PDGFR, and FGF receptor auto-phosphorylation [143]. At this time there are no available data for this compound in the treatment of patients with HRPC, although phase I/II clinical trials are underway.

9.5. Anti-VEGF MA

MAs against VEGF inhibit tumour neo-angiogenesis and have been evaluated in combination with chemotherapy in phase I/II clinical trials in patients with colorectal and renal cancers, with very promising results including tolerable toxicity profiles [144,145].

Bevacizumab is a recombinant humanised MA (rhuMA) against VEGF, inhibiting its interaction with the cognate receptor. Unfortunately, primary clinical results in patients with HRPC revealed low efficacy [146]. However, clinical trials using this agent in combination with chemotherapeutic drugs and other new treatment strategies are underway [147,148].

9.6. TNP-470

The TNP-470, o-(chloroacetylcarbamoyl)fumagillid, is a synthetic analogue of fumagillin, an antibiotic produced by the fungus *Aspergillus fumigatus fresenius*. It has been shown to cause dose-dependent inhibition of angiogenesis and tumour growth in many tumours, including prostate cancer, both as single-agent and in combination with other cytotoxic drugs [149]. The mechanism of action is not fully understood, while recent experimental data suggest modulation of cell cycle through various mechanisms [122]. Preclinical studies with TNP-470 suggested no major side effects and a relatively tolerable toxicity profile [123]. A phase I dose-escalation trial of TNP-470 in patients with HRPC reported reversible neuro-psychiatric dose-limiting side effects, and transient PSA increases with a subsequent decline [150]. Phase II clinical trials have been initiated to assess its therapeutic potential.

9.7. Endostatin, Angiostatin

Endostatin, a 20-kDa carboxy-terminal fragment of collagen XVII (COL17A1), is currently in clinical development as a novel anti-angiogenic agent. Another inhibitor of endothelial cell proliferation that has entered clinical testing is angiostatin [151]. Mechanisms of action of both agents have not yet established, but preclinical testing showed regression of established prostate xenografts. Phase I clinical trials are underway in patients with HRPC [152], while their combinatorial use with cytotoxic drugs has been evaluated in animal models [153].

10. ANTI-METASTATIC AGENTS

Cancer cell metastatic potential requires tissue boundaries distraction, circulation, interaction with vascular endothelium and, finally, proliferation and formation of distant lesions. This multi-step process involves cell-to-cell and cell-to-extracellular matrix interplay [154]. The role of zinc-dependent MMPs and adhesion molecules in cancer cell invasion and migration has been studied and has led to the development of suggested pharmacological interventions (Fig. 1D).

10.1. MMPs Inhibitors

MMPs comprise a large family of zinc-dependent endopeptidases that are normally synthesised as inactive progenitors and are activated by proteinases for normal tissue maintenance and repair in order to enable physiological function. Their activity is normally regulated by non-specific protease inhibitors and a family of tissue-specific inhibitors of MMPs (TSIMMPs) [155]. MMPs up-regulators along with down-regulators are normally present in all normal connective tissues and almost every type of human cancer, including prostate cancer. Their up-regulation may be associated with poor repair in association with a poor outcome [156]. The strategy of using super-induction of endogenous TSIMMPs in the treatment of HRPC although appearing to be somewhat rational is not applicable, because they are large glycoproteins requiring a significant portion of

their molecule for biological activity [157]. To overcome these limitations, small synthetic TSIMMPs such as batimastat (BB94) [158], marimastat (BB2516) [159,160], prinomastat (AG3340) [161], BB3644 [162] and BMS-275291 [163] have been developed and are under evaluation in cancer clinical trials, including patients with HRPC. Despite the promising preclinical and early clinical results, the interim analysis of a recent phase III trial of prinomastat in combination with mitoxantrone (M)/prednisone (P) versus M/P, failed to show any significant differences among the two treatment regimens in PSA response rate, progression-free survival as well as 1-year and overall survival [164]. However, more definite conclusion about the value of this class of agents awaits results of further clinical trials and a better understanding of the need for normal MMP function.

