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NOVEL THERAPEUTIC MOLECULAR TARGETS FOR PROSTATE CANCER: THE MTOR SIGNALING PATHWAY AND EPIDERMAL GROWTH FACTOR RECEPTOR

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ABSTRACT

Purpose: The scientific rationale and existing evidence for the use of novel molecular targets in the chemoprevention of cancer are reviewed, with special attention to prostate cancer.

Materials and Methods: A search for relevant literature on basic science and clinical trials was conducted using PubMed/MEDLINE.

Results: The emergence of molecularly targeted therapies for advanced malignancies creates an important opportunity to examine these agents for the chemoprevention of prostate cancer. Two critical targets in the proliferation and malignant transformation of normal cells, the PI3/Akt signal transduction pathway and the epidermal growth factor receptor, are currently the focus of several novel investigational therapies that are in late stage phase II and phase III studies.

Conclusions: Research to date supports consideration of these novel molecular targets as future agents in the chemoprevention of prostate cancer.

KEY WORDS: rapamycin; receptor, epidermal factor; prostatic neoplasms

Advances in molecular genetics have identified several pathways of cellular proliferation and diminished apoptosis that represent interesting molecular targets for future chemoprevention studies. A pragmatic approach, based at least in part on the long interval between drug discovery and drug approval for advanced disease and subsequent evaluation for prevention, would view the drugs most worthy of discussion being those that are already in late stage clinical studies for advance disease. As such, 2 important molecular targets that mediate cellular proliferation, the epidermal growth factor receptor and the PI3 (phosphatidylinositol-3) kinase/Akt pathway, and the classes of agents that target these pathways and are in late stage clinical development are reviewed.

THE MTOR SIGNALING PATHWAY IN CANCER

The PI3K/Akt signal transduction pathway is an attractive target for chemoprevention drug development. The Akt/PI3 kinase pathway mediates the proliferative signals of several ligands and transmembrane receptors including insulin-like growth factor, neuron growth factor, platelet derived growth factor and immune cytokines such as interleukin-6 and 8.[1–8] Based on epidemiological evidence suggesting that insulin-like growth factors have a role in the proliferation and development of prostate cancer and on circumstantial evidence and emerging data that suggest that the inflammatory process may also contribute to prostate duct proliferation and malignant transformation, this pathway may be a strategic target for the abrogation or inhibition of malignant change. Aberrant proliferative signals from either over expression of the receptor or ligands, or inactivation within PTEN gene (phosphatase and tensin homolog deleted on chromosome 10) lead to increased cellular proliferative signals and diminished apoptosis.

The PI3 kinase pathway is regulated at least in part by functional PTEN. Inactivation of the PTEN gene has been documented with high frequency in a broad spectrum of malignancies, including prostate cancer, and results in unregulated stimulation of the Akt/PI3 kinase pathway.[9–11] Stimulation of Akt/PI3 kinase ultimately leads to translation of proteins critical for G_2/S migration and synthesis of ribosomal and growth related proteins.[12] In addition to these proliferative signals, phosphorylation of pro-apoptotic members of the Bcl-2 family, notably BAD, is a downstream even secondary to activation of the Akt/PI3 kinase pathway, thereby diminishing apoptosis.

PTEN mutations, or mutations within the 10q 23 region, occur in approximately 49% of prostate carcinomas.[13] A surge in phosphorylated-Akt (activated) is found in prostate intraepithelial neoplasia (PIN) compared to adjacent normal prostate epithelia.[14] However, not all investigators have demonstrated conclusively that phospho-Akt expression is present in high frequency in PIN. In 1 pathological series using immunohistochemical staining only 10% of PIN specimens stained positive for activated Akt.[15] In another tumor model system increased Akt expression closely demarcated cells that possess PTEN inactivation from adjacent normal cells.[16] Taken together, this evidence suggests that mutations within PTEN and increased activation of the PI3 kinase/Akt pathway may represent an attractive target for the chemoprevention of prostate cancer.

