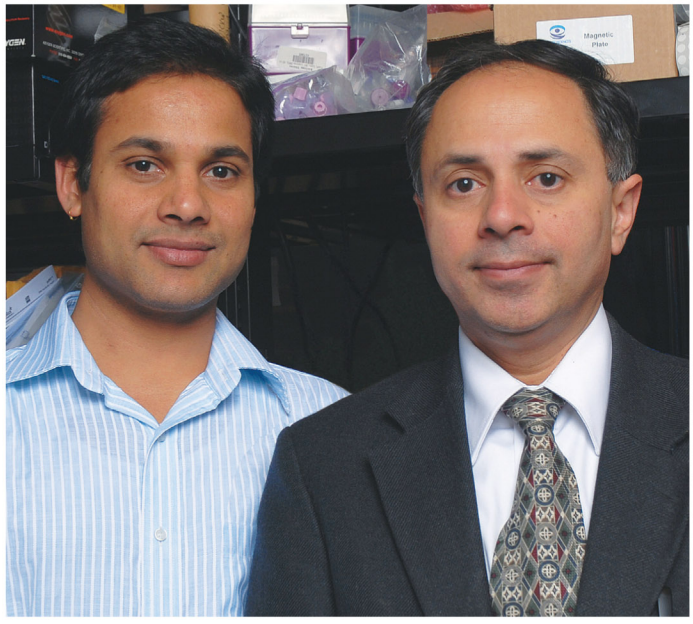
Prostate Cancer: The Challenge in Detection

STANFORD SCHOOL OF MEDICINE

Stanford University Medical Center

Please note that in all cases the text under *Original Proposal* is significantly reduced from the four to six page proposals submitted by the institutions. Please contact the Foundation if you would like to read the original full research proposals.



Principal Investigator - above right:
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Original Proposal

We intend to revolutionize prostate cancer management based on *Theranostics* – the combination and consequent synergy of state-of-the-art, multimodality molecular imaging for *diagnosis* with high specificity molecular-targeted *therapeutics*.

Photoacoustic molecular imaging is an emerging technology that overcomes, to a great extent, the spatial resolution and depth limitations of whole-body optical imaging. Photoacoustic imaging is capable of monitoring molecular levels of cancer-specific proteins in a living subject. In this technique, a conventional photoacoustic imaging instrument is coupled with an imaging agent (contrast agent) targeted to a cancer-specific protein to achieve a specific imaging signal. The concept is that light goes into the body and sound comes out. Nanoengineering is employed to develop the imaging agent--nanoparticles specifically designed to seek and identify prostate cancer at the cellular level. The imaging agent is introduced into the body and the nanotubes attach to prostate cancer cells wherever they may be, in the gland or out if the cancer has metastasized. The laser light is passed over the subject causing the nanotubes to emit an ultrasound that is captured and imaged outside of the body. The next step would be to design the nanotubes to carry a pay load. Then an audio sound could be sent back into the body that triggers the nanotubes to destroy the cancerous cells only.

Progress Report

Nearly 200,000 cases of prostate cancer are diagnosed in the US yearly from more than 800,000 biopsy procedures[1,2]. Despite the huge number of biopsies performed, current prostate biopsy schemes miss approximately 15-25% of incident cancers[1]. Prostate cancer is the only malignancy diagnosed by blind biopsy – usually prompted by elevated serum PSA levels. Because of the inaccuracies of early detection methods, many men without prostate cancer receive unnecessary prostate biopsies, with the attendant morbidity[3].

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New imaging approaches may provide a more sensitive and more accurate approach for the detection of prostate cancers reducing the number of missed diagnoses.