10.2. Anti-Adhesion Agents

On the road to metastases development, cell-adhesion molecules, such as integrins, cadherins and immunoglobulin-like cell-adhesion molecule (Ig-CAM), participate in cell-cell and cell-matrix interactions [165]. Various studies have documented expression differences of these molecules in various histological entities during carcinogenesis. Moreover, it is now accepted that these molecules directly modulate intracellular signalling pathways, thereby interfering with other physiological and pathological processes, e.g. apoptosis [166]. Based on the increasing knowledge of the crucial role of adhesion molecules, researchers are now focusing on the development of specific inhibitors that might also prove effective in the treatment of HRPC. However, these adhesion molecules may have an important role in overcoming neoplastic diseases as a normal component of the host response.

11. DIFFERENTIATION AGENTS

A great number of agents are being clinically tested for the purpose of inhibiting prostate cancer cell proliferation and induction of differentiation and/or apoptosis (Fig. 1E).

11.1. Calcitriol

A metabolite of the vitamin D hormone, calcitriol, induces differentiation and exhibits anti-proliferative activity in both AR-positive and AR-negative human prostate cancer cell lines, mainly by bonding to vitamin D receptors (VDRs) [167]. These receptors are a member of the nuclear receptor superfamily. Calcitriol represents a specific ligand, which causes activation and heterodimerisation of VDR with retinoid X receptor (RXR), resulting in transcriptional regulation of multiple target genes [168]. VDRs have been identified in a variety of prostate cancer cell lines and preclinical data support the activity of vitamin D in prostate cancer [169]. Dose-escalation studies of calcitriol in patients with prostate cancer and increasing PSA values produced significant decreases in the rate of PSA increase [170]. Although the activity of vitamin D seems to be dose-dependent, its hypercalcemic effect limits dose-escalation of calcitriol [171]. Calcitriol can also significantly increase cytotoxic drug-induced antitumour effects. As a result, phase I and II trials in combination with carboplatin, taxanes, or dexamethasone have been initiated in patients with both

androgen-dependent and androgen-independent prostate cancer [172-175]. Patients were evaluated for toxicity, maximum tolerated dose (MTD), schedule efficacy and PSA response. Results from these studies suggest that the use of high-dose calcitriol is feasible, on an intermittent schedule. The MTD is still being delineated and taxanes or dexamethasone seems to ameliorate toxicity. Other novel selective calcitriol analogues with less hypercalcemic effects are being pursued in clinical tests for patients with HRPC [171].

11.2. Retinoids

Retinoids are derivatives of vitamin A that have pivotal roles in many biological processes, including differentiation, proliferation and apoptosis. They exert their pleiotropic effects through two classes of nuclear receptors, the retinoic acid receptors (RARs) and RXRs. Like other members of the nuclear receptor superfamily, retinoid receptors act as ligand-activated, DNA-bonding transcription factors through bonding as RAR/RXR heterodimers to *cis*-acting retinoic acid (RA)-response elements present in relevant genes [176]. The expression of retinoid receptors in normal, premalignant and malignant prostate tissues has been studied and examined with regard to down-regulation of some isotypes, in the context of the so-called "field cancerisation" concept, which hypothesises that the entire organ has an increased risk for the development of premalignant lesions because of multiple genetic abnormalities in the whole tissue region [177-179].

Pharmacologically, retinoids have been shown to suppress carcinogenesis in a variety of tissue types, e.g. skin, lung and oral cavity in many animal models, while clinically they are able to reverse premalignant lesions in the respiratory epithelium [177]. Based on these findings, phase I and II clinical trials evaluated the role of currently available retinoids in the treatment of prostate cancer, hormone-dependent and HRPC, either as monotherapy [180,181] or in combination regimens [182,183]. Up to now, there is no evidence that retinoids are effective in prostate cancer chemoprevention or treatment strategies. Extensive research efforts are still ongoing to shed light on the molecular and cellular mechanisms of retinoids action and to design new more potent and selective synthetic retinoids.

11.3. Peroxisome Proliferator-Activated Receptors (PPAR)- γ Agonists

The PPAR family of nuclear receptors includes various subtypes (α , β and γ) encoded by separate genes and displaying different tissue distribution and distinct ligand selectivity [184]. PPAR γ is a crucial transcription factor in adipocyte differentiation and glucose metabolism, whilst its ligands have also been demonstrated to induce differentiation in human breast, lung and colon carcinoma cell lines [185]. The ability of PPAR γ to modulate gene activity requires the presence of its heterodimerisation partner RXR [186].

