Lonidamine: Basic Science and Rationale for Treatment of Prostatic Proliferative Disorders

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Normal and hyperplastic prostatic tissues concentrate citrate within the epithelium; however, a unique biochemical property within prostate epithelial cells renders them dependent on glycolysis, rather than the citric acid cycle, for energy production. Lonidamine, an orally administered small molecule that inhibits glycolysis by the inactivation of hexokinase, may represent a unique and novel approach to the treatment of benign prostatic hyperplasia (BPH). Results of a phase II trial of lonidamine in BPH (described elsewhere in this supplement) are encouraging. Lonidamine is already used in the treatment of several cancers in other countries. Its target-specific nature renders it a safe compound for administration; in cancer therapy, patients have been treated with 40 times the daily dose used in the BPH trial, with negligible toxicity.

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It has been said that surgery exists because of our inability to control the processes leading to disease. This is perhaps nowhere more apt than in the management of progressive proliferative disorders. Many of these disorders are part of the "normal" process of aging, and benign prostatic hyperplasia (BPH) is a prime example.

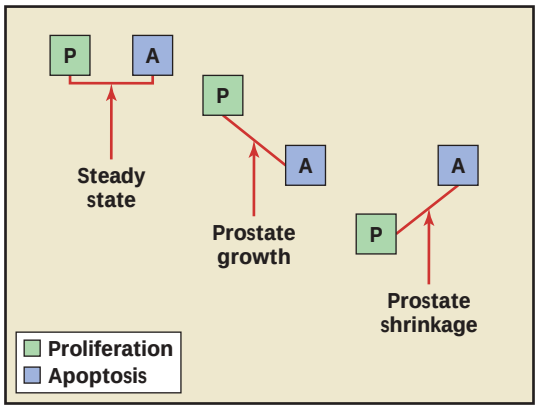


Figure 1. *α-Blockers and 5α-reductase inhibitors are highly effective for managing the symptoms of BPH but do nothing to permanently halt or reverse the hyperplastic process. An ideal agent would tip the balance to increase apoptosis and decrease proliferation of prostatic cells. BPH, benign prostatic hyperplasia.*

Over the last 2 decades we have seen unparalleled changes in the management of BPH. This is covered, in part, in the article by Kaplan in this supplement. Previously, BPH was treated with surgical ablation (primarily transurethral resection), whereas today it can usually be successfully managed pharmacologically. The α-blockers have been the primary agents and are often used in a diagnostic/therapeutic manner. The 5α-reductase inhibitors also have a significant role, either alone or in combination with α-blockers. The development of these agents has revolutionized the management of this most common male proliferative disorder.

Although α-blockers can lead to significant reductions in symptomatology, they do nothing to change the proliferative nature of BPH. Five-α-reductase inhibitors and other hormonal manipulations result in significant involution of the gland owing to the exquisite dependence of normal prostatic epithelium on circulating androgens. However, restoration of normal hormonal levels within the prostate results in regrowth. Consequently, neither of these approaches addresses the critical issue of BPH—reduction of the hyperplastic process. An ideal agent would tip the balance to increase apoptosis and decrease proliferation of prostatic cells, as shown in Figure 1.

Metabolic targeting is an evolving form of drug development in which small molecules alter fundamental properties specific to selected organ sites. One approach is the development of compounds that affect glucose metabolism, which is being studied most vigorously in cancer therapy. This approach to cancer therapy is exciting because it may well result in prolonged remissions and prevention of relapse, with limited toxicity. This stems from the specific metabolic differences found in transformed tissue. It has long been established that malignant tissues have abnormal biochemical pathways favoring glycolysis owing to their relatively anaerobic milieu. Compounds that change glucose metabolism in malignancies could be used in concert with conventional therapy, including radiation and chemotherapy. The advantage of metabolic targeting of glucose metabolism is that it affects not only rapidly dividing cells in areas of normal oxygen tension but also cells in areas of relative hypoxia, where traditional therapies are less effective.

