



Translational Research in Prostate Cancer

Paradigms in the therapy of prostate cancer have evolved significantly over the last twenty years. While previously prostate cancer was more commonly diagnosed at an advanced stage, introduction of improved diagnostic techniques and use of screening have contributed to a reduction in morbidity and mortality due to our ability to identify early stage malignancy when intervention has a high likelihood of being curative. Nevertheless, each year, thousands of men either present with advanced stage or progress after initial therapy, and over 180,000 men died of prostate cancer in 2006.¹ A new approach in cancer therapy is development of small molecule inhibitors and neutralizing antibodies designed to block expression or function of specific proteins thought to contribute to processes of carcinogenesis, including vascular endothelial growth factor, epidermal growth factor and members of the endothelin family of proteins. It is hypothesized that this approach will lead to greater therapeutic success and a reduction in adverse effects caused by cytotoxic chemotherapeutic regimens commonly used in treatment of solid tumors.

Introduction

The hallmark of initial therapy for extensive or progressive prostate cancer has been the use of androgen deprivation.² Removal of the androgen growth stimulus; achieved either with bilateral orchiectomy or gonadotropin releasing hormone analogue injection with or without concomitant oral anti-androgen therapy, has a long established response rate of greater than 80%. These therapies are not given with curative intent and median response durations range up to approximately 18 months.

The use of cytotoxic chemotherapies in prostate cancers which progress following androgen deprivation has only recently been demonstrated to be beneficial.^{3,4} Interpretation of analyses obtained from the previous

studies of cytotoxic chemotherapy in this group of patients have been a challenge due to the limitations with the primary endpoint, which was measurable visceral disease as an indicator of response. Abnormal bone scans could not be followed with certainty to indicate response. Subsequent to these studies, in 2006, the use of PSA as a surrogate marker for response was accepted.⁵ This has significantly contributed to clinical trial design and conduct in this cohort.

Mitoxantrone and docetaxel have been shown to have significant response rates in androgen independent prostate cancer (AIPC) and, docetaxel has been reported to prolong survival in this cohort of patients.⁶ Clinical studies have demonstrated the benefit of docetaxel based therapy in first and second line interventions for AIPC. Studies in the second line setting demonstrated a PSA response in 50–85% of men and a reduction in pain in approximately 60% of men previously treated with mitoxantrone.^{7,8} Randomized clinical trials in the first line setting have established docetaxel as the standard for frontline therapy in men with AIPC. Tannock, et al, reported a randomized trial comparing docetaxel with prednisolone to mitoxantrone with prednisone in 1006 men with AIPC.⁹ Overall survival and pain responses were significantly improved in men receiving docetaxel in an every 3-week schedule. The Southwest Oncology Group has published results of a trial comparing docetaxel with estramustine to mitoxantrone plus prednisolone.⁶ The docetaxel and estramustine group had a significantly longer survival. Toxicities, however, were greater in the docetaxel and estramustine group. Cytotoxic agents have improved outcome for subgroups of men with AIPC. But clearly, there is a great need for improved, safe treatments with efficacy in a broader group of patients.

Research in the molecular biology of prostate cancer has indicates that some of the same aberrant

molecular signaling pathways identified in other malignancies, such as breast or lung cancer, are important in prostate carcinogenesis as well. Protein targets which are components of these pathways, such as transcription factors, kinases and recruitment enzymes, have been identified; and manipulation of those targets has demonstrated a significant clinical impact in other malignancies, most notably breast cancer. Current clinical trials are seeking to take advantage of this knowledge to impact the care of patients with AIPC. In this article we will review several of the new targeted inhibitors being investigated in prostate cancer patients, including epidermal growth factor receptors (EGFR), vascular endothelial growth factor and their receptors (VEGF), and the endothelin pathways. For each agent we will discuss the preclinical laboratory findings which characterized the molecular mechanisms that lead to development of the new drugs in a clinical setting; and reported clinical findings and ongoing trials using the targeted agents will be presented. The educational goal of this piece is to present examples of successful translational research; with laboratory findings directly leading to development of novel agents for promising cancer therapies.