PPAR γ is also highly expressed in prostate cancer cell lines compared with normal prostate [187]. Most experimental observations concerning the effect of PPAR γ on tumour growth have utilised the anti-diabetic agent troglitazone, a synthetic ligand of the receptor. When

exposed to troglitazone, prostatic carcinoma cells exhibit growth inhibition and induction of differentiation and/or apoptosis [188]. A phase II trial was conducted in patients with metastatic prostate cancer and demonstrated PSA decrease in 30% of them [189]. Ongoing large clinical trials will illuminate the role of troglitazone and other synthetic PPAR γ ligands in the treatment of HRPC.

11.4. Cyclo-oxygenase (COX)-2 Inhibitors

Cyclo-oxygenase (COX)-2 expression has been found to be consistently up-regulated in high grade intraepithelial prostatic neoplasia and carcinoma compared to benign epithelium, as measured by immunohistochemistry, COX-2 mRNA and Western-blot analysis [190], albeit opposite results have also been reported [191]. Treatment of human cancer cell lines with selective COX-2 inhibitors (COXIs) led to down-regulation of bcl-2 protein and increased apoptosis both *in vitro* and *in vivo* [192]. The *in vivo* results also showed that COX-2 inhibition decreased tumour microvessel density and angiogenesis and prevented hypoxic up-regulation of VEGF [193], while a recent pilot trial revealed a clinical benefit for COXIs on serum PSA levels in patients with biochemical progression after definitive radiation therapy or radical prostatectomy [194]. All these results indicate that COXIs may serve as effective preventive and therapeutic agents in prostatic carcinogenesis, although their role in HRPC is still uncertain. It is also anticipated that in the future a selective COXI may be combined with other agents, such as antioxidants, receptor TK modulators, anti-angiogenic modulators, anti-proliferative/differentiating agents and NF- κ B modulators in combination chemoprevention [195].

11.5. Phenylacetate and Analogues

Phenylacetate and analogues represent a new class of pleiotropic growth regulators that alter tumour cell biology by affecting gene expression at both the transcriptional and post-transcriptional levels [196]. Phenylacetate and phenylbutyrate have been evaluated clinically in phase I studies in patients with prostate cancer suggesting that therapeutic levels can be achieved with no significant toxicities, while preliminary promising results have been reported [197,198]. Ongoing clinical trials are intended to evaluate their combination with other agents for potentially synergistic effects. However, it should be noted that tumour markers, such as PSA, might not be the most accurate measurement of progressive disease in patients treated with these and similar agents, as a rise in tumour markers may signal cell differentiation rather than disease progression.

11.6. Zinc and Copper

In the normal human prostate, epithelial cells contain more zinc and copper levels in comparison with the cancerous prostate cells [199]. In experimental studies zinc deficiency-induced prostate neoplasia has been documented [200]. *In vitro* physiologic concentrations of zinc inhibit growth of both androgen-sensitive and androgen-independent prostate cancer cell lines, possibly through the induction of cell-cycle arrest and programmed cell death [200].

Copper- and zinc-containing superoxide dismutase is an antioxidant enzyme that is down-regulated in prostate cancer [201]. Moreover, reports are available demonstrating that copper complexes cause differentiation of neoplastic cells and have anti-carcinogenic activities *in vivo*, as a result of the facilitation of normal repair processes, normal antioxidant activities and DNA repair mechanisms [202]. Recent studies revealed that organic copper complexes can potently and selectively inhibit the chymotrypsin-like activity of the proteasome *in vitro* and *in vivo* [203]. Certain types of organic ligands could bind to tumour cellular copper, forming potent proteasome inhibitors and apoptosis inducers at copper concentrations found in the prostate.