Standard screening methods used by physicians - such as blood screening for prostate specific antigen (PSA), digital rectal examination, and transrectal ultrasound (TRUS) biopsy – are not accurate in early diagnosis of prostate cancer. Other techniques such as magnetic resonance imaging (MRI), computed tomography (CT), and positron emission tomography (PET) use ionizing radiation, lack clarity and/or are prohibitively expensive for routine use. While TRUS has great utility in guiding biopsy needle placement, its utility for screening and detecting prostate cancer, particularly aggressive prostate cancer, is very limited[4] primarily due to its limited ability to differentiate between tissue types. Despite the limitations, it is important to note that TRUS is used by virtually all urologists in practice in a clinical setting and is therefore an ideal platform for adaptation as an improved imaging tool with great promise for applications involving imaging agents and new technologies for distinguishing aggressive from essentially benign disease.

Photoacoustic imaging (PAI) is a newly emerging strategy for applications in cancer detection, diagnosis and therapy monitoring, which essentially lets us "hear" light (Figure 1). Photoacoustic strategies allow deeper tissue penetration while preserving the spatial resolution advantages of ultrasound. Therefore, objects within a human body can be more clearly visualized at a depth that is clinically relevant. Previous work has shown the utility of photoacoustics for imaging various endogenous signals from tissues (i.e. signal from molecules found naturally in cells)[5]. We are currently investigating a potential way to use photoacoustic imaging in combination with TRUS in the clinic, PAI/TRUS. PAI/TRUS enables visualization of not just the prostate but any objects within the organ that absorb light differently from the rest of the tissue. Because the primary light absorber is hemoglobin, most of the differential light absorption comes from areas within the prostate that are more highly vascularized, a common property of tumors. In addition, injection of a dye already approved by the FDA for human use, such as Indocyanine Green (ICG), can increase the photoacoustic image contrast within the blood vessels and add sensitivity to the PAI/TRUS technique. Therefore, PAI/TRUS could be used to target biopsies to areas

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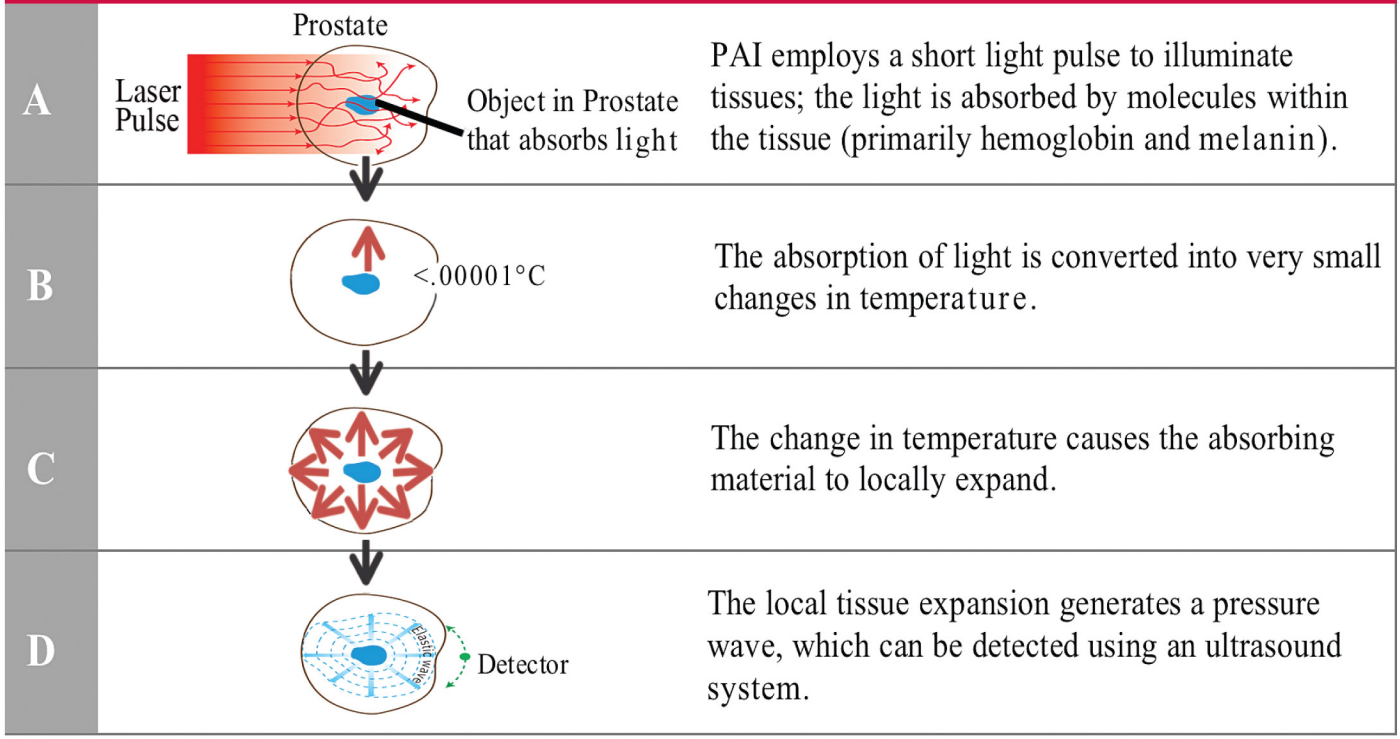
Figure 1: How do we "hear" light?