TARGETED THERAPY FOR PROSTATE CANCER

The Epidermal Growth Factor Receptor

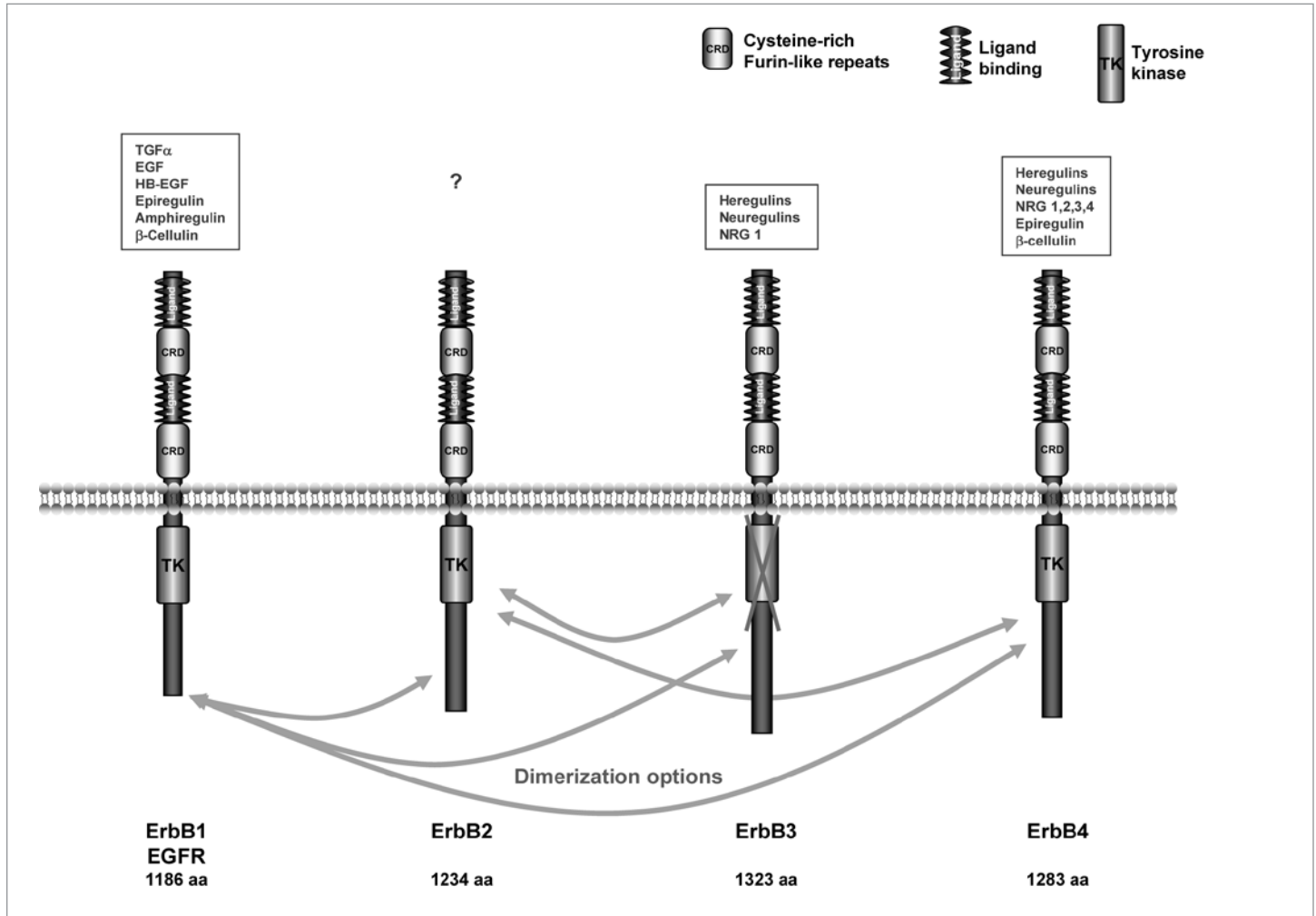
The epidermal growth factor receptor (EGFR) is a transmembrane receptor tyrosine kinase belonging to the Erb2 family of proteins. Overexpression and aberrant activation of these receptors in epithelial tumors contributes to carcinogenesis. The protein family is comprised of four related receptors including EGFR, ErbB2, ErbB3 and ErbB4; also referred to as EGFR/ErbB1, HER2/neu, HER3 and HER4, respectively. (Figure 1) Downstream signal transduction from the activated receptors is non-linear. The horizontal cross-connection of the signaling network lends itself to intricate, combinatorial cellular responses. (Figure 2) The receptors, when inactive, exist as single subunits. Once a ligand binds to an EGFR subunit on the extracellular domain, there is a conformational change allowing dimerization of two subunits. Subsequently, recruitment and phosphorylation events of intracellular substrates results in the downstream actions contributing to mitogenic signaling; including activation of transcription factors which regulate cell proliferation, control of invasive capacity and apoptosis.¹⁰⁻¹³ While full comprehension of the detail