THE LONG ROAD TO THE DISCOVERY OF RAPAMYCIN

The development of specific inhibitors of the PI3 kinase/Akt pathway date back to the 1960s when a Canadian expedition traveled to Easter Island (Rapa Nui) to gather plant and soil samples. These soil samples were initially examined by what is now Wyeth Laboratories and were found to have interesting biological properties, including immunosuppressive and anticancer effects. The active agent was identified and isolated by Wyeth from the streptomyces hydroscopicus and was named sirolimus.[17] Unfortunately, due to corporate priorities the project was not fully developed for more than 10 years. Because of the signal transduction inhibitory properties observed with this agent, the immunosuppressive mechanism of rapamycin (sirolimus) was first recognized in cells dependent on the interleukin-2 receptor.

Sirolimus binds intracellularly to the immunophilin

* Financial interest and/or other relationship with AstraZeneca.

FK506 binding protein 12 (FKBP 12), and the resulting complex inhibits the protein kinase activity of mammalian target of rapamycin (mTOR). Inhibition of mTOR affects the activity of 2 separate downstream pathways that control the translation of specific mRNAs required for cell cycle traverse from G1 to S phase. Inhibition of mTOR affects the activity of the 40S ribosomal protein S6 kinase (p70s6k) and function of the eukaryotic initiation factor 4E-binding protein-1 (4E-BP1), leading to growth arrest in the G1 phase of the cell cycle. Furthermore, rapamycin prevents cyclin dependent kinase activation, inhibits retinoblastoma protein phosphorylation, and accelerates the turnover of cyclin D1 that leads to deficiency of the active cdk4/cyclin D1 complexes, all of which inhibit G1/S traverse.[17] In 1987 research into sirolimus was resurrected following the merger of Wyeth and Ayerst, and was developed as an immunosuppressant agent. Concurrently, the role of Akt/PI3 kinase pathway and mTOR in the growth and proliferation of cancer cells was further delineated and this agent was selected for anticancer development. Noteworthy, preclinical data indicate that PTEN null cells have enhanced sensitivity to mTOR inhibition in vivo.[18]

In addition to the antiproliferative effects on malignant cells, antiangiogenic properties were associated with this class of compounds as first described by Guba et al.[19] Rapamycin leads to inhibition of endothelial cell proliferation and decreased vascular endothelial growth factor expression. The impact of the antiproliferative effects on malignant cells and the anti-invasive and antiangiogenic effects appear to vary with dose and schedules.[19]

There are several mTOR inhibitors in clinical development for cancer therapy. CCI-779 is a rapamycin derivative developed by Wyeth-Ayerst which has completed phase I studies as a single agent using intravenous formulation and oral formulation. It is currently in combination studies with other anticancer agents and in a broad spectrum of phase II single agent studies. A testament to the interest in this class of compounds, several analogues are currently being clinically developed by large and small pharma.

The first clinical trials of patients with CCI-779 were performed in the United States and Europe. The daily times 5 intravenous schedule was used at The Cancer Therapy and Research Center in San Antonio along with the Mayo Clinic, whereas the weekly schedule of intravenous rapamycin was used in Europe. Remarkably, antitumor activity was seen across a broad spectrum of doses. Notable in the phase I studies were bonafide partial responses seen in renal cell carcinoma, nonsmall cell lung cancer, breast cancer and a neuroendocrine tumor. The toxicity observed is considered moderate in relation to other anticancer agents. This agent also has an extensive safety database from organ transplantation cases and the use of rapamycin coated stents for the prevention of coronary artery stenosis.

No nausea or vomiting was seen and, therefore, no premedication was required. Opportunistic infections were not observed in either of the phase I studies. There was only a modest amount of hematological toxicity noted at most dose levels. Some central nervous system effects, including excitation and depression, were noted at high doses, and testosterone levels decreased in some male patients. In addition, skin toxicity was commonly observed and described as small erythematous papules and folliculitis observed on the trunk and face of some patients, as well splitting at the base of nail was. In the weekly study a maximum tolerated dose was not determined. Doses of 7.5 to 220 mg/m^2 could be administered weekly without dose limiting toxicity and without exceeding the threshold for dose limiting toxicity. In the United States study 15 mg/m^2 administered daily for 5 days was administered safely in patients who were heavily pretreated, whereas 24 mg/m^2 intravenously daily times 5 days could be administered to patients who were minimally pretreated. Dose limiting toxicity included grade 3 elevation of liver function abnormalities at 19.1 mg/m^2 and grade 3 hypocalcemia. There was no obvious relationship between dose and observed activity with tumor regression observed across the entire spectrum of doses.[20]