within the prostate where there is a potential problem. Because it is built upon widely available ultrasound technology and FDA approved dyes, combined PAI/TRUS technique could be translated to the clinic relatively quickly.

To study PAI capabilities in prostate cancer screening, we first developed a transrectal photoacoustic imaging device (Figure 2). The dimensions of this hand held device are in accordance with standard dimensions of clinical transrectal ultrasound (TRUS) probe. We validated deep tissue imaging capabilities of the device using prostate phantoms that simulate ultrasound and optical properties of prostate tissue. Currently we are testing the device on surgically removed human prostates. Internal Review Board (IRB) approval has been obtained for a study of the device in human prostate cancer screening. In a few months we will initiate clinical studies using a combined transrectal ultrasound and photoacoustic imaging device.

References

1. Singh, H. et al. Predictors of Prostate Cancer After Initial Negative Systematic 12 Core Biopsy. *J. Urol.* **171**, 1850–1854 (2004).

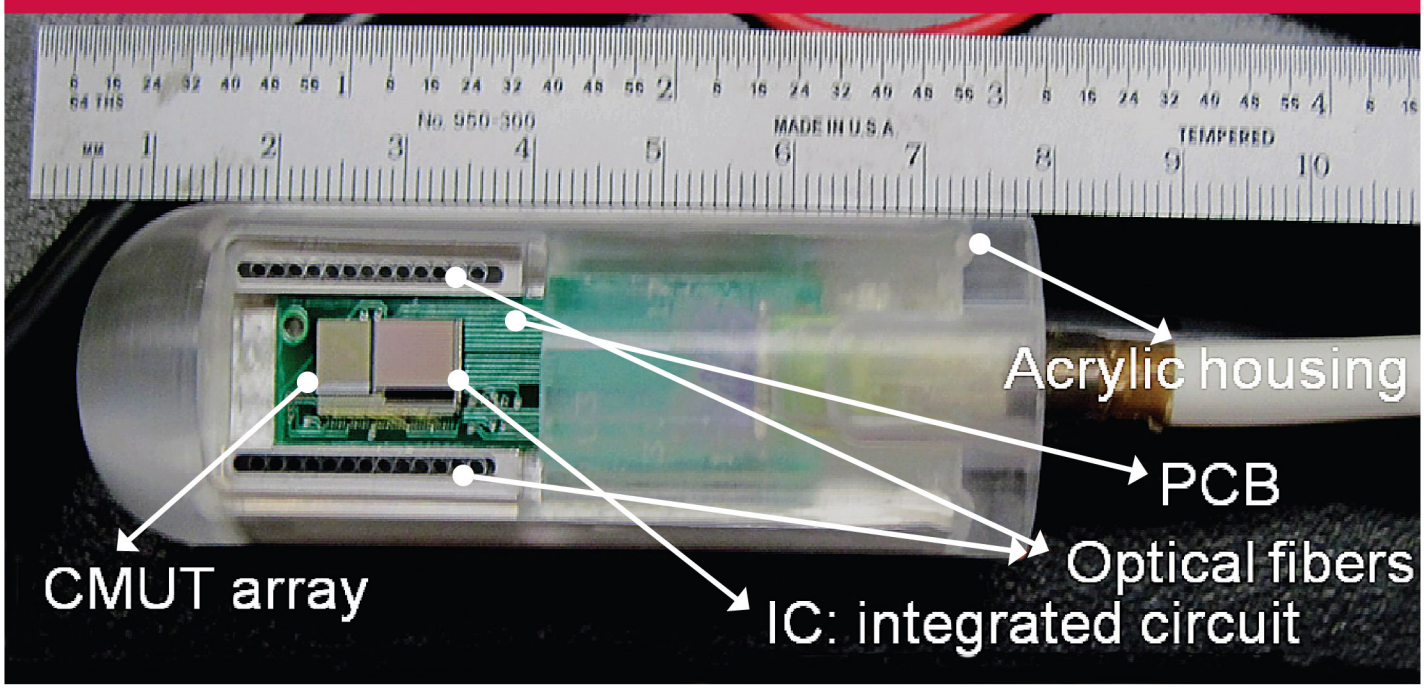
2. Siegel, R., Ward, E., Hao, Y. & Xu, J. Cancer statistics, 2009. CA: *a cancer journal* … (2009).

