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Even as new next-generation hormonal agents in metastatic castration-resistant prostate cancer approach approval, clinicians struggle to identify the optimal sequencing of these agents in the management of their patients. Dreicer provides a brief overview of the development of predictive biomarkers, in combination with prospective data, which will lead to optimal treatment of these patients.

BREAST CANCER

Clinical Decision Making in Stage I and II Breast Cancer Patients Based on Gene Profiling
Masood Pasha Syed, MD; Shalini Kolluri, MD; Janeiro Valle Goffin, MD; and Debu Tripathy, MD

The authors review the clinical series and trials upon which commercially available gene profiling assays are based, and available data on the utility of these assays.

CASTRATION-RESISTANT PROSTATE CANCER

Targeting Bone Metastatic Castration-Resistant Prostate Cancer
Leah M. Cook, PhD; and Conor C. Lynch, PhD

Cook and Lynch provide a brief review of current standard of care therapies, ongoing trials, and novel therapies for the treatment of metastatic castration-resistant prostate cancer.

STEREOTACTIC BODY RADIOTHERAPY

Stereotactic Body Radiotherapy for Oligometastases: An Opportunity for Cure?
Greg Kauffmann, MD; Jeffrey Lemons, MD; Steven J. Chmura, MD, PhD

The authors discuss the proposed state of limited metastatic disease (commonly referred to as oligometastases) and the growing role of stereotactic body radiation therapy in the management of these patients.

CME

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Non-Small Cell Lung Cancer

Assessing Current and Emerging Data Sets to Optimize Sequencing Considerations in ALK-Positive NSCLC
Ross Camidge, MD, PhD, of the University of Colorado Denver School of Medicine reviews the current sequencing options in patients with ALK-positive non-small cell lung cancer (NSCLC), assesses the potential impact of ongoing clinical trials/emerging data with respect to treatment sequencing, and discusses the impact on clinical decision making that central nervous system metastases has on treatment and sequencing choices in ALK-positive NSCLC.
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In this month’s issue of The American Journal of Hematology/Oncology®, we provide a brief overview of gene profiling studies with commentary on the commercially available assays. Prospective validation has been carried out on many of these assays, especially for shorter-term recurrences and mortality. The authors note that given the long natural history of breast cancer, it will be critical to await additional data from prospective controlled trials linked to gene profiles and other bioassays to further optimize and personalize therapeutic decision-making.

Sequencing and cross-resistance continues to remain a thorny clinical issue in treating castration-resistant prostate cancer (CRPC), despite the improvement in patient outcomes afforded by next-generation hormonal therapies such as abiraterone and enzalutamide. Robert Dreicer, MD, MS, author of “Sequence and Cross-Resistance: Challenges for Optimal Use of Next-Generation Anti-Androgen Therapies,” reports that 15% to 25% of patients are unresponsive to both of these agents up front, 20% to 30% have transient responses of 2 to 3 months, and the remainder have significant benefit, with median responses of 9 to 15 months. He calls for a nuanced approach in patients managed with either abiraterone/prednisone or enzalutamide as initial therapy.

Dreicer looks forward to several ongoing clinical trials that will hopefully inform some of the many ongoing management questions. Notably, The US Intergroup study A031201 (NCT01949337) recently completed enrollment of more than 1200 men with metastatic CRPC (mCRPC) who were randomized to receive enzalutamide or the combination of enzalutamide plus abiraterone/prednisone. Another important study is a randomized phase II study led by investigators of the British Columbia Cancer Agency in Canada, where patients are randomly assigned to abiraterone or enzalutamide and then switched to the alternative agent at time of disease progression.

Cook and Lynch, in “Targeting Bone Metastatic Castrate-Resistant Prostate Cancer,” describe how understanding the process in which metastatic prostate cancer cells grow and interact with the surrounding tumor microenvironment can identify key circuits driving the progression of the disease. They note that research in this area has revealed targets for therapeutic intervention, the translation of which should enhance the overall survival of patients with mCRPC.

