Editorial

Addressing Apoptosis to Tumor Zip Codes

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INTRODUCTION

Currently, as we approach the 50-year anniversary of Dr. Charles Huggins' Nobel Prize for describing the hormonal dependence of prostate cancer (1966), we reflect on the developments that have been made in the systemic management of this disease. Androgen-deprivation therapy (ADT), one of the first examples of targeted cancer therapy, continues to be the main pillar for systemic therapy of prostate cancer. By delving deeper into the pituitary-gonadal axis, extragonadal steroidogenesis, and androgen receptor function, we have broadened our understanding and arsenal with the recent additions of abiraterone and enzalutamide, while several novel agents targeting different aspects of androgen receptor signaling are undergoing evaluation.[1,2] Classic cytotoxic chemotherapies were attempted, and the microtubule was validated as a target, with 2 taxane family agents (docetaxel, cabazitaxel) demonstrating survival benefit in metastatic, castrate-resistant prostate cancer; and, more recently, remarkable survival benefits have been demonstrated with docetaxel in combination with ADT in newly diagnosed metastatic disease.[1,3]

Although there are many critical questions across the disease landscape, the clinical challenge continues to be the management of the metastatic stage of the disease, which is terminal for the vast majority of patients. Clearly, major therapeutic improvements for the metastatic population would have a large, immediate, and very palpable impact, because it is estimated that 27,540 men will die from prostate cancer in 2015.[4]

Because metastatic disease is the driver of morbidity and mortality, defining its origin is crucial. A metastatic deposit must survive release from the primary site into the blood stream, tolerate the circulatory environment, avoid the immune system, cross a vascular endothelium, adhere to the extracellular matrix in a new site, and develop a dedicated blood supply to support growth.[5] Building from this basic biologic paradigm, efforts to diminish growth factor availability and to interrupt cell-extracellular matrix binding along with antiangiogenic therapies have been attempted.

Tumor Microenvironment

The *seed and soil* interaction has been a focus area for cancer therapy development in general and for prostate cancer in particular. Bone, being the hallmark of prostate cancer metastasis, is a dynamic structure that undergoes constant destruction and rebuilding to manage an individual's physical demands and metabolic needs. This homeostasis in part depends on growth factors (insulin-like growth factor, transforming growth factor-β), chemotaxic factors (stromal cell-derived factor 1, C-X-C chemokine receptor type 4), and the maintenance of specific cell-cell and cell-extracellular matrix interactions. Prostate cancer (among others) uses this nurturing environment and can reside in both dormant and active states. To date, targeting some of the growth factor environment by inhibiting the insulin-like growth factor-1 receptor has not been effective.[5] The adherence of prostate cancer to the extracellular matrix is based on the interaction between integrin proteins on the cell surface and the matrix. Targeting this interaction also has not been successful to date.[6,7]

Angiogenesis

Angiogenesis and its role in creating and supporting the tumor microenvironment was first proposed by Judah Folkman in 1971[8] and has grown to both biologic and clinical relevance for a variety of solid tumors with US Food and Drug Administration-approved indications. The therapeutic premise is that, if the blood supply to the tumor is halted, then the tumor should die or, at a minimum, stop growing, spreading, and seeding. Tumor blood vessels arise through the stimulation of native vessel endothelium to proliferate through the vascular endothelial growth factor (VEGF) pathway. This

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pathway begins with extracellular ligand (ie, VEGF-A, VEGF-B) activating one of several VEGF receptors (ie, VEGFR1, VEGFR2). This causes receptor heterodimerization and/or homodimerization with autophosphorylation of the intracellular domain. The active kinase amplifies its original signal by triggering a multitude of signal pathways, such as phosphatidyl-inositol 3-kinase (PI3K), proto-oncogene tyrosine-protein kinase (SRC), or phospholipase Cγ-protein kinase C (PLC-γ-PKC) pathways. These lead to the activation of migratory proteins and proliferative pathways while down-regulating apoptotic pathways.[9]

Drugs have targeted several steps in the angiogenic process. The classic example is bevacizumab, a monoclonal antibody that functions as a ligand sink for VEGF-A by binding it in circulation. Bevacizumab was tested in a blinded phase 3 trial by adding it to docetaxel.[10] The bevacizumab/docetaxel combination had no impact on overall survival compared with docetaxel (22.6 months vs 21.6 months). Aflibercept, an antibody-based VEGF receptor mimetic, also acts upstream of receptor activation by binding a variety of receptor ligands (ie, VEGF-A, VEGF-B, and placental growth factor). The hope was that depletion of more ligands would enhance the antitumor effect. However, it also was disappointing, because it produced no overall survival benefit (22.2 months vs 21.2 months).[11] Rather than depleting the ligand, another approach was to inhibit the ligand-receptor interaction. The monoclonal antibody ramucirumab binds VEGFR2 directly, hence sterically inhibiting it from ligand. This drug was recently approved in gastric cancer and had preliminary, potentially promising data based on a noncomparative phase 2 trial in combination with mitoxantrone.[12] Inhibiting the initiating ligand-receptor interaction may yield results, but the challenges lie in the redundancy in both ligand and receptor pool; and, when 1 pathway slows, another amplifies to replace its mitogenic effect.

The VEGF pathway was also targeted by stopping downstream intracellular phosphorylation events. These are the tyrosine kinase inhibitors. The best studied in prostate cancer is the nonselective kinase inhibitor sunitinib, which stops VEGFR-2 phosphorylation. In a phase 3 trial, it did not improve survival (13.1 months vs 11.8 months).[13] Other inhibitors have been investigated (sorafenib, cediranib, cabozantinib), yet none produced confirmed, meaningful clinical activity or benefit.