3. Ghani, K. R., Dundas, D. & Patel, U. Bleeding after transrectal ultrasonography-guided prostate biopsy: a study of 7-day morbidity after a six-, eight- and 12-core biopsy protocol. *BJU Int.* **94**, 1014–1020 (2004).

4. Carter, H. B. *et al.* Evaluation of transrectal ultrasound in the early detection of prostate cancer. *J. Urol.* **142**, 1008–1010 (1989).

5. Wang, X. *et al.* Noninvasive laser-induced photoacoustic tomography for structural and functional in vivo imaging of the brain. *Nat Biotechnol* **21**, 803–806 (2003).

Figure 2: Transrectal photoacoustic probe



Transrectal photoacoustic probe. IC and CMUT are flip-chip bonded and placed on PCB (printed circuit board). PCB is rested in between two parallel fiber optic light guides that focus light 0.5 inch above the CMUT surface. The entire PCB-light guide assembly is housed in an acrylic probe that has similar dimensions as conventional transrectal ultrasound probe.

Imaging of PSMA in Prostate Cancer and Beyond

Memorial Sloan-Kettering Cancer Center

Principal Investigator:

Hedvig Hricak, MD, PhD, DrHC

Chairman of Department of Radiology

Carroll and Milton Petrie Chair

Peter Michael Postdoctoral Fellow:

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Original Proposal

The overall goal of the project is to develop new tools to detect prostate cancer. Using the same imaging agent, we aim to furthermore detect cancer independent of the tumor type by their neovasculature. The objective of this proposal is to gain insights into the function of prostate specific membrane antigen (PSMA) in prostate cancer and tumor neovascularization utilizing novel imaging probes and to exploit these to monitor current and create future therapies. PSMA can provide several valuable advantages over other antigens, because i) the expression on prostate cancer and neovasculature of other tumors (and lack thereof on normal vasculature) makes it an ideal tumor target; ii) dose-dependent internalization of PSMA results in accumulation of the targeted agent in the tumor; and iii) the enzymatic activity can be used to image PSMA funciton with an activatable probe and to treat tumors with a prodrug.

Progress Report

As the 2011 Peter Michael Fellow at Memorial Sloan-Kettering Cancer Center (MSKCC), Dr Hebert Alberto Vargas has worked closely with other members of the prostate cancer management team to investigate the value of conventional and novel magnetic resonance imaging (MRI) techniques for prostate cancer management. His research efforts are summarized below by subject area.

