SEATTLE PROSTATE INSTITUTE

PCa Commentary
Vol. 69: May-June 2011

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BASIC SCIENCE & PROSTATE BIOLOGY: Prostate Cancer's Hidden Biology

The unseen biology of the early spread of prostate cancer hides sobering facts. *Prior to surgery* 45% (8/19) of men with "localized cancer" exhibited circulating tumor cells (CTC); less surprisingly, 64% (23/36) of men with metastatic disease carried blood born cancer cells. (Stott et al., *ScienceTranslationalMedicine*, March 2010)

Kollerman et al. (*J Clin Oncol* 26:2008) studied 193 men with cT1-3 cancer *prior* to surgery and found disseminated tumor cells (DTC) in 44.6% of *pretreatment* bone marrows. Of the men with Gleason scores of ≤6, 43.2% were marrow positive; of those with stage pT2 stage, 43.7% were positive; and of those with a baseline PSA of <10ng/ml, 44.3% showed cancer. They found no correlation between tumor grade, Gleason score, nor clinical risk category, making DTCs a significant independent predictor of PSA relapse. The marrow positive cohort had a 47.7% PSA relapse rate vs. 28% of the marrow negative men at a median follow-up of 16 months. Surgery had been preceded by 8 months of androgen deprivation and the authors concluded that the marrow DTCs survived unaffected by treatment.

These findings are both of concern and rather puzzling - of concern because many of these men by standard staging classification are deemed good candidates for "curative" primary local therapy but relapse early compared to men lacking these CTCs; and, puzzling because some men who harbor these disseminated cells proceed live on to become "clinical cures."

What is known about the certainty of identifying these "CTCs" as actually cancer cells and about the frequency of their successful engraftment as active metastases? To what extent are CTCs predictive of treatment outcome? What is the mechanism of this early spread and what explains their preferential homing to bone marrow? Which begs the question, what is meant by tumor "dormancy?"

First things first. For information about CTCs to be clinically useful it is mandatory that these cells are positively identified as prostate cancer and not leukocytes or epithelial cells from other origins. This challenge is being met by various combinations of surface markers coupled with the basic tumor-specific biomarker, PSA. In the article by Stott et al., "Isolation and Characterization of Circulating Tumor Cells from Patients with Localized and Metastatic Prostate Cancer," the detection method was essentially microfluidic antibody capture of cells expressing EpCAM, a common epithelial cell surface marker, followed by selection of PSA positive cells. The CellSearch System (Veridex, LLC) is FDA approved as a validated detection platform for identifying CTCs and has been cleared for use as a prognostic indicator for *metastatic* prostate, breast and colorectal cancers.

CTCs are very rare - about 1 in 10 billion. In Stott's study the threshold for positive detection was set at ≥14 CTCs/ml and in the 42% of men with *localized prostate cancer* the median was 95 cells/ml with a range of 38-222. The captured cells were heterogeneous with some presumed to be metastatic precursors with proliferative and engraftment potential (positive for Ki67 staining - range 1% to 81%) and others nonproliferating, possibly having entered the circulation through leaky tumor vasculature.

By enumerating the number of CTCs prior to prostatectomy and at intervals after surgery, Stott established the likely half-life of CTCs at less than 24 hours. The explanation of some CTCs persisting up to 3 months after surgery is that these cells "are derived from extraprostatic sites of disease that continue to shed CTCs into the circulation for a limited time after resection of the primary tumor."

Technical improvements in automated imaging and standardized scoring algorithms enable serial testing, veritable "liquid biopsies," to monitor disease progression, response to treatment, sensitivity to drugs, and the biologic properties of metastasizing cells. In Stott's study, as with the Kollerman analysis, "The presence or absence of CTCs was not correlated with preoperative serum PSA concentrations or with standard measures of tumor grade (Gleason score)" confirming CTCs as an independent variable, pointing up that much is yet to be learned about which cells "leave home early". Fortunately a very low percentage of CTCs - possibly only 1% - with metastatic potential are thought to successfully accomplish all the demanding feats essential for successful engraftment.

The preferential spread of prostate cancer cells to the bone marrow is well recognized, but until recently the biologic explanation for this has been poorly understood. Meticulous and technically sophisticated research over more than 5 years by Russell Taichman, his lead researcher, Yusuke Shiozawa, and colleagues at the Universities of Michigan and Harvard have provided key insight: "Human prostate cancer metastases target the hematopoietic stem cell niche to establish footholds in mouse bone marrow," *J Clin Invest.* 2011, April.