in this figure is not crucial in the context of this review, the complexity outlining the molecular events that induce activation of the EGFR reveals the challenges in identifying an effective targeted cancer therapy. The pleiotropic events downstream of the receptor lend itself to crosstalk between pathways, which can allow cancer cells to incur resistance. Furthermore, toxicities are unpredictable since numerous cellular responses result from receptor activation.

Because of the broad spectrum of downstream cellular responses observed to be mediated through this receptor in the preclinical studies, optimal inhibition of carcinogenic effects may best be achieved at the level of the ligand or receptor. Over 20 years ago, the EGFR was proposed as a cancer therapy target. In 1983, Sato and colleagues demonstrated that monoclonal antibodies designed to be competitive with the epidermal growth factor (EGF) ligand inhibited proliferation of human cancer cell lines *in vitro*.¹⁴⁻¹⁶ It is of interest that, although the response *in vitro* was cytostatic and not cytotoxic, the subsequent studies in an athymic nude mouse model demonstrated that the same antibodies completely prevented tumor growth.¹⁷ This provided further evidence that inhibitors of the EGFR would be effective in treatment of solid tumors.

Those early efforts have led to development of the EGFR inhibitors currently used in cancer patients. Two major classes of EGFR inhibitors are being studied; monoclonal antibodies, and low molecular weight tyrosine kinase inhibitors. Each class of agents functions through different mechanisms of action. The monoclonal antibody cetuximab is directed against the extracellular EGFR ligand binding domain and inhibits the conformational alteration required for receptor dimerization. It is also of interest that cetuximab induces a downregulation of EGFR expression.¹⁸ The low molecular weight inhibitors act within the cell by binding the tyrosine kinase regions of the intracellular domain of EGFR.^{12,13,19} This abrogates downstream mitogenic signaling. Because EGFR and HER2 share high sequence and structural identity, some agents can block function of both types of receptors making this class of agents potentially more effective. Gefitinib (Iressa™ (AstraZeneca, New York, NY)), an agent currently in clinical development, acts by preventing autophosphorylation of the intracellular domain of the EGFR.²⁰ This blocks the recruitment of cofactors which mediate downstream activity. However, although initial data have shown promise, recent *in vitro* data have suggested that tumor cells can become gefitinib-resistant due to crosstalk with an alternative pathway