MRI for pretreatment assessment of clinically low-risk prostate cancer: In a recently submitted article, Dr. Vargas reported results from the first prospective study evaluating the performance characteristics of MRI in a large cohort of patients with clinically low-risk prostate cancer (B1). The study showed that tumors with volumes <1 cc and Gleason scores ≤6 could not be reliably detected on prostate MRI, but that MRI performed well in

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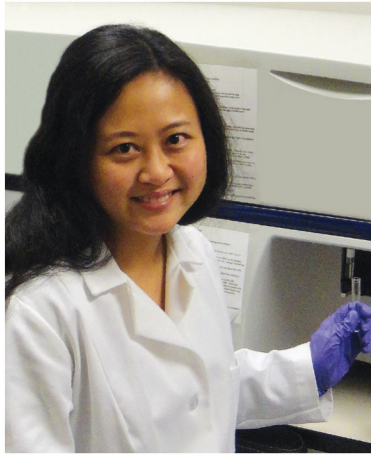
Next Generation Therapeutic Cancer Vaccines for Prostate Cancer

UCSF Helen Diller Family Comprehensive Cancer Center



Principal Investigator:

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Peter Michael Postdoctoral Fellow:

Serena Kwek, PhD

Original Proposal

We have discovered that the immune systems in some prostate cancer patients can recognize their own cancer. Tumors, however, can produce different substances and create an environment within the host to dampen these immune responses. By introducing a treatment that can stimulate the immune system, we can drive the immune system to overcome this immunosuppressive milieu, thereby inducing anti-tumor responses. One such approach involves treating prostate cancer patients with both a bone marrow growth factor, GM-CSF, as well as an anti-CTLA4 antibody. The former drug serves to expand the number the cells that serve to educate the immune system, instructing it on what to target. The latter drug releases the brakes on the immune system by blocking one of the crucial immune system checkpoints.

We propose to define the immune targets (antigens) to which patients receiving CTLA4 blockade treatment are responding. Because some patients have dramatic clinical responses and others do not, we can determine whether immune responses to particular antigens are associated with clinical responses or, alternatively, side effects. Moreover, we can determine whether preexisting immune responses to particular antigens could predict who will respond to this treatment. These results could help guide us to select the patients who would derive a clinical benefit from this treatment. Rather than identifying these antigens in animal models, the approach by which most immunotherapies are developed, our proposal focuses on the antigens relevant for prostate cancer patients. As a result, the antigens that we discover should be immediately relevant for humans.

Progress Report

Treatment with anti-CTLA4 antibody (ipilimumab), which is now FDA approved for melanoma, can lead to clinical responses in prostate cancer patients. Using arrays bearing over 8000 different human proteins, we are profiling the immune responses of patients receiving this treatment to identify the target proteins, or antigens, which the

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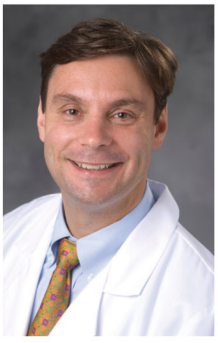
Cell-specific Interference Strategies for Prostate Cancer

Duke Cancer Institute

UNC ESHELMAN SCHOOL OF PHARMACY

Principal Investigators:



Daniel George, MD, Duke

Rudy Juliano, PhD, UNC

Bruce Sullenger, PhD, Duke

Peter Michael Postdoctoral Fellow:

Jennifer Freeman, PhD

Original Proposal

Interference RNA (RNAi) strategies represent a potentially new targeted approach to silencing specific genetic pathways within cancer, however, efficient delivery of small interfering RNA (siRNA) molecules into target cells is a major obstacle to developing this modality into effective therapy. RNA aptamers represent another novel form of RNA therapeutic – one which binds specifically to unique peptide sequences. Using an RNA aptamer for prostate specific membrane antigen (PSMA), we have created a delivery mechanism that not only results in cell specific binding, but also rapid and efficient endocytosis.