These cumulative failures with agents targeting angiogenesis and related pathways likely reflect the diverse signaling environment driven by advanced-stage disease, plasticity of the cell to be stimulated in a variety of ways, selective amplification of weak signals, lack of predictive biomarkers to allow for better patient selection, and potentially the disease setting/burden. It is within this context that the article in the current issue of *Cancer* by Pasqualini and colleagues is of interest.[14] These investigators report their data on another novel mechanistic class by targeting the endothelial vascular signature of prostate cancer metastasis. It is believed that these unique vascular surface signatures ("zip codes") are created when tumor cells seed a site and stimulate new vasculature to grow. Through an earlier in vivo phage library screen developed by the group, the interleukin 11 receptor α (IL-11Rα) was up-regulated in vascular endothelium associated with prostate cancer.[14] By creating a chimeric peptide with the IL-11Rα–binding domain fused to an apoptosis-inducing peptide motif, they sought to selectively target the intratumor vasculature while sparing nontumor-associated vessels. This is similar to antibody-drug conjugates currently in use for other malignancies except with a smaller recognition moiety and protein cascade of apoptosis compared with direct cytotoxins.

After developing this chimeric peptidomimetic drug (bone metastasis-targeting peptidomimetic-11 [BMTP-11]), the investigators first characterized its preclinical efficacy. By using 3 cell lines that reflected different clinical scenarios (androgen-sensitive, castrate-resistant, and bone-predominant disease), intravenous BMTP-11 was given weekly, and tumor volume was monitored. BMTP-11 stalled the growth in each scenario, and the strongest response was observed in the bone-predominant cell line, which had the highest expression of IL-11Rα. Tissue distribution, serum stability, and pharmacokinetic parameters were determined in preparation for toxicity evaluation in nonhuman primates. Renal injury became apparent, with discoloration of the kidneys and tubule destruction, but BMTP-11 was deemed safe to continue.

The first-in-man studies with dose escalation were done in patients with widely metastatic bone disease, and biopsies were used to confirm the presence of bone metastases. The expression of IL-11Rα in initial confirmatory bone marrow biopsies revealed enrichment of the receptor by immunohistochemistry. A bone marrow biopsy was repeated after the first infusion, and immunohistochemistry and mass spectrometry confirmed the presence of BMTP-11. Terminal deoxynucleotidyl transferase dUTP nick-end labeling confirmed apoptosis-mediated death, hence completing the localization and predicted effect loop. By using the 6 patients on study, an escalation dose model was done. This began at 18 mg/m^2 and then was increased to 36 mg/m^2 after the first dose was tolerated

well by 2 patients for 4 treatments each. At this higher dose, patient 3 had grade 3 renal toxicity; therefore, the protocol was amended for a 27 mg/m^2 dosing cohort. Three more patients were treated at this intermediate dose but did not complete the 4 planned cycles because of either progression or renal toxicity. With the small sample size, efficacy cannot be established. It is interesting that 1 patient had symptomatic and biochemical improvement, but his disease relapsed quickly when the medication was withdrawn. That patient had the least renal toxicity, suggesting the possibility of pharmacogenomic variability; however, the data is limited at this time.

Renal dysfunction seems to be the primary adverse event, because 33% of patients had grade 2 or 3 glomerular filtration rate changes. More intravenous fluids were added but still did not negate the effect. The injury was reversible but clearly raises concern about longer durations of treatment and feasibility in the prostate cancer population. This effect is not common among other peptidomimetics (argatroban, human immunodeficiency virus-protease inhibitors), arguing against a class affect. The researchers observe the same tubular injury with their other engineered peptidomimetic drugs, which share the apoptotic motif.[16] They discuss engineering a different chemical moiety to avoid this while also suggesting dose or schedule modifications. Clearly these are important options to pursue.

Although bone metastasis is the "clinical" hallmark of prostate cancer, autopsy data highlight the promiscuous nature of the disease and scope of its spread, stressing the need for more than just targeting of bone.[17] It will be critical to evaluate the same technology in the context of lymph node metastases and other organ metastasis to determine whether or not the "zip code" signatures and targeting methods described here are applicable and effective.

Pasqualini and colleague's study is extensive in its scope and novel in its scientific base. It builds on previous rational, mechanistic drug-development strategies, yet is in a new mechanistic realm, hopefully devoid of the redundancy observed in the other vascular-targeted therapies. However, a key issue that needs to be addressed and optimized is the feasibility of human application with the toxicities mentioned above.

It is clear from the prior clinical research on angiogenesis and other pathways in metastatic, castration-resistant prostate cancer that these tumors are *smart*: they adapt, and they cannot be forced to become dependent on a single pathway, nutrient, or environment. Despite the disappointing results to date, targeting tumor angiogenesis is still logical in this disease; however, it is clear that more critical research is needed to better understand the biologic context of tumorigenesis, metastasis/seeding, and the microenvironment in prostate cancer to better guide our drug development and clinical research.

Performing high-quality, informative clinical trials is not easy but very critical as is the case in this trial. It is ultimately the brave patients and families; like the brain dead patient who underwent the peptide library infusion and biopsy and the patients who participated in this phase 0 trial and underwent multiple bone biopsies that allow us to find the next best therapies.

It is challenging to predict, at the onset of a new field, whether the pathway is worthwhile; however, this report clearly highlights an interesting and novel target and a directed strategy that need further exploration. After more than 7 decades of androgen-signaling research and over 4 decades of angiogenesis research, we are still learning. We continue to be puzzled by the frequent mismatch between preclinical observations and clinical outcomes. Therefore, *addressing apoptosis to tumor zip codes* as an overarching principle, exemplified in this case by Dr. Pasqualini and her team's approach, continues to be a goal that we all (and no doubt Drs. Huggins and Folkman) would appreciate.

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