One of the characteristic features of cancer is the establishment of neovascularity through angiogenesis. Absent this, a tumor quickly outstrips its blood supply. This has been thoroughly documented by Folkman and others.[1,2] Although established cancers show an ability to induce angiogenesis by up-regulation of angiogenic promoters and down-regulation of angiogenic inhibitors, there are areas of relative hypoxia within virtually all tumors. Brizel and colleagues,[3] for example, demonstrated in head and neck tumors an oxygen tension of 11.8 mm Hg, whereas that of the surrounding normal tissue was 51.9 mm Hg. It has been confirmed that cellular proliferation is greatly inhibited within areas of ischemia and resulting hypoxia. This is a problem in conventional chemotherapy, which depends on proliferating cells for efficacy.

Normal and hyperplastic prostatic tissues concentrate citrate within the epithelium. In contrast, transformed epithelium in the prostate has much lower citrate levels (Table 1). Cooper and Farid[4,5] demonstrated that citrate levels in early prostatic carcinoma were significantly (36%) lower than in BPH. Advanced prostate cancer showed citrate levels 86% lower than normal. Utilizing lactate as a comparator, the researchers noted that the differences were even more dramatic, with no overlap in the lactate/citrate levels of early or advanced prostate cancer and BPH. Costello and Farid[6] confirmed that citrate levels were even lower when controlling for the stromal component of prostate tissue, which has inherently low levels of citrate.

There are areas of relative hypoxia within virtually all tumors.

These observations had little clinical impact until the development of

Table 1
Citrate and Zinc in the Human Prostate

Tissue	Citrate, nmol/g	Zinc, μg/g
NL Mixed	8000	209
NL (CZ)	4000	—
NL (PZ)	13,000	—
BPH	8000–15,000	589
CAP (Mixed)	1000–2000	55
CAP (Malig)	500	—
Other soft tissue	150–450	30
Plasma	90–110	1
Prostatic fluid	40,000–150,000	590

NL, normal; CZ, central zone; PZ, peripheral zone; BPH, benign prostatic hyperplasia; CAP, prostate cancer; Malig, malignant.
Reprinted with permission from Costello and Franklin.[29]

magnetic resonance imaging technology, specifically magnetic resonance spectroscopy (MRS). Sillerud and co-workers[7,8] established that prostate citrate levels could be measured noninvasively with MRS. Narayan and coworkers[9-13] developed an MRS technique utilizing endorectal coils. These researchers demonstrated that MRS, utilizing quantification of citrate levels, could differentiate cancer from benign tissue in the prostate.

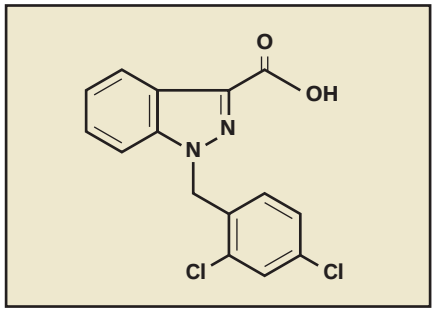
Otto Warburg, winner of the 1931 Nobel Prize in Physiology, demonstrated early in the last century that cancer cells have an increased dependence on glycolysis. This stems from the decreased oxygen tension within most neoplasms, which renders generation of adenosine triphosphate through the Krebs cycle impossible. Transformed epithelium adapts to this anaerobic environment by upregulating glucose transport proteins on the cell surface.[14-18] Subsequently, there is an increase in the initial enzymatic step in glycolysis with upregulation of hexokinase and glucose transport protein.[19-21] The resulting accumulation of glucose in the cancer is the basis for the use of positron emission tomography (PET), an emerging imaging modality for neoplasms, which capitalizes on the increased transport of glucose-based imaging agents to the cancer cell. In addition there is down-regulation in cancer of glucose-6-phosphatase, which reverses glycolysis.[22] This is the basis for using the glucose-based imaging agent fluorodeoxyglucose in PET scanning, which has significantly improved staging of a number of neoplasms.[22-28]

A unique biochemical property within prostate epithelial cells makes metabolic targeting of glucose catabolism applicable.

