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 tohormone-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.

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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.

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-indendent 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

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

Agent	*Target*	*Primary conclusion*	*Phase*	*Ref*
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.....

Agent	*Target*	*Primary conclusion*	*Phase*	*Ref*
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-*trans*-retinoic acid (ATRA)		ATRA is not active against HRPC.	II	180
All-*trans*-retinoic acid (ATRA)		ATRA has minimal activity against HPRC.	II	181
13-*cis*-retinoic acid (Isotretinoin) + Androgen Blockade		Isotretinoin does not impair PSA response or cause significant toxicity.	II	182
13-*cis*-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	PPARγ [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] PPARγ, Peroxisome Proliferator-Activated Receptor γ.

for the subgroup of HRPC patients exhibiting HER-2 over- regimens. Finally, several anti-prostate-specific membrane

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 *vise 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] 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

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