This month’s CME article informs physicians about current and emerging data sets in the context of optimizing sequencing considerations in patients with ALK-positive non-small cell lung cancer (NSCLC). Ross Camidge, MD, director of Thoracic Oncology at the University of Colorado, provides his insights and point-of-view, noting that most cases present with advanced disease. Treatment of advanced NSCLC has gone through a substantial paradigm shift in recent years as our understanding of “targetable” driver oncogenes has continued to grow. The presence of key oncogenic alterations, such as activating mutations and chromosomal rearrangements, can now help physicians predict responsiveness to specific targeted therapies.
Staying Ahead of the Curve of Anti-Androgen Therapy Resistance

Blockade of the androgen receptor (AR) pathway remains the mainstay of systemic therapy for prostate cancer. Not only have newer anti-androgen agents been approved in recent years for clear benefits in the advanced setting, but there are also increasing roles for androgen deprivation earlier in the course of the disease. From the time that Huggins initially demonstrated that androgens are the drivers of prostate cancer and showed a clinical benefit of castration, to the subsequent discovery of mutations in genes involved in the AR pathways and in AR itself, the central role of this pathway continues to drive new treatment paradigms. By the same token, clinical resistance to AR inhibitors has been an active area of research—in fact, the definition of true “castration-resistant” prostate cancer (CPRC) is becoming a moving target as newer drugs are addressing strategies that attack different elements of the AR machinery in CPRC.

In this issue of The American Journal of Hematology/Oncology®, Dr Dreicer navigates us through some of the clinical dilemmas that arise in the sequencing of hormonal therapy, and using principles of cross-resistance among these agents to optimize strategies over time. Importantly, clinical observations of efficacy of abiraterone before or after the anti-androgen receptor inhibitor enzalutamide given in sequence have ramifications that are detailed in the context of specific clinical scenarios. Additionally, insights gained from biological studies of CPRC have informed future strategies—for example, the fact that resistance results from the abilities of prostate cancer cells to synthesize its own androgens for precursors has provided the basis for the development of drugs such as abiraterone, which inhibits the cytochrome P450 enzyme, CYP17, which participates in the activity of both 17α-hydroxylase and 17,20-lyase, and thus provides activity in “CPRC.” However, more specific inhibitors of this biosynthetic pathway, particularly those that act more distally and immediately prior to generation of the more potent androgens will be important areas of progress that may extend the benefits that might still be derived in seemingly hormonally resistant disease. Dr Dreicer’s review also delves into consequences of activating mutations in one of these enzymes (3βHSD1) and strategies to overcome this anomaly that would naturally lead to hormonal resistance. Read on to integrate biological nuances in addressing decisions in the clinic and framing future drug and biomarker development strategies emerging for refractory prostate cancer.

REFERENCES:
Our entry into “next-generation” hormonal therapy for metastatic castration-resistant prostate cancer (mCRPC) following the FDA approvals of abiraterone and enzalutamide has matured enough for some important, albeit still early, observations: 15% to 25% of patients are unresponsive to both of these agents up front; 20% to 30% have transient responses of 2 to 3 months; and the remainder have significant benefit, with median responses in the 9- to 15-month range. A high degree of cross-resistance between abiraterone and enzalutamide has also been observed, limiting routine sequential use of these well-tolerated drugs.

Key words: anti-androgens, metastatic castration-resistant prostate cancer, enzalutamide, abiraterone

Mechanisms of Resistance to Androgen Receptor–Directed Therapies

The androgen receptor remains a key target in mCRPC, and many investigative groups are pursuing hypotheses to explain de novo and acquired resistance.