Benign prostatic hyperplasia, strictly speaking, is not a neoplasm. However, a unique biochemical property within prostate epithelial cells makes metabolic targeting of glucose catabolism in the normal prostate applicable. High concentrations of citrate are diverted from the epithelial cells to the seminal fluid to nourish the sperm; therefore, these cells cannot produce energy through the citric acid cycle. This process is mediated by the extraordinarily high concentrations of zinc in the prostate, which block citrate metabolism and disable the citric acid cycle in prostate cells. Consequently, the inability to metabolize citrate renders the cells of the prostate dependent on glycolysis for energy production.[29]

Lonidamine (Figure 2) is an orally administered small molecule that inhibits glycolysis by the inactivation of hexokinase. Hexokinase is an enzyme that catalyzes glucose, the first step in glycolysis (Figure 3). The inhibition of hexokinase by lonidamine is well established and has been described by a number of prominent biochemists, including Albert Lehninger.[30,31] In addition, there is evidence that lonidamine may increase programmed cell death. This stems from the observation that mitochondria and mitochondria-bound hexokinase are crucial for induction of apoptosis; agents that directly effect mitochondria may, therefore, trigger apoptosis. Indeed,

Figure 2. *Lonidamine, a derivative of indazole-3-carboxylic acid, is an orally administered small molecule that inhibits glycolysis by the inactivation of hexokinase, and is used as a cancer therapy in a number of countries. Originally used as an antispermatogenic agent, it is currently being investigated for use in treating benign prostatic hyperplasia.*



