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# Chemoprevention of Prostate Cancer with the Polyamine Synthesis Inhibitor Difluoromethylornithine

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## Abstract

In vitro and in vivo preclinical results suggest that inhibition of polyamine synthesis inhibits the progression of prostate cancer. These findings has led to two clinical trials in patients at risk for invasive prostate cancer with difluoromethylornithine which specifically and irreversibly inhibits ornithine decarboxylase which catalyses the conversion of ornithine to putrescine the rate limiting step in polyamines synthesis. We have conducted a phase IIa one month and placebo randomized phase IIb 12 months trials in patients at increased risk for invasive prostate cancer. Favorable reduction in prostate polyamine levels and prostate volume was documented with no difference in clinical hearing changes. Patients with Gleason's VI lesions in a surveillance cohort would be appropriate candidates for a definitive risk reduction trial although the unavailability of validated biomarkers for invasive progression would require a large and lengthy study.

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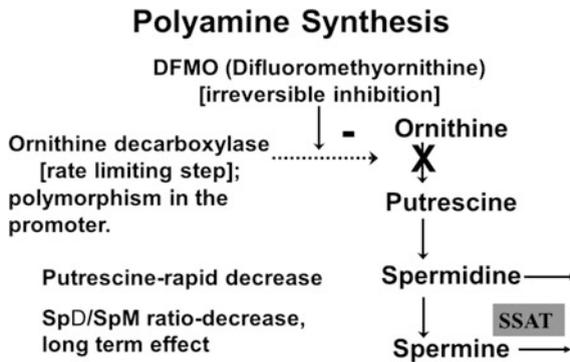
## 1 Introduction

Polyamines are small cationic molecules that have diverse roles in the cellular management of normal and malignant cells (Nowotarski et al. 2013). The biochemical pathways which intersect with polyamine metabolism are complex and a simplified version is shown in Fig. 1. Extensive discussions of the polyamine network of synthetic and catabolic enzymes and transport systems are available elsewhere (Gerner et al. 2004).

A key regulatory step involves the decarboxylation of ornithine to yield putrescine by the rate-limiting enzyme ornithine decarboxylase (ODC). This gene contains several single nucleotide polymorphisms (SNPs); under certain circumstances, one ODC SNP at +316 from the transcription start site is associated with cancer prognosis and response to certain therapies (Zell et al. 2010). In 1976 difluoromethylornithine (DFMO), a specific irreversible inhibitor of ODC, was synthesized. It is important to note that inhibition of ODC by DFMO results in a rapid decrease in changes in the levels of putrescine, while a decrease in the Spd/Spm ratio reflects long-term alterations. Therapeutic studies of DFMO in hematopoietic malignancies and solid tumors were negative (Seiler 2003), but a series of preclinical prevention studies in the late 1980s were positive, particularly in colon and prostate cancers, which led us to explore the activity of DFMO in patients with these organ site malignancies. A summary of the early findings particularly relevant to prostate cancer are summarized in Table 1 and include *in vitro* and animal model findings (Kadmon 1992). Based on these encouraging preclinical results, we began a series of trials involving patients at significant risk for progression of low-grade (Gleason's VI) prostate cancer. Until recently selection of at-risk patients were driven by clinical parameters, Gleason scores, and prostate-specific antigen (PSA) levels. We began our studies of prostate cancer chemoprevention in 1995 and have reported results from a 1-month phase IIa trial in 2001 and a 12-month phase IIb trial in 2008 (Simoneau et al. 2001, 2008).

The phase IIa trial (1 month duration) was designed as follows:

- Objective: to evaluate the effects of DFMO on polyamine levels in the prostate.
- Methodology:
  - Prospective nonrandomized study of men aged 50–85 who required prostate needle biopsy. Four additional cores were taken at time of initial contact.
  - If surgery or rebiopsy was indicated, subjects started DFMO 0.5 gm/m<sup>2</sup> orally each day for 28 days prior to a second procedure. Four additional cores were taken for analysis and analyzed for polyamine levels.



**Fig. 1** Synthesis of Polyamine: Major Pathway. The amino acid ornithine is rapidly converted by ornithine decarboxylase into the polyamine putrescine and its levels are a reliable measure of short-term changes in tissue polyamines. A series of enzymatic steps lead to formation of spermidine and the terminal polyamine spermine. Changes in the spermidine/spermine ratio reflect long-term effects. Acetylation (SSAT) of the polyamines occurs which enhances export; some NSAIDs (such as Sulindac) enhance this step

**Table 1** Historical perspective of preclinical studies of polyamines and the prostate (1978–1992)<sup>a</sup>

- ODC activity and polyamines are higher in prostatic tissue compared to other tissues
- Rats given DFMO had reduction of ODC activity to 10 % of controls by 4 h
- The prostate was more sensitive than other tissues to DFMO polyamine suppression
- DFMO can affect rat prostate weight
- ODC activity is higher in hormone unresponsive prostate cancer cell lines (G<sub>3</sub>)
- DFMO inhibited prostate cancer cell lines in vitro and in vivo

<sup>a</sup>Adapted from Kadmon (1992)

- Participants: 49 signed consent, 18 who did not have extra biopsies; 22 with first biopsy only; 10 who took DFMO; and 9 who completed pre- and post-biopsy.