RAPAMYCIN ANALOGUES AS POTENTIAL CHEMOPREVENTIVE AGENTS

Rapamycin analogues are attractive chemoprevention agents. They are orally bioavailable, have an established toxicity profile culled from many years of use in the transplantation setting, are active compounds leading to apoptosis and tumor regression in advanced disease, and target a critical signal transduction pathway used in many malignancies. The clinical development of targeted therapies has created new challenges for the interpretation of efficacy and successful drug approval. The template for most oncology registration strategies has been tumor site specific, irrespective of the molecular heterogeneity that ultimately leads to malignant transformation. This design has also been applied to the limited number of prevention studies performed to date, including tamoxifen for breast cancer prevention and finasteride for prostate cancer prevention. However, it is probable that with rapamycin, an agent that specifically targets tumor growth mediated by dysregulated activation of Akt/PI3 kinase pathway, will have activity only in cells in which this pathway is critical for malignant transformation and proliferation. Therefore, the rational clinical development of this drug for chemoprevention may be limited to patients who are at high risk for prostate cancer and who demonstrate evidence of PTEN inactivation or increased Akt activation in prostate or PIN cells detected at biopsy. This niche market may represent an opportunity for rational chemoprevention but also a potential limitation to future market size—an issue critical to the acceptance of chemoprevention agents as viable "products" by manufacturers.

THE EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) AS A TARGET FOR CHEMOPREVENTION

EGFR over expression and the ligand transforming growth factor-α have been identified in pathological specimens containing high grade prostatic intraepithelial hyperplasia, whereas less frequent and lower expression has been noted in low grade PIN and normal prostatic epithelia.[21–23] Furthermore, tumor transforming growth factor-α expression is increased in specimens containing PIN, a coexisting carcinoma, suggesting a role in the proliferation of early transformed prostate epithelia.[21] EGFR is a member of a family of erbB receptors and ligands. ErbB2 or Her2/neu is the prototypic member of this family associated with poor prognosis in breast cancer, and is the target for the first successful development of a molecularly targeted agent in solid tumor oncology (trastuzumab). Other EGFR family members, erbB3 and erbB4, do not have a defined role in the transformation and proliferation of prostate cancer. Members of the erbB receptor family undergo homo-dimerization and hetero-dimerization in response to relevant ligand binding, resulting in tyrosine kinase activity at the intracellular receptor domain, phosphorylation and signaling of the MAP kinase pathway.[24]

There are several EGFR targeting therapies in clinical development. Monoclonal antibodies, chimeric (Imclone C225) and human (Abgenix EGF), are currently in late phase III clinical development for advanced solid tumors including colorectal and nonsmall cell lung cancer. Small molecule tyrosine kinase inhibitors, such as ZD1839 and OSI-774, are also in late stage clinical studies, with ZD1839 pending regulatory approval for the treatment of nonsmall cell lung cancer.[25] Characteristic toxicities associated with monoclonal antibodies include an acneiform rash that forms on the

upper body and face that is dose dependent, which is consistent with folliculitis and is treated with topical steroids and minocycline.

Albeit infrequent, hypersensitivity reactions are associated with use of the chimeric monoclonal antibody C225.[26,27] ZD1839 and OSI-774 have elimination half-lives that approximate 24 hours and are administered once daily. Toxicity profiles associated with small tyrosine kinase inhibitors is similar, although, in addition to the acneiform rash, diarrhea is dose-limiting. Diarrhea is manageable with the use of simple antidiarrheal agents such as imodium. In contrast to the small molecule tyrosine kinase inhibitors in which daily dosing is appropriate, the monoclonal antibodies all have relatively long elimination half-lives that extend from 10 to 20 days and can be administered weekly or potentially longer.[25] All of these agents have demonstrated single agent antitumor activity.[28]