12. EPIGENETIC THERAPEUTICS

Epigenetic events, such as histone acetylation/deacetylation and aberrant DNA methylation represent crucial steps in cancer development, causing alterations in gene expression without changes in the DNA coding sequence that are heritable through cell division [204]. Such changes occur throughout all stages of prostate carcinogenesis and are increasingly encountered as major mechanisms involved in silencing tumour suppressor genes [205]. Epigenetic changes can be reversed by the use of small molecules. Thus, such changes are promising targets for future cancer chemopreventive drug development (Fig. 1F).

12.1. Histone Deacetylase (HDAC) Inhibitors

Histones are core protein components of nucleosomes and their acetylation status regulates, in part, gene expression. Histone acetylation is determined by two enzymatic activities, histone acetyltransferases (HATs) and histone deacetylases (HDACs). Deacetylated histones are generally associated with silencing gene expression [206]. Several compound classes have been identified, including short-chain fatty acids such as 4-phenylbutyrate and valporic acid, hydroxamic acids such as suberoylanilide hydroxamic acid (SAHA), pyroxamide, trichostatin A and oxamflatin, cyclic tetrapeptides such as trapoxin, apicidin and depsipeptide (also known as FK-228 or FR 901228) and benzamides such as MS-275 [207]. HDACs inhibitors have been shown to induce expression of genes linked to growth inhibition and cellular differentiation. Several phase I trials with these agents are ongoing in cancer patients, including patients with HRPC [208-210].

12.2. Hypomethylating Agents

Tumour suppressor genes are an important class of genes that are required to regulate and control cell growth. These genes can be switched off by being deleted or mutated, thus triggering a normal cell to become a cancer cell. An alternative mechanism to switch off tumour suppressor genes is accomplished by a chemical modification known as DNA methylation. DNA methylation is a normal cellular process whereby certain bases, cytosines, in the DNA become methylated to give 5-methylcytosine by the enzyme DNA methyltransferase [211]. However, in cancer cells the methylation process is deregulated and many genes,

including tumour suppressor genes, become abnormally methylated at cytosine bases. Moreover, it seems that aberrant methylation causes recruitment of HDACs resulting in a more potent transcriptional inhibition of target genes [212]. Many studies have demonstrated epigenetic silencing of crucial genes, e.g., AR, PTEN, RAR β , during prostate carcinogenesis [213]. The hypomethylating agent 5-aza-2'-deoxycytidine has been tested in several solid tumours including prostate cancer. A phase II clinical trial has been completed in HRPC, demonstrating significant myelotoxicity [214]. Novel hypomethylating agents are in various stages of experimental and clinical development.

13. TELOMERASE INACTIVATORS

Telomerase is a specialised reverse transcriptase found at the ends of linear chromosomes, contributing in cellular proliferative immortality. It is expressed in essentially all cancer cells but not in most normal human cells. The crucial role of telomerase activation in cancer progression has been established in several studies [215]. It has been reported that 66% to 92% of prostatic carcinomas display telomerase activity, while it has also been suggested to be an early marker of progression to HRPC [216]. Various tested telomerase inactivators demonstrated the validity of telomerase inhibition in HRPC therapy, as this approach resulted in apoptosis, differentiation, or senescence of cancer cells, although months of treatment were required for the detection of these beneficial effects [217]. A new optimised antisense oligonucleotide telomerase inhibitor, ISIS 24691, induced anti-proliferative effects in prostate cancer cell lines after relatively shorter treatment periods [209]. However, there were no synergistic effects with standard chemotherapeutic drugs. Extensive research efforts are underway for the development and evaluation of effective telomerase inactivators.

CONCLUSIONS AND FUTURE PERSPECTIVES

Prostate cancer is commonly treated with drugs that lower serum testosterone levels often in combination with competitive AR antagonists. Although these therapies are initially effective in slowing tumour growth, these cells eventually become resistant, resulting in HRPC. Late-stage prostate cancer, unresponsive to hormonal manipulations, sustains AR signalling through a diverse array of molecular changes. Identifying these crucial events has been difficult because most of the prostate tumours available for study only represent the disease at the time of diagnosis and it is not considered standard practice to take biopsies of recurrent disease.