The major features of the phase IIb trial (12 months) included:

- Objective: to evaluate the effects of DFMO on polyamine levels in the prostate, prostate volume, PSA levels, and toxicity.
- Methodology:
  - Men diagnosed with prostate cancer before the age of 70 who also had a first-degree relative with prostate cancer, or men diagnosed before the age of 55 designated as a proband. Their brothers and first cousins under 70 years were eligible for the study.
  - Participants had an AUA history, PSA determination, prostate ultrasound, and prostate biopsies.

**Table 2** Major findings in phase IIa and phase IIb chemoprevention trials of DFMO for prostate cancer<sup>a</sup>


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 Phase IIa (1 month, pre–post comparison)
 

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Marked reduction of putrescine, spermidine, and spermine levels in all nine participants and decreased Sp/Spm ratio in eight of nine patients

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 Phase IIb (12 month, pre–post comparison, randomized)
 

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- Prostate volume: DFMO ( $\uparrow$  0.14 cm<sup>3</sup>), placebo ( $\uparrow$  2.95 cm<sup>3</sup>);  $p = 0.03$

- Prostate putrescine: DFMO ( $\downarrow$  60.8 %), placebo ( $\uparrow$  139 %);  $p = 0.001$

The changes in volume and putrescine levels occurred in the AA and AG but not the GG ODC genotype

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- Clinical ototoxicities: no difference between arms, but subclinical changes documented by audiometry in the AA/AG group (Zell et al. 2010)

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<sup>a</sup>Summarized from Simoneau (2001, 2008)

- Prostate tissue examined for histology, polyamine content, and tissue markers.
- Participants received placebo or 500 mg/day of DFMO.
- One year later repeat studies were performed. Analysis of differences before and after DFMO, each man served as his own control.
- Participants: 140 men enrolled (consented); 81 underwent an initial biopsy, 76 men were randomized; 66 completed two sets of biopsies of which 62 finished within 12 months of study drug and an end of study biopsy.

The overall results of both trials are summarized in Table 2. The results from these trials clearly demonstrated that a low nontoxic dose of DFMO suppressed putrescine levels in the prostate rapidly and long term led to a significant decrease in prostate size that was more pronounced in patients with the AA/GA ODC allele. There was also a trend in decreasing PSA doubling time.

We and others have gained extensive clinical experience with DFMO in the prevention of colorectal (Meyskens et al. 2008) and nonmelanoma skin cancers (Kreul et al. 2012). At the doses used, this drug was nontoxic, although sub-clinical changes in hearing in a few patients were detectable by audiometry (McLaren et al. 2008).

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## 2 The Future

The results from these trials and the increasing recognition of the important role that polyamines play in cellular regulation (Agostinelli et al. 2010) have encouraged us to re-examine a potential role for DFMO in the chemoprevention of prostate cancer. Exciting new work with polyamine transport inhibitors has also refocused attention on the polyamine pathway (Samal et al. 2013). Negative results in the large PCPT and SELECT trials (Thompson et al. 2003; Algotar et al. 2013) as well as the failure of toremifene and 1  $\alpha$ —hydroxyvitamin D<sub>2</sub> (Gee et al. 2013; Taneja et al. 2013) in HGPIN has also refocused attention on DFMO. A wide

range of preclinical studies are examining the role of natural compounds in prostate cancer prevention (Horie 2012; Ozten-Kandaş and Bosland 2011; Cimino et al. 2012; Thapa and Ghosh 2012), but to date the results have been unconvincing and clinical trials have not been forthcoming (Horie 2012).

The major question then about expanding studies with DFMO is: Which group of patients should be the targeted population? Only about 20 % of patients with Gleason's 6 tumors progress to aggressive cancers. Until biomarkers are developed that can identify these patients with considerable accuracy, large definitive trials are unlikely to be undertaken. Alternatively, one might consider a trial in patients with Gleason's 7 or even 8/9 tumors, but ethical considerations might limit such a trial. Overall, definitive trials of DFMO as a chemopreventive agent await more accurate classification of risk based on a better understanding of the natural history of low-grade prostate cancer (e.g., see Earnshaw et al. 2013; Pan et al. 2012).

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