Two terms are basic to understanding the findings. The *niche,* in this case the *metastatic niche or osteoblastic niche*, refers to the combined unit of the cancer cell(s) and the surrounding, supporting stroma, i.e the tumor microenvironment. The cancer cells and the nurturing stroma (fibroblasts and osteoblasts are key here) carry out a brisk interchange of chemical messages each significantly influencing the other. In the case of prostate cancer

the niche is located on the bone surface surrounding young osteoblasts, the same nest where hematopoietic stem cells reside.

The second set of terms of importance is *chemokine*, a chemical message - in this case a chemoattractant (CXCL12), issued from the osteoblasts in the metastatic niche; and prostate *cell surface receptors* (CXCR4 and CXCR7), uniquely receptive to CXCL12, which beckon the malignant cells to leave the circulation and home to the niche (oddly enough, displacing the hematopoietic stem cell (HSC), an observation key to unravelling the biology of this system). As prostate cancer cells become more aggressive, they increasingly express surface CXCR4. Putative prostate cancer stem cells expressed the highest levels of CXCR4.

Once in the niche the malignant cells may be driven to proliferation by messages within the niche or lulled into a state of *dormancy* - possibly for periods of many years - only to be awakened subsequently by chemical kisses - from cytokine growth factors and mutations rousing the cells to proliferate and releasing them from immune surveillance.

One function of this osteoblastic niche as regards HSCs is to hold off proliferation until required. The microenvironment of the niche may likewise suppress proliferation of metastatic prostate cells, sedating them into dormancy in the same say the niche keeps HSC from dividing. Like HSC, prostate cells from the niche, responding to the appropriate cues, can move into the circulation, return to the original niche or take root elsewhere.

Breast and lung cancers also respond to the same CXC4/CXCL12 axis and also home extensively to bone, whereas the cells of gastric, colon, and esophageal cancers largely lack the specific receptors for homing to bone.

Taichman's research has shown that antibodies that neutralize CXCL12 can to some extent prevent the homing of CXCR4+ cells to the osteoblastic niche, enabling a potentially new therapeutic approach.

Tumor dormancy is a possible explanation for the seeming paradox of metastatic deposits in residence in bone but no objectively demonstrable "metastases." These early metastasis may be as small as 0.4 mm and remain dormant and undetectable.

What is the mechanism underlying dormancy? What therapeutic opportunities lie in understanding how to control it - i.e. to maintain dormancy? General agreement, however, converges on one point - dormancy is poorly understood. Dormant cells are metabolically active and resistant to cell death signals (apoptosis), but in them the program for replication is turned off. And without proliferation these cells may be unaffected by chemotherapy.

Many men in objective "remission" harbor dormant malignant cells - micrometastases - in their bone marrow. In this poorly understood phenomenon the messaging from the stroma plays a pivotal role, alternately suppressing or activating cells, thus offering a fertile field for chemoprevention and measures to potentiate immune surveillance. There must be some explanation for dormancy that might last for a man's life span.

The presence of circulating tumor cells generally predicts a worse outcome. Although this relationship is well documented in many studies on prostate, breast, and colon cancer, contrary findings have been reported.

In a small study by Ali, Moul et al, "Assessment of circulating tumor cells (CTCs) in prostate cancer with low-volume tumors," *Pathology International* 2010, CTCs were detected in 35 of 64 patients, and 7 of 9 low-volume tumors were marrow positive. All of these low-volume

cancers had Gleason scores of 6 and CTCs ranged from 1 - 16. At a median follow-up of 52 months, however, the presence of CTCs did not predict PSA failure, possibly a result of dormancy.

In contrast, De Bono, Montgomery et al. (*Clin Cancer Research* 2008 Oct) studied 233 men with metastatic CRPC prior to starting chemotherapy. The 57% with ≥5 CTC/7.5 ml showed a median overall survival of 11.5 months vs. 21.7 for those men with <5 CTCs, and "These data led to the Food and Drug Administration clearance of this assay for the evaluation of CRPC."

Okegawa *et al*., *International Journal of Urology, 2010 May,* reported similar results from 76 men with CRPC 62% of which had >5 CTCs/7.5 cc blood using the CellSearch system. At a median follow-up of 19.3 months the median survival of these men was 12.0 months as compared to 26 months for those with fewer than 5 CTCs.

BOTTOM LINE: Understanding the biology and the clinical significance of circulating tumor cells and disseminated malignancy in the bone marrow is a work in progress which, when further developed, will influence the practice of oncology. Even now sequential "liquid biopsies" identifying CTCs can be used to monitor response to therapy. In the future analyzing CTCs will allow characterizing metastasizing cells, predicting their behavior, and indicating sensitivity to chemotherapeutics.