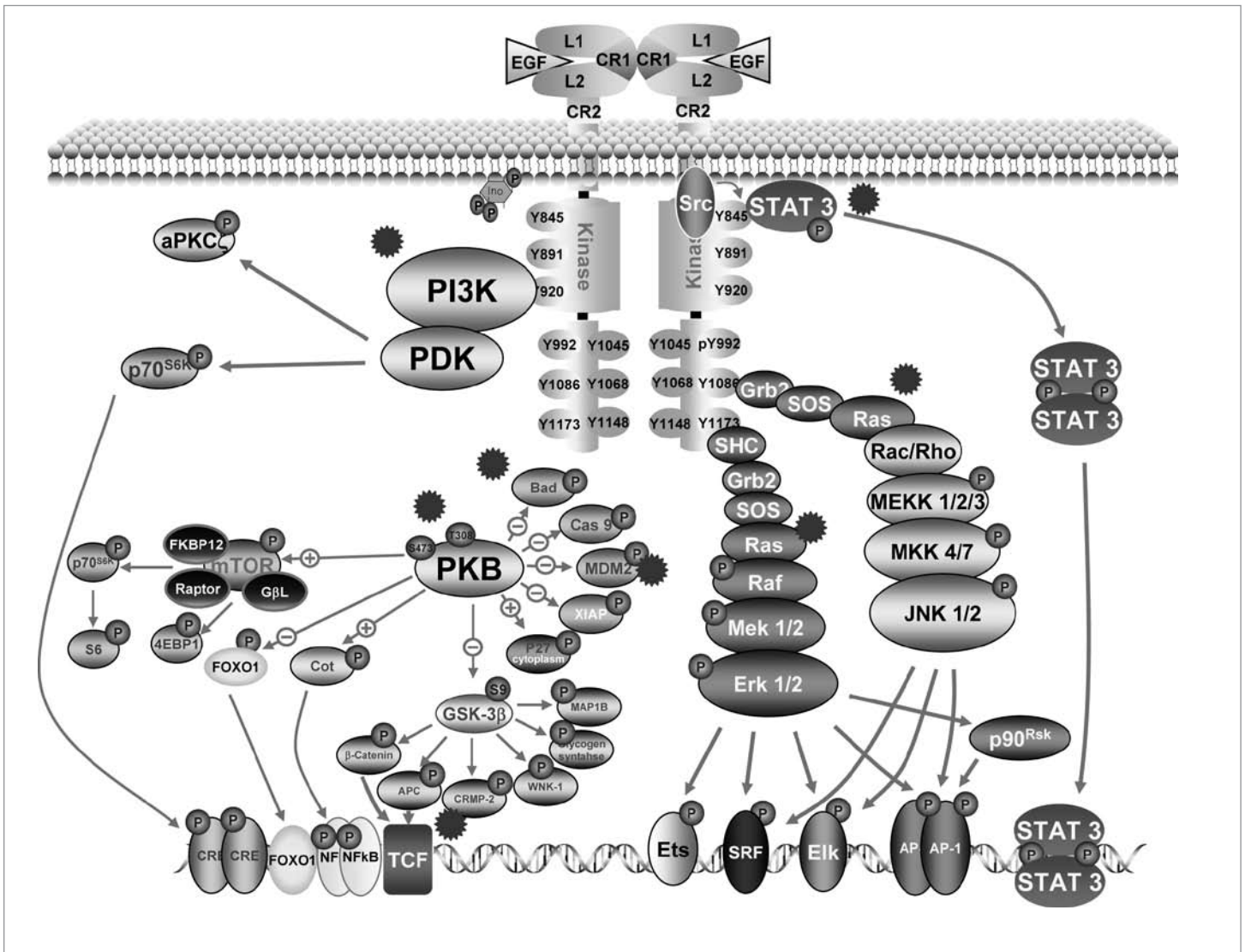
Figure 1. EGF receptor family signaling specificity (Human). These are transmembrane cell surface receptors. Ligands are members of the ERB class of molecules. Key regions and dimerization partners are labeled. Specificity of ligand binding is determined by the heterodimeric active receptor.



regulated by the protein ras.²¹ (Figure 2) Further research is underway to circumvent development of resistance. Some strategies include combining several targeted agents in one treatment or a combination of agents against the same target with the hypothesis that there may be a synergistic or additive effect. Lapatinib (Tykerb™ (GlaxoSmithKline, Philadelphia, PA)) is a low molecular weight small molecule designed to inhibit the tyrosine kinase domains of both EGFR and HER2. Preclinical studies show promise for the agent; and human xenograft studies in the athymic mouse model suggest that lapatinib is cytotoxic by inducing apoptosis. Furthermore, *in vitro* and *in vivo* data indicate that lapatinib may increase the sensitivity of cancer cells to cytotoxic agents making it a likely agent to be tested in combination drug studies. Early clinical studies also have shown an increase in apoptosis in tumor samples of human subjects.