Therefore we propose to develop a novel RNA chimera, combining our PSMA aptamer with an AR-specific siRNA. We hypothesize that a PSMA aptamer-AR (androgen receptor) siRNA chimera will demonstrate cell-specific inhibition of AR that is more potent than known AR antagonists. The specific aims of the research are to:

- Create and demonstrate the in vitro specificity and efficacy of the PSMA aptamer-AR siRNA Chimera.
- Demonstrate in vivo activity of the PSMA
- aptamer-AR siRNA Chimera

Progress Report

Men diagnosed with early stage prostate cancer undergo primary therapies of surgery or radiation, but 20-40% of patients experience recurrence of cancer following therapy. Advanced prostate cancer is treated with hormone therapy, which reduces the levels of male hormones (androgens) in the body. Androgens play a critical role in the growth and survival of prostate cancer cells. Hormone therapy is initially effective, but within 2-3 years most patients develop resistance to this type of therapy. Treatment of disease that continues to progress with chemotherapy results in only modest improvements in survival. It is estimated that 1 in 36 American men will die of prostate cancer. Clearly, a desperate need exists to better understand why patients develop resistance to hormone therapy and to develop novel therapeutic agents to treat patients with prostate cancer. Our team is focusing on developing novel agents to reduce the function of the protein that is required for androgens to exert effects on prostate cancer cells (the androgen receptor). In addition, we are also focusing on selectively delivering these novel therapeutic agents to the compartment within prostate cancer cells where such agents will have their biologic effect, the cell nucleus. This targeted delivery should improve the efficacy of the therapy while reducing its cost and potential side effects. Successful completion of this

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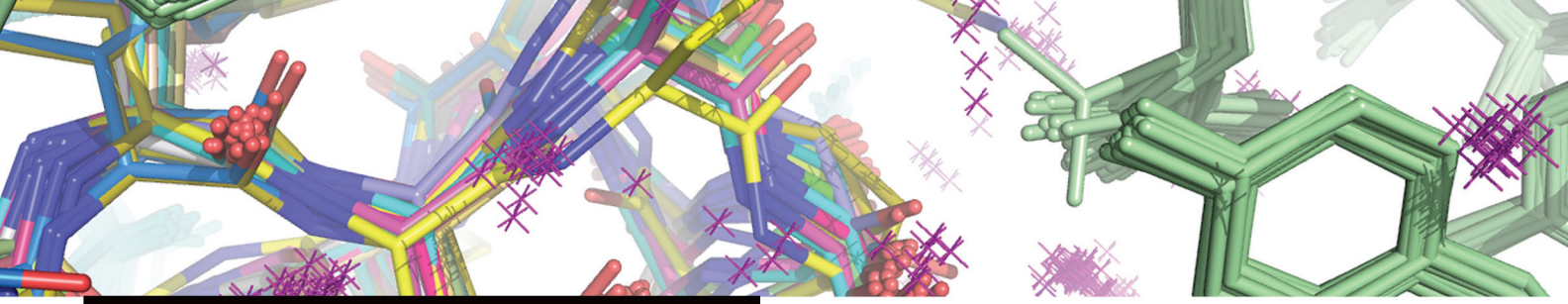

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detecting lesions ≥1 cc of any Gleason score and lesions ≥ 0.5 cc with Gleason scores ≥7. These findings have important implications for the use of MRI in prostate cancer management, particularly in active surveillance and guidance of focal therapy.

Because the extent of prostate cancer is often underestimated on initial biopsy, confirmatory biopsy is sometimes performed in patients with clinically low-risk cancer before active surveillance is begun. A study led by Dr. Vargas assessed the value of MRI in predicting confirmatory prostate biopsy findings. The study found that, when read by experienced radiologists, prostate MRI was a strong predictor of confirmatory biopsy findings and could potentially obviate the need for confirmatory biopsy (B2).