Treatment of advanced prostate cancer with medical or surgical castration eventually leads to the development of CRPC, which evolves in part as a consequence of the ability of prostate cells developing the capability of synthesizing its own testosterone and/or dihydrotestosterone from precursors, as well as other mechanisms of stimulating the androgen receptor (AR).\(^3\)

Silberstein and colleagues have divided these resistance mechanisms into three broad groups: persistent androgen/AR-signaling, AR bypass pathways and androgen/AR-independent mechanism. Ferraldeschi and colleagues\(^1\) have identified a gain-of-stability mutation that leads to a gain of function in 3\(\beta\)HSD1, an enzyme that catalyzes the initial rate-limiting step in converting the adrenal-derived dehydroepiandrosterone to the most potent androgen, dihydrotestosterone. The population frequency of this is approximately 22% but appears to vary widely by ethnicity. Efforts are ongoing to develop a competitive small-molecule inhibitor of 3\(\beta\)HSD1, and a sensitive and specific molecular assay for detection of 3\(\beta\)HSD1 mutations.\(^5\)

Androgen receptor splice variants encode for truncated AR proteins that cannot bind to the ligand, but retain activity as transcription factors that are capable of promoting activation of target genes. Antonarakis and colleagues\(^6\) prospectively evaluated the AR splice variant 7 (AR-V7) in circulating tumor cells from patients receiving enzalutamide or abiraterone, with the goal of predicting response or resistance to these agents. Endpoints of their evaluation included PSA response, clinical or radiographic progression, and both progression-free survival (PFS) and overall survival (OS).

A total of 62 patients (31 patients for each therapy) received enzalutamide or abiraterone, of whom 39% and 19%, respectively, had detectable AR-V7 in circulating tumor cells. Men whose tumors were AR-V7-positive had lower PSA response and time to PSA progression, as well as shorter clinical or radiographic PFS following treatment with either abiraterone or enzalutamide, with the goal of predicting response or resistance to these agents. Endpoints of their evaluation included PSA response, clinical or radiographic progression, and both progression-free survival (PFS) and overall survival (OS).

Men whose tumors were AR-V7-positive had lower PSA response and time to PSA progression, as well as shorter clinical or radiographic PFS following treatment with either abiraterone or enzalutamide. For patients in both groups, OS was shorter in men with detectable AR-V7 at baseline than among those with undetectable AR-V7. Of note, no AR-V7-positive patient had any meaningful clinical benefit from enzalutamide or abiraterone therapy.

Other proposed mechanisms of resistance include glucocorticoid activation of the AR, and the presence of non-AR splice variant other AR mutations.\(^7,8\)
Clinical Implications of Resistance

The initial enthusiasm generated by approvals of the next-generation AR-targeted agents abiraterone and enzalutamide has been tempered somewhat by the limited efficacy when these agents are used sequentially.

Nadal et al reported on 126 patients with mCRPC treated following progression with either enzalutamide or abiraterone with the alternative agent. The majority of patients received enzalutamide (87%) in this setting. PSA responses were seen in only 22.4% of patients with a median progression free survival of 3.6 months.

Schrader et al recently reported on 35 patients with mCRPC treated with enzalutamide following therapy with abiraterone/prednisone and docetaxel. In this group, the median duration of prior abiraterone treatment was 9 months (range, 2-19 months), with 16 patients demonstrating a greater than 50% decline in PSA as their best response. The median duration of subsequent enzalutamide therapy was 4.9 months. Seven of 16 patients (44%) who were initially abiraterone-sensitive and 3 of 19 patients (16%) who were initially abiraterone-insensitive experienced a greater than 50% PSA decline while taking enzalutamide.

Noonan and colleagues recently reported on 30 patients from a number of centers treated with enzalutamide in the phase III AFFIRM study who were subsequently managed with abiraterone/prednisone. Of 27 evaluable patients, the median enzalutamide treatment duration was 41 weeks (range, 6-95 weeks). Subsequent abiraterone/prednisone treatment duration was 13 weeks (range, 1-52 weeks). No objective radiographic responses were observed, and the median abiraterone time to progression was 15.4 weeks, with a median OS of 50.1 weeks.

The mounting evidence of cross-resistance of abiraterone/ prednisone with enzalutamide has a number of important clinical implications. In patients managed with either abiraterone/prednisone or enzalutamide as initial therapy, the selection of therapy at time-of-disease progression may require a more nuanced decision process. In patients who are asymptomatic or minimally symptomatic, crossover to the alternative agent may be reasonable, as the cross-resistance observed is not absolute, and some patients may in fact benefit from this approach, given the tolerability of these agents. In patients with symptomatic disease progression, in the opinion of the author, it may be preferable to select what appear to be more active agents, such as docetaxel, or in patients with bone-only disease, radium-223.