The first EGFR blocking agent to receive FDA approval was gefitinib. This drug was approved for use in non-small cell lung cancer following analyses of a clinical trial demonstrating higher rates of response in patients with adenocarcinoma who were non-smokers or women. In 2007, Boccardo, et al, presented the results of a trial comparing the use of prednisone with gefitinib or placebo in patients with AIPC.²² This was a randomized double-blind study; although patients were unblinded in the event of progression and were given the opportunity to crossover onto gefitinib regimen if they had previously been on the placebo. An interesting and unanticipated result was the observation that there was a rise in PSA during the first eight weeks before a decrease in PSA those subjects who responded. The mechanism for this has not been elucidated but will be a consideration in the design of future trials of these agents. The crossover

Figure 2. EGFR signal transduction. The molecular signaling pathway elicited downstream of EGFR is complex. Blue stars indicate components which have been shown to play a role in carcinogenesis. This suggests that inhibition of more than one downstream effector would be required for optimal inhibition.



design of the trial makes true assessment of survival outcomes difficult. The authors, however, report that those patients receiving gefitinib at anytime in the trial had a longer median survival, 26.5 months as opposed to 17.5 months in those patients never receiving gefitinib. There may be a significant confounder in these analyses if there is a presupposition that a selection bias in the crossover group was a factor, and that subjects progressing on placebo and not crossing over to gefitinib treatment arm were at a more advanced stage of disease; however, the results are compelling and merit further investigation.

Lapatinib is a more recently FDA approved EGFR blocking agent. It is a dual inhibitor of EGFR and ErbB2 tyrosine kinase activity. It has been demonstrated to

have significant activity in patients with breast cancer who have progressed after therapy with trastuzumab (Herceptin™ (Genentech, San Francisco, CA)). Side effects have been tolerable and include a rash similar to acne along with gastrointestinal symptoms and fatigue. Preclinical studies demonstrated that EGFR blockage does not show benefit in prostate cells that have progressed to become independent of androgens but may be helpful in earlier stages of disease when androgens still stimulate growth. The Eastern Cooperative Oncology Group (ECOG) has established an ongoing clinical trial looking at the use of Tykerb in men with recurrent prostate cancer prior to the use of androgen deprivation therapy. The primary endpoint is a reduction in the rate of decline in PSA

of greater than 50%. The study will also evaluate the time to progression of disease and the number of patients free from progression after two years of follow up. If men diagnosed with early stage, organ confined prostate cancer could safely delay initiation of androgen deprivation therapy, a significant impact in quality of life and survival may result.

Vascular Endothelial Growth Factor

Vascular Endothelial Growth Factor (VEGF) is a critical ligand mediator of tumor angiogenesis. Its expression further contributes to cancer progression by increasing vascular permeability facilitating dissemination of malignant cells; and, by recruiting circulating endothelial cells which provides protection for newly intravasated tumor cells.²³⁻²⁶ Its overexpression in solid tumors is stimulated, in part, by the transcription factor, hypoxia inducing factor-1 (HIF-1).²⁷ HIF-1 expression is upregulated in tumors when they become necrotic as a result of growth past the available blood supply.^{28,29} (Figure 3) Therefore, VEGF activity initiates the cascade of molecular events which form blood vessels to provide tumors with necessary factors for growth. Downstream effects of the VEGF ligand are mediated through a family of three receptor tyrosine kinases, VEGFR-1, -2 and -3.^{23,24} Because of its central role in angiogenesis and other mechanisms of cancer progression, inhibition of VEGF activity shows great promise as a strategy for therapy of solid tumors. Agents in development include monoclonal antibodies directed against the ligand, mutant VEGF receptor antagonists and small molecule tyrosine kinase inhibitors which antagonize receptor function by binding the intracellular domain of the receptor.