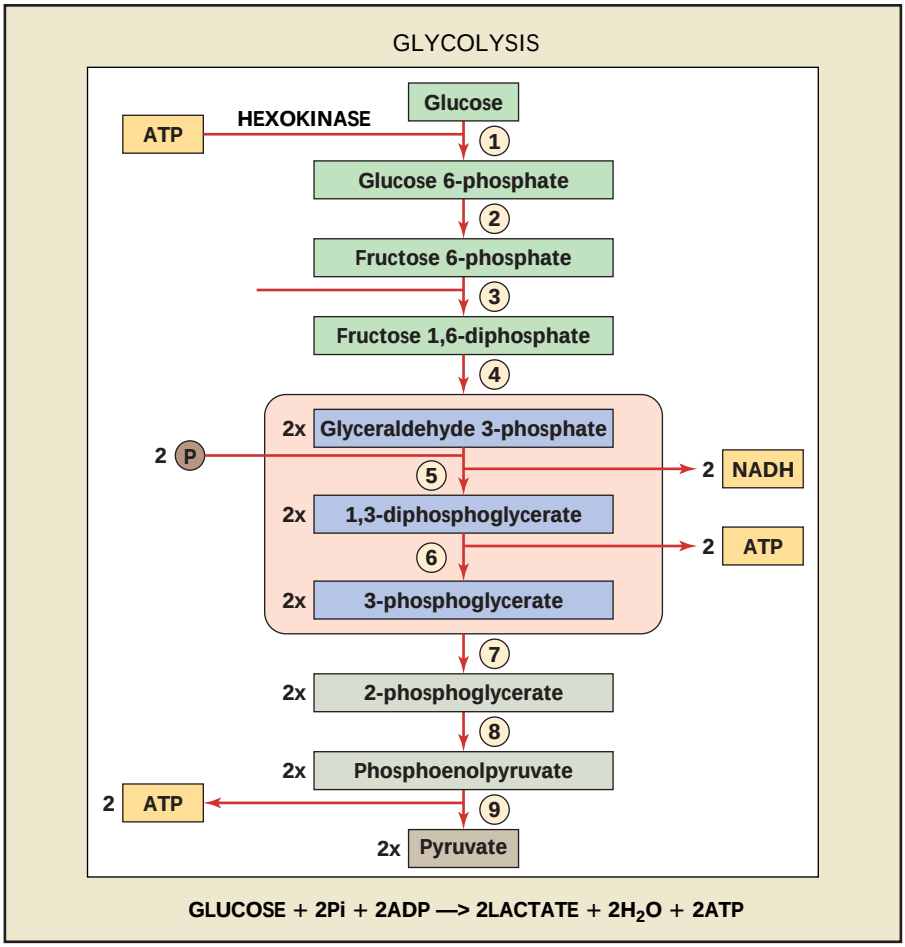


Figure 3. *Hexokinase, an enzyme that catalyzes glucose as the first step in glycolysis, is inhibited by lonidamine. ADP, adenosine diphosphate; ATP, adenosine triphosphate; NADH, nicotinamide adenine dinucleotide (reduced form).*

in vitro models with lonidamine exhibit the hallmarks of apoptosis, including mitochondrial membrane depolarization, release of cytochrome C, phosphatidylserine externalization, and DNA fragmentation.

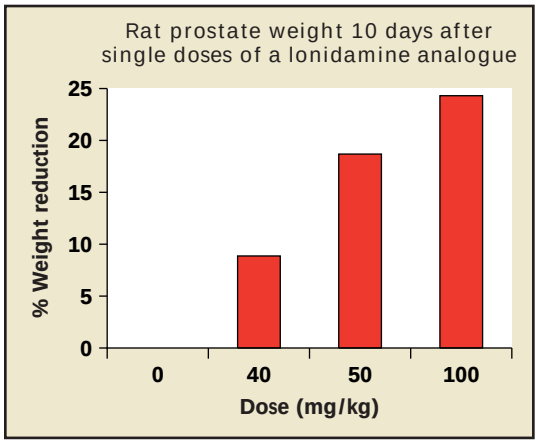
First developed for cancer therapy, lonidamine has been shown to stop the glycolysis on which relatively hypoxic tumor cells are dependent. This results in involution of the cancer. Lonidamine has been widely investigated throughout the world and is approved for use in Europe for cancer therapy.

Because of the chemical quirk rendering the prostate epithelium dependent on glycolysis, it stands to reason that lonidamine may be an effective therapy for the treatment of BPH. Preclinical data demonstrate in vitro tumor cell inhibition in prostate epithelial cells. In a Long-Evans rat model, lonidamine doses of 800 mg/kg led to prostate weight reductions of 50% in a 30-day continuous dosing trial; further investigation showed a reduction in prostate weight of up to 24% within 10 days of a single dose of a lonidamine analogue (Figure 4).[32]

Lonidamine has been the subject of a phase II trial for the treatment of BPH, described elsewhere in this supplement. The target-specific nature of lonidamine renders it an extremely safe compound for administration. In cancer therapy, patients have been treated with 40 times the daily dose used in the BPH trial, with negligible toxicity.

Investigators at Threshold have carried out an interesting investigation in prostate cancer cell lines.[33] In order to further investigate the effect of lonidamine on prostate cells dependent on glycolysis, they compared the LNCaP and PC3 cell lines. The former, which maintains androgen dependence and thus is a suitable model in BPH, produces citrate like normal prostatic epithelium; PC3 is an anaplastic cell line that no longer has androgen dependence. PC3 cells are able to metabolize citrate because they lack the ability to accumulate zinc. The LNCaP line, like normal human prostate cells, concentrates zinc, thus inhibiting the

Figure 4. *A single 100 mg/kg dose of a lonidamine analogue led to a 24% reduction in prostate weight in the Long-Evans rat model. Data from Lobl et al.*[32]