HORMONE INTERVENTION / ANDROGEN SURPRESSION: Intermittent Androgen Suppression: Findings From A Major Review

Despite the American Society of Clinical Oncology's current official position that intermittent androgen suppression (IAS) is still to be regarded as "investigational," i.e."insufficient evidence to support its use," IAS has gained wide acceptance as an alternative to continuous androgen deprivation in the treatment of high-risk, relapsing, and metastatic prostate cancer. This article is a based on an extensive review, "Intermittent androgen suppression for prostate cancer" by Buchan and Goldenberg (Vancouver Prostate Center, UBC) in *Nat.Rev.Urol.*7, (2010). Dr. Goldenberg is Professor and Head, Department of Urologic Services, a urologic surgeon and clinical scientist; Dr. Buchan, a uro-oncology fellow; and both have written widely on the subject of IAS.

Their baseline summary: "Overall, these trials suggest that IAS is neither inferior nor superior to continuous androgen suppression in respect to time to castration resistance and cancer specific survival, but has significant advantages in terms of adverse effects, quality of life, and cost."

Important issues in the application of this strategy remain unresolved but may be clarified by the results of two major ongoing cooperative Phase III trials of IAS v. continuous therapy, one conducted jointly by SWOG and the National Cancer Institute of Canada involving 1512 men with metastatic disease, and another by SWOG/NCIC focusing on 1386 men with rising PSA values after radiotherapy for localized disease.

As yet, possibly pending the outcome of the two major ongoing trials, since there is no consensus regarding the optimal regimen for administering IAS. It is worth noting the recommendations and conclusions Goldenberg and Buchan draw from their review. (The details of IAS were reviewed in the PCa Commentaries Jan/Feb and July/Aug 2010.)

Selection of appropriate candidates for IAS: In general, "Factors that affected outcome were the initial PSA level [and the] PSA nadir reached during the initial cycle." Goldenberg

concluded that if the initial PSA exceeded 10 ng/ml a satisfactory target nadir was <4 ng/ml, and for a baseline PSA of <10 ng/ml "a nadir of less that 0.2 - 0.5 ng/ml can be considered acceptable." Those men who do not achieve these low nadirs "demonstrate shorter time to progression to androgen-independent growth," and are likely best treated with continuous therapy.

IAS for men at high risk for PSA failure after primary therapy: This involves the controversial issue of "early androgen deprivation (ADT) versus "late", a challenging trade-off between treatment toxicity balanced against benefit. Examples of early ADT yielding survival benefit are the Messing trial using immediate ADT in the men after surgery with positive lymph nodes, and the Early Prostate Cancer trial in which immediate use of 150 mg Casodex yielded a survival advantage for men with locally advanced disease. IAS might be used with less toxicity in any situation in which continuous ADT had been shown to offer a survival advantage.

Duration of therapy: The two SWOG/NCIC ongoing trials use induction and subsequent treatment durations of 7 and 8 months. Supporting these choices for treatment duration is the theory that "a short period of treatment might result in sub-optimal tumor regression and less-effective therapy, whereas longer treatment might hasten the development of castration-resistant cells." However, a large study by the South European Uroncological Group employed a 3-month treatment cycle and demonstrated equivalence in disease progression and overall mortality between IAS and continuous androgen suppression.

PSA set-point for restarting IAS: There is a wide variety of choices for the PSA restart level ranging from 3 ng/ml to as high as 20 ng/ml. The Canadian protocol arbitrarily suggests that for men with a pretreatment PSA of >20 ng/ml the next cycle of suppression would start at PSA 10-20 ng/ml; for men with initial PSA levels of 10-20 ng/ml, restart at PSA levels 5-15 ng/ml. "After failure of radical treatment, patients resumed ADT when their PSA reached a level of 4-6 ng/ml."

5alpha-reductase inhibitors during the off-treatment period: This in a area of evolving research currently being studied in a "Canadian trial comparing 0.5 mg/day of dutasteride (Avodart) to placebo in men receiving IAS." Two small studies each with 6 men (Sholz *et al*.,*J.Urol,* 2006; Locke and Bruchovsky, *Can.J.Urol,* 2010) using finasteride in the off-periods showed significant lengthening of the PSA doubling time during the "holidays."

BOTTOM LINE: A regimen of intermittent androgen suppression has shown equivalence in efficacy to continuous therapy in a variety of clinical settings. It results in prolonged intervals of freedom from treatment and less associated toxicity.