Thalidomide, most widely known for its teratogenic effects discovered in the 1950s and 60s, was shown to be a breakthrough therapy for leprosy and other conditions of acute inflammation. However, the molecular mechanism of action was not elucidated until the 1990s when Sampaio and colleagues demonstrated that thalidomide selectively inhibited expression of the cytokine, Tumor Necrosis Factor-alpha (TNF α), in monocytes stimulated by lipopolysaccharide.³⁰ This cytokine is commonly overexpressed in patients with solid tumors and pathological conditions where the inflammatory cascade is dysregulated such as rheumatoid arthritis and Crohn's disease.³¹ In addition, in vitro and in vivo preclinical studies demonstrated that thalidomide had a strong inhibitory effect on angiogenesis.³² These findings lead to interest in testing thalidomide as a therapeutic agent for solid tumors. In 1994, the US

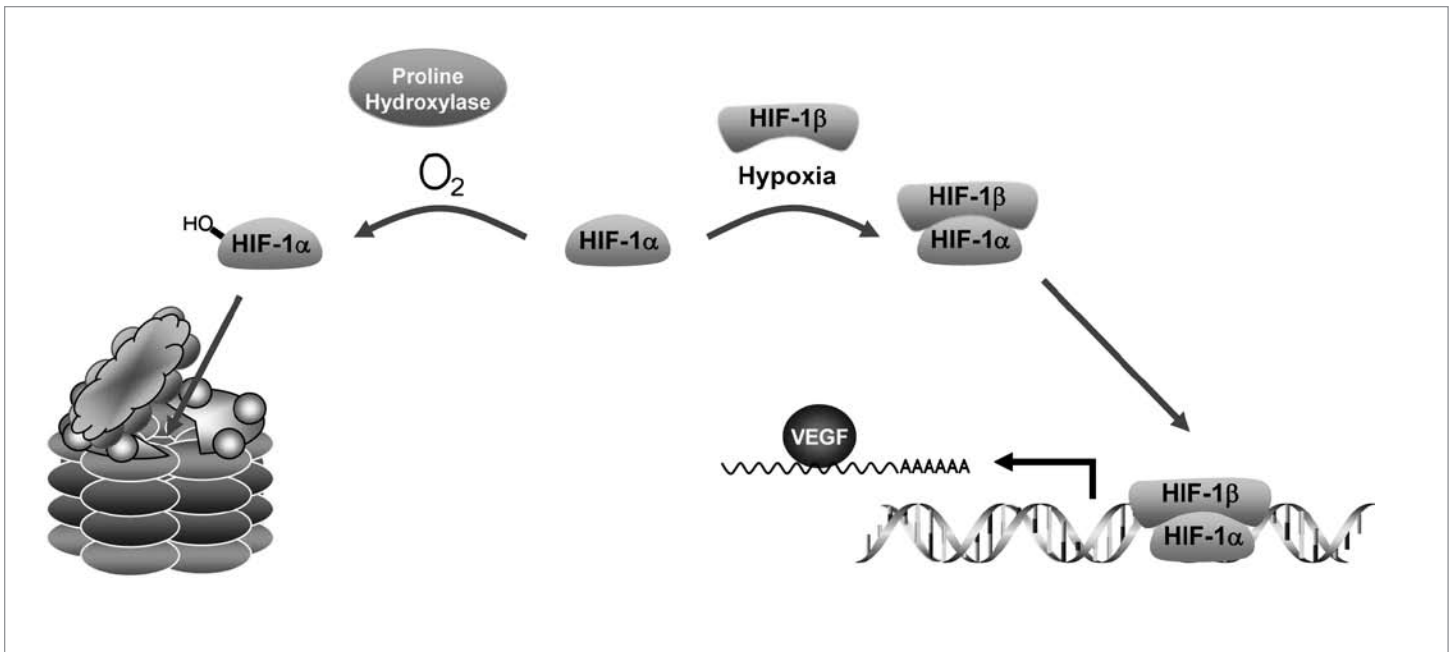
Food and Drug Administration formed the thalidomide working group in order to test this agent under close scrutiny.³³ Currently, clinical studies in several solid tumor types are underway. It is promising that recent data have suggested that the teratogenic effects of thalidomide were most likely the result of its activity as a DNA intercalator in genes critical for embryonic development.³⁴ Therefore, as thalidomide continues clinical evaluation to treat solid tumors, new analogs may be developed with a more favorable safety profile with respect to teratogenicity.