Up to now, there is no curative treatment for HRPC and the principal aim of the existing treatment options is supportive care. Thus, the development of new therapeutic strategies with significant impact regarding progression-free survival and overall survival is of great importance. Molecular knowledge of prostate cancer natural history is rapidly expanding, revealing new potential therapeutic targets. Together with the emerging technologies of "omics" (genomics, proteomics, pharmacogenomics and metallomics), the potential of appropriate design of selective, narrow spectrum-targeted therapies appears realistic. A large

volume of new therapeutic agents is now under development and are gradually entering clinical testing in all phases of the prostate carcinogenesis process.

This array of targets and agents comes to a field already crowded with unanswered clinical questions, such as the optimal application of hormonal therapy and the definite role of chemotherapy in prostate cancer therapeutic armamentaria. Given the fact that carcinogenesis represents a multi-step process, of paramount importance is the characterisation of crucial candidate molecular target differences during various stages of prostate cancer progression. Better elucidation of the timing of these changes would establish whether novel biological agents are effective in either preventing or treating prostate cancer, and the numerous clinical trials that are underway should provide additional information about this. In most preclinical studies, these new agents reduced the growth rate of established tumours rather than causing tumour regression. This suggests that their use will be most beneficial when administered in combination with standard therapy. This concept is supported by several experimental studies in which the efficacy of chemotherapy and radiotherapy was enhanced by co-treatment with some of these novel agents.

Surrogate endpoints of clinical trials, evaluating the role of novel biological agents in the treatment of prostate cancer, are another hot issue. For example, many doubts have aroused concern regarding the applicability of toxicity as a phase I endpoint or PSA as a phase II endpoint. Many scientists pose that premature optimism or disappointment suggested for many of these new agents is based on suboptimal clinical trials.

The demand for new treatment options for prostate cancer is pressing. However, it seems that the key to the successful introduction of these biological agents in prostate cancer therapeutics might be the accurate stratification of patients based on molecular, rather than clinical, characteristics, in order to define the subset of patients who will most benefit from each agent, as well as the optimal sequencing of the developing and standard therapeutics options.

LIST OF ABBREVIATIONS

API	=	apoptosis proteins inhibitor
APSMMA	=	anti-prostate-specific membrane MA
AR	=	androgen receptor
CAT	=	carboxyamido-triazole
CDK	=	cyclin-dependent kinase
CDKI	=	cyclin-dependent kinase inhibitor
COX	=	cyclo-oxygenase
COXI	=	cyclo-oxygenase inhibitor
DR5	=	death receptor 5
EGFR	=	epidermal growth factor receptor
ET-1	=	endothelin-1
ET _A	=	endothelin A
ERK	=	extracellular signal-regulated kinase

FGF	= fibroblast growth factor
FPTase	= farnesyl-protein transferase
FPTI	= farnesyl-protein transferase inhibitor
HAT	= histone acetyltransferase
HDAC	= histone deacetylase
HRPC	= hormone-refractory prostate cancer
IGF	= insulin-like growth factor
IGFR	= insulin-like growth factor receptor
Ig-CAM	= immunoglobulin-like cell-adhesion molecule
IAP	= inhibitor of apoptosis proteins
JNK	= c-Jun amino-terminal kinase
M	= mitoxantrone
MA	= monoclonal antibody
MAPK	= mitogen-activated protein kinase
MMP	= matrix metalloproteinase
mRNA	= messenger RNA
MTD	= maximum tolerated dose
mTOR	= mammalian target of rapamycin
P	= prednisone
PDGF	= platelet-derived growth factor
PKC	= protein kinase C
PPAR	= peroxisome proliferator-activated receptor
PSA	= prostate-specific antigen
PSMA	= prostate-specific membrane antibody
PTEN	= phosphatase and tensin homologue deleted on chromosome 10
RANKb	= receptor activator of nuclear factor-Kb
RAR	= retinoic acid receptor
rhuMA	= recombinant humanised MA
RXR	= retinoid X receptor
SAHA	= suberoylanilide hydroxamic acid
SAPK	= stress-activated protein kinase
TK	= tyrosine kinase
TNF	= tumour necrosis factor
TSIMMP	= tissue-specific inhibitors of MMP
VDR	= vitamin D receptor
VEGF	= vascular endothelial growth factor
VEGFR	= vascular endothelial growth factor receptor

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