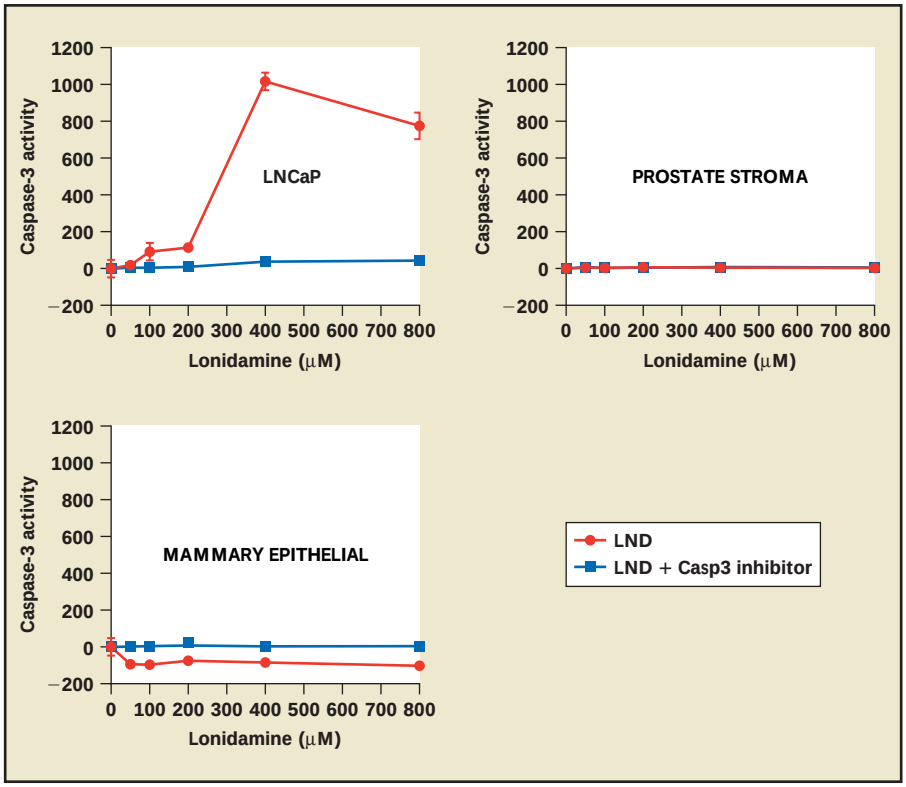


Figure 5. *Caspase-3 (Casp3) activity and lonidamine (LND) in the prostate LNCaP cell line, stroma, and mammary cells.*

citric acid cycle. Figure 5 illustrates the activity of caspase-3 in the prostate LNCaP cell line, prostate stroma, and mammary epithelium. Caspase activity was dramatically enhanced by lonidamine in LNCaP cells but not in PC3 cells. The caspase assay is a measure of apoptosis. In addition to illustrating the prostate epithelial–specificity of lonidamine, these data further illustrate the novel mechanism of action of lonidamine by inhibiting glycolysis in cells such as LNCaP, which are dependent on glycolysis, as opposed to PC3 cells, which can rely on the citric acid cycle for generation of energy.

In summary, lonidamine may represent a unique and novel approach to the treatment of the most ubiquitous proliferative process in men—BPH. Its novel mechanism of action with true specific metabolic targeting could be of potentially great benefit in the treatment of this progressive condition. Preliminary data based on in vivo studies and animal models provide proof of principle. Results of the phase II trial presented in this supplement are encouraging. If such data are confirmed in large-scale phase III trials, the paradigm for treatment of BPH may shift. ■

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Main Points

- Metabolic targeting is an evolving form of drug development in which small molecules alter fundamental properties specific to selected organ sites. Lonidamine shows promise as a treatment for benign prostatic hyperplasia (BPH) by virtue of its ability to inhibit glucose metabolism in prostatic tissue.
- The advantage of metabolic targeting that focuses on glucose metabolism is that it affects not only rapidly dividing cells in areas of normal oxygen tension but also cells in areas of relative hypoxia, where traditional therapies are less effective.
- A unique biochemical property within prostate epithelial cells makes them dependent on glycolysis, rather than the citric acid cycle, for energy production. Lonidamine inhibits glycolysis by the inactivation of hexokinase, an enzyme that catalyzes glucose.
- The target-specific nature of lonidamine renders it a safe compound for administration. In cancer therapy, patients have been treated with 40 times the daily dose used in the BPH trial, with negligible toxicity.

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