Bevacizumab (AvastinTM (Genentech, San Francisco, CA)) is a humanized monoclonal antibody directed against the VEGF ligand. Binding of the antibody to the ligand prevents VEGF from interacting with its respective receptors. This is the most well studied VEGF inhibitor currently undergoing clinical testing in solid tumors. In 2005, Wang and colleagues characterized the downstream effects of bevacizumab in vitro in a human endothelial cell line.³⁵ Bevacizumab inhibited VEGF stimulated cell proliferation in a dose dependent manner. An in vitro assay measuring vascular permeability demonstrated that bevacizumab also attenuated the increase in vascular permeability induced by VEGF. These data support the hypothesis that bevacizumab activity is mediated, in part, by decreasing invasive capacity. This was further supported by laboratory studies which showed a marked decrease in cell migration through matrigel in an in vitro transwell assay in the presence of bevacizumab.

Several antiangiogenic agents are already FDA approved for a range of diseases. Thalidomide has an unfavorable history, but has now been demonstrated to have activity in several tumor types, such as multiple myeloma. Bevacizumab was FDA approved for use in non-small cell lung cancers as well as colorectal cancers following successful pivotal clinical trials. While both bevacizumab and thalidomide are inhibitors of VEGF, their mechanisms of action are dissimilar at the molecular level. It is now being hypothesized that combination of the two inhibitors would act in an additive or synergistic manner. Thus, in a clinical trial, they were combined with docetaxel, arguably the most active cytotoxic chemotherapy in use against prostate cancer. In subjects with AIPC enrolled on the trial, 87% had a greater than 50% fall in PSA. Sixty-seven percent of subjects had a greater than 80% fall in PSA. In men with otherwise objectively measurable disease, the overall response rate was 59%.³⁶ This regimen shows great promise for men with AIPC.

The Cancer and Leukemia Group B (CALGB) studied bevacizumab along with docetaxel and

Figure 3. HIF-1 induces expression of VEGF. Hypoxic conditions within a solid tumor upregulate activity of the HIF-1 transcription factors. HIF-1 α and β induce expression of VEGF, a key factor in tumor angiogenesis.



estramustine in men with AIPC. Although PSA responses were seen in 81% of subjects, significant dose limiting toxicities were encountered that were likely related to the use of estramustine, including deep venous thrombosis, pulmonary embolus, and mesenteric vein thrombosis. Although the toxicities related to the use of estramustine are established, the benefits of its use in this combination are not clear. As a result, the CALGB opened a trial looking at docetaxel and prednisone with either bevacizumab or placebo. By eliminating the estramustine component of the regimen, a larger dose of docetaxel can be used. The primary objective will be to demonstrate whether the addition of bevacizumab lengthens overall survival, but the study will also compare rates of PSA decline, time to progression in the two groups, and the rates of significant toxicities. Favorable results in this randomized, placebo controlled, Phase III trial, should help firmly establish the benefit and toxicities of adding bevacizumab to cytotoxic chemotherapy.

Endothelin-1 (ET-1)

Endothelins are a family of peptides with vasoconstriction activities involved with maintenance of vascular homeostatic.^{37,38} There are two major receptor types; ETA and ETB, which are widely expressed in endothelial cells. Because preservation of normal vascular pressure requires a delicate balance between vasoconstriction and vasodilation, over expression

of endothelins can result in pathological conditions such as pulmonary hypertension for which endothelin inhibitors such as bosentan and sitaxsentan can be effective therapies.

The ET family of signaling molecules was subsequently found to have other biochemical and physiologic effects in addition to maintenance of vascular homeostasis. While the mechanism of action has not yet been elucidated, ET-1 also has been characterized as a potential contributor to carcinogenesis in solid tumors. Given the nature of pathways mediating protein expression; with significant crosstalk, this is not unanticipated. In 1990, Kusuvara and colleagues demonstrated that cell lines derived for several human tumors expressed ET-1.³⁹ This was also reflected *in vivo* with immunohistochemical methods which showed increased ET-1 expression in cancer of the prostate,⁴⁰ breast⁴¹ and colon⁴² compared with adjacent normal tissue. Subsequent *in vitro* studies indicated that ET-1 may modulate key processes of carcinogenesis including angiogenesis, invasion and apoptosis.^{43,44} Furthermore, ET-1 is over expressed peripherally in some patients with solid tumors. These data suggest that ET-1 may play a role in cancer progression; however, the precise molecular mechanisms have not been identified.