MRI for detecting prostate cancer recurrence: Dr Vargas facilitated the publication of a study begun in 2010 showing that the addition of dynamic contrast-enhanced (DCE) MRI to anatomic MRI improves the detection of post-treatment recurrence of prostate cancer, especially by relatively inexperienced readers (A1). Thus, DCE-MRI could potentially facilitate the use of prostate MRI at centers lacking experienced genitourinary MRI specialists.

Factors influencing MRI interpretation: On T2-weighted (T2W) MRI (the standard MRI sequence for prostate cancer detection), post-biopsy changes can hamper cancer identification and lead to false positive results. Areas of prostate cancer have been found to show less post-biopsy changes than areas of benign changes at pathology. Furthermore, outlining of tumors by post-biopsy change, dubbed the "hemorrhage exclusion sign," has been anecdotally reported on T1-weighted imaging. Dr. Vargas collaborated on the first study showing that the presence of the hemorrhage exclusion sign on T1-weighted MRI, along with a finding of cancer in a corresponding location on T2W MRI, is highly predictive of prostate cancer (A2).

MRI before biopsy for suspected prostate cancer: The initial biopsy detects prostate cancer in only 22% of men suspected to have the disease. Given the frequency of negative biopsies, it is understandable that many men choose not to undergo biopsy after weighing the benefits and risks. A noninvasive diagnostic tool that reliably identified normal prostate tissue without missing cancer could be used to prevent unnecessary biopsies. Such a diagnostic tool would need to combine high negative predictive value with high sensitivity. Dr. Vargas collaborated with researchers in Germany on a study examining the value of MRI performed before 12-core systematic biopsy in 90 men suspected to have prostate cancer (B3). MRI ruled out high-grade prostate cancer on subsequent biopsy with high negative predictive value (91.9%-92.4%), suggesting that it could be of value in patients unwilling or unable to undergo biopsy. However, sensitivity was low, indicating that such patients would need to undergo close follow-up despite negative MRI findings.

A) Work published/accepted for publication in 2011

A1. Wassberg C, Akin O, **Vargas HA**, Shukla-Dave A, Zhang J, Hricak H. The incremental value of contrast-enhanced MR imaging in the detection of biopsy proven local recurrence of prostate cancer after radical prostatectomy: effect of reader experience. Am J Roentgenology (In press)

A2. Barrett T, **Vargas HA**, Goldman D, Akin O, Hricak H. The value of the "Hemorrhage exclusion" sign on T1-weighted prostate MRI for the detection of prostate cancer. Radiology 2012 (In press)

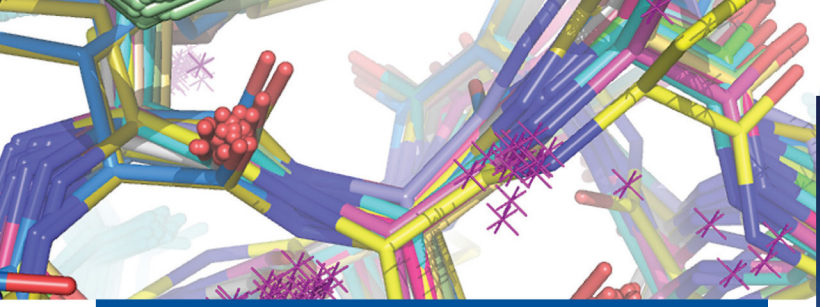
B) Work submitted for publication

B1. **Vargas HA**, Akin O, Shukla-Dave A, Zhang J, Zheng J, Kanao K, Goldman D, Moskowitz CS, Reuter V, Eastham J, Scardino P, Hricak H. Performance Characteristics of MRI in the Evaluation of Clinically Low-Risk Prostate Cancer: A Prospective Study

B2. **Vargas HA**, Akin O, Afaq A, Goldman D, Zheng J, Moskowitz CS, Shukla-Dave A, Eastham J, Scardino P, Hricak H. The Value of Magnetic Resonance Imaging in Predicting Confirmatory Prostate Biopsy Findings in Patients Being Considered for Active Surveillance of Clinically Low-risk Prostate Cancer.