Abnormal growth factor receptor targeting agents may be attractive for chemoprevention studies. Two or three agents may be approved in the next 2 to 3 years, including C225, Abgenix EGF antibody and Iressa. Based on intriguing evidence that EGFR is over expressed in PIN, the good tolerability profile of these agents in chronic dosing schedules, and evidence of apoptosis induction and regression of tumors that EGFR expression is critical for cell proliferation and survival, the selection of these agents for chemoprevention studies represents a rational "next step". The identification of the appropriate subgroups at high risk for prostate cancer and in which EGFR expression is a pivotal driving molecular pathway remains an important challenge for this class of molecularly targeted agents.

CONCLUSIONS

Although several molecular targets are attractive for chemoprevention, those pathways with late stage clinical development represent the most practical agents to consider for prevention studies. These include agents that target EGFR and mTOR signaling pathways, which are associated with modest toxicities in phase I and phase II studies, can be administered for prolonged periods to patients and, therefore, are well suited to chemoprevention strategies. The key to these molecularly targeted agents will be identification of predictive biomarkers so that appropriate patients are selected as candidates for studies to determine the efficacy of these agents.

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DISCUSSION

Dr. Ian M. Thompson. When we consider chemoprevention, we think about giving the patient an agent on a daily basis. If we really believe that premalignant intraepithelial lesions ultimately invade, I wonder whether we could possibly pulse some of these agents, allowing the patient to shed the so-called "bad" epithelial cells. The kinds of agents you described, especially those with long half-lives, might be very amenable to this approach.

Dr. Anthony W. Tolcher. One of the nice things about some of the antibodies is that they have a tremendously long half-life. HER-2 over expression is found commonly in carcinoma in situ of the breast. The question is can one target that with an antibody such as herceptin or an anti-EGFR antibody, which has an elimination half-life of 21 days, potentially allowing monthly administration for 6 months followed by 3 additional months of coverage. That may be all that is necessary. If the cells are dependent on that pathway for proliferation or cell survival, once you take away that cell proliferative pathway, the cells will undergo apoptosis, which clearly does occur in certain dependent cells. You would actually have a brief period of intervention instead of lifelong drug therapy.

Dr. Leslie G. Ford. The rash is clearly a limiting factor. Is it dose dependent or are some people just reactors?

Doctor Tolcher. The rash is certainly dose dependent. Currently, we actually like to see the rash because we are still testing toxicity. In the model we use for the cytotoxic therapy, if you do not see decreasing blood counts, you think you are not giving enough chemotherapy.

Doctor Ford. Given the redundancy, when you block 1 part of the pathway or hit 1 target, everything just finds another pathway. Is it realistic to think you could give a single agent?

Doctor Tolcher. I will tell you why I think so. Although I agree that there is redundancy, I think that we sometimes become too negative. We take it for granted that it will just be compensated but patients actually have tumor regression with some of these agents, thereby demonstrating critical reliance on 1 single pathway for some cells.

Doctor Ford. But it isn't disappearance; the tumor regresses and then comes back at some point.

Doctor Tolcher. The multitargeted, multistep process of malignancy may be such that when we are targeting it at its earliest form, we are actually looking for 1 or 2 mutations and not 7 or 8. We are not looking at redundant systems that are already in place.

Doctor Ford. Some of the agents being developed for treatment should actually be developed for prevention. However, it is difficult to get a pharmaceutical firm to think that way.

Dr. Neil Fleshner. Given that prevention is such a long business and patent lives are shorter than that, unless we have an intermediate end point, how will any pharmaceutical company invest in prevention in the real world? There may be agents that have no efficacy in end stage disease or in established disease, but may be useful as preventive agents, and we will never see them developed.

Doctor Tolcher. We have to change the way we develop and approve drugs. You currently see advertisements promoting an analogue of sirolimus as a nonsteroidal cream for eczema. It is interesting that we can get access to a drug like that to treat eczema but cannot get a drug like sirolimus approved for oncology. Currently, sirolimus is only approved for transplants.