Inhibitors of ET-1 have been shown to decrease growth in multiple *in vivo* models of carcinogenesis of solid tumors. In a mouse prostate cancer model of bone metastases, the combination of taxotere and the ET-1

receptor antagonist, Atrasentan (Xinlay™ (Abbott Laboratories, Abbott Park, IL)), reduced tumor growth and increased apoptosis.⁴⁵ At termination, a 90% reduction in tumor volume was observed in the combination treatment group compared to the untreated control group. Most importantly, the antitumor activity was associated with the down-regulation of molecular markers in tumor tissues that were similar to those observed *in vitro*.

Atrasentan is not yet approved by the FDA but continues to be extensively studied. A benefit of this agent is that it is orally bioavailable. This is favorable impacting both quality of life of patients and healthcare costs by circumventing the need for in-clinic drug infusion. Studies of atrasentan alone versus placebo in men with AIPC have not shown overwhelming results; however, results are of interest and warrant further study in this clinical setting. Markers of bone remodeling were clearly suppressed in men taking atrasentan compared to placebo. Although the overall results of these studies were not statistically significant, small subgroups of men promising results were observed. Men with confirmed bony metastases had a delay in the time to progression of their disease, as did men with independently confirmed metastatic disease at study entry. In addition, quality of life measures were significantly improved in men with bony metastases. Preclinical studies on ovarian cancer cell lines suggest that atrasentan pretreatment can sensitize malignant cells to the effects of taxanes *in vitro*.⁴⁶ Therefore, future combination regimens in some solid tumors types may be beneficial.

The culmination of these data have lead to the consensus that atrasentan should be mainly studied in men with AIPC and established bony metastases. It has also been determined that additional trials should include an agent with established activity in soft tissue disease. The Southwest Oncology Group initiated a clinical trial evaluating the combination of docetaxel and atrasentan versus placebo this cohort. Although the primary endpoint to be measured will be survival effects of adding atrasentan, PSA and quality of life effects with the addition of atrasentan will be evaluated. A significant translational component will be quantification of bone resorption and formation markers to evaluate correlation with responses and survival.

Summary

Knowledge advances in the molecular biology of malignant cells have been translated into the therapies of a number of diseases including chronic myeloid

leukemia, and breast cancer. The use of this knowledge base for the advancement of prostate cancer therapies is near the beginning but targeted therapy shows promise, and represents the archetype of translational research. Not only has preclinical knowledge lead to clinical therapy, but clinical and laboratory findings on initial trials have lead to an adjustment of therapeutic approaches. Identification of molecular targets that regulate the processes of prostate carcinogenesis could ultimately lead to breakthroughs in treatment for all stages of disease.

Suzanne Stratton, PhD is the Vice President of Research at Carle Foundation Hospital, Urbana, IL.

David Graham, MD is a medical oncologist and the Medical Director for Clinical Research at Carle Clinic Association, Urbana, IL.

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These references are presented in Endnote format. They do not represent standard Carle Selected Papers style.

CME Questions 4a-d

Please select the best answer for the following:

- 4a. Previous studies suggest that which of the following is the most appropriate approach for studies of endothelin-1 inhibitors?
 - a. ET-1 inhibitors are best used as a single agent
 - b. Toxicities will require use of multiple supportive therapies with ET-1 inhibitors
 - c. Lack of efficacies suggest that no further studies of ET-1 inhibitors should be undertaken
 - d. Primary effects on bone suggest that ET-1 inhibitors should be combined with agents having effects on visceral disease
- 4b. Best effects of lapatinib (Tykerb) have been seen in men with androgen independent prostate cancer.
 - a. True
 - b. False
- 4c. Which inflammatory cytokine has been shown to be inhibited by thalidomide?
 - a. Interkeulin-1 β
 - b. Meeplokinase-7
 - c. Platelet derived growth factor
 - d. Tumor necrosis factor- α
- 4d. Activation related to EGFR ligand binding does not include which of the following?
 - a. Cell proliferation
 - b. Apoptosis
 - c. Telomere shortening
 - d. Control of invasive capacity