B3. Franiel T, **Vargas HA**, Mazaheri Y, Böhmer S, Schröder J, Hricak H, Akin O, Beyersdorff D. Prostate Cancer Screening: Role of MRI in Ruling out Prostate Cancer

Memorial Sloan-Kettering Cancer Center



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work will lead to a more thorough understanding of mechanisms of resistance to hormone therapy as well as yield novel highly targeted therapeutic agents to treat patients with prostate cancer.

Our team is focusing on developing and selectively delivering agents to prostate cancer cells that inhibit the function of the androgen receptor in ways that are fundamentally different from all current strategies of hormone therapy. All current strategies of hormone therapy target either androgen or the interaction between androgen and the androgen receptor. The work we are doing will use a common platform to develop agents that can repair altered forms of the androgen receptor that are mutated and over-active in prostate cancer cells or interfere with the domain of the androgen receptor that is absolutely required for exertion of its biologic effects. To do this, we will utilize splice-switching oligonucleotides, small nucleic acids that can correct production of aberrant variants of genes and produce novel variants of genes. A member of our team, Rudy Juliano, PhD at the UNC School of Pharmacy is a world expert in splice-switching oligonucleotides. In addition, our work will use an innovative strategy to selectively deliver these novel therapeutic agents to prostate cancer cells and localize them to the compartment within the cancer cells where such agents will have a biologic effect, the cancer cell nucleus. To do this, we will utilize a nucleolin aptamer, a small nucleic acid that specifically binds a protein that shuttles between the cell surface and the nucleus. Another member of our team, Bruce Sullenger, PhD at Duke University Medical Center, is a world authority in this field of research.

We have demonstrated binding of the nucleolin aptamer to a panel of androgen-dependent and androgen-independent prostate cancer cell lines. In addition, we have designed and synthesized splice-switching oligonucleotides to repair altered forms of the androgen receptor that are mutated and over-active in prostate cancer cells and to interfere with the domain of the androgen receptor that is absolutely required for exertion of its biologic effects. Currently, we are performing assays to test the potency of these splice-switching oligonucleotides. Once potency of these splice-switching oligonucleotides is established, we will append these splice-switching oligonucleotides onto the nucleolin aptamer and deliver these nucleolin aptamer-therapeutic oligonucleotide chimeras to prostate cancer cells. Ultimately, such a strategy could allow us to further elucidate the mechanisms of resistance to hormone therapy as well as create novel agents that can serve as potential new therapies for treating prostate cancer patients.

Helen Diller Family Comprehensive Cancer Center / UCSF

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immune system is recognizing following this treatment. So far, we have validated one antigen, Pak6, that is expressed in prostate cancer to which patients who clinically responded to the treatment developed an immune response. We have found that this protein is mislocalized in certain prostate cancer cells, including cells that are no longer androgen sensitive. We are studying whether this protein is mutated or altered in the cancer cells and whether this protein contributes to the proliferative capacity of cancer cells. This work would lay the ground work for whether we can use this protein as a treatment target in prostate cancer.

We have also taken this protein candidate into in vivo immunogenicity studies in animal models. We can show that vaccination with this new antigen not only induces an immune response, but can also protect mice against tumor challenge in both the TRAMP and Myc-CAP mouse models of prostate cancer. We are now trying to optimize our vaccine approach so that we can induce a maximal immune response to this antigen. The goal would be to translate this vaccine back into patients with prostate cancer. In addition, work continues on the validation of other vaccine candidates that we have identified.

Publications

Kwek SS, Cha L, Fong L. Unmasking the immune recognition of prostate cancer with CTLA-4 blockade. Nature Reviews Cancer 4/2012 in press.

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