Several ongoing clinical trials hopefully will inform some of the many ongoing management questions. The US Intergroup study A031201 (NCT01949337) has recently completed enrollment of more than 1200 men with mCRPC who were randomized to receive enzalutamide or the combination of enzalutamide plus abiraterone/prednisone. This trial will address the issue of concomitant targeting of different AR pathways, as well as allow analysis of subsequent AR-directed therapies in patients randomized to enzalutamide alone.

Another important study is a randomized phase 2 study led by investigators of the BC Cancer Agency in Canada, where patients are randomly assigned to abiraterone or enzalutamide and then switched to the alternative agent at time of disease progression. This trial will provide prospective evidence of the true rate of resistance and has a number of potentially informative correlative studies embedded in the trial (NCT02125357).

A number of novel agents with the potential to overcome the resistance seen with abiraterone and enzalutamide are currently under evaluation. Although a randomized trial of the novel agent galecterone was stopped early for lack of efficacy, other agents such as a VT-464, a lyase-selective inhibitor of CYP17, and EPI-001, a novel compound that interferes with the transactivation domain of the androgen receptor are currently being studied in abiraterone- and enzalutamide-resistant prostate cancer patients.

Over the next several years, we can look to the potential development of predictive biomarkers to inform clinicians regarding optimal drug selection, in combination with prospective data generated from randomized trials to better enable optimal management of patients with mCRPC.

Affiliations: Robert Dreicer, MD, MS, FACP, FASCO, is from the University of Virginia School of Medicine, Charlottesville.

Disclosure: Dr Dreicer has provided consulting services for Astellas, Medivation, Asana, Sanofi-Genzyme, Chugai Pharma and Genentech.

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Clinical Decision Making in Stage I and II Breast Cancer Patients Based on Gene Profiling

Masood Pasha Syed, MD; Shalini Kolluri, MD; Janeiro Valle Goffin, MD; and Debu Tripathy, MD

Introduction
Breast cancer is a complex disease with heterogeneous presentation and clinical course. Factors that are prognostic for recurrence and mortality risk and predictive of magnitude of risk reduction attributable to specific systemic treatment options are critical to personalize management. The anatomic TNM staging system was used for decades to prognosticate risk and guide treatment. More recently, tumor characteristics like the nuclear grade and the proliferative index as measured by Ki67 immunohistochemical staining also provided risk assessment. However, as specific treatments for breast cancer have evolved, the characterizations of predictive markers that identify the degree of benefit to therapy are more relevant and useful. Oophorectomy was shown to benefit some patients around 100 years ago but the basis for its activity - the estrogen receptor was discovered many decades later. The estrogen, progesterone and human epidermal growth factor receptors (ER, PR, and HER2) are examples of validated factors that are both prognostic and predictive. The estrogen, progesterone and human epidermal growth factor receptors (ER, PR, and HER2) are examples of validated factors that are both prognostic and predictive. For example, HER2 expression predicts a higher risk of recurrence independent of treatment and is also predictive of response to the HER2 antibody trastuzumab. Higher grade and Ki67 score are associated with higher risks independent of treatment and larger relative reduction in recurrence with chemotherapy.

Predictive markers for chemotherapy have been elusive, yet represent a high priority given the significant short and long-term effects of therapy. While tumor grade and hormone receptor negativity are somewhat predictive of chemotherapy benefit, they have not been sufficiently discriminating, as evidenced by consensus recommendations for chemotherapy for most patients with node-negative breast cancer. With the development of multigene expression profiling, the first applications sought was to identify patients with lower risk breast cancer who would most benefit from chemotherapy. The last decade has seen the development, commercialization, and increasing utilization of multigene assays, designed to better assist physicians and patients to make high-quality decisions in early-stage breast cancer.

Recently, the American Society of Clinical Oncology (ASCO) published their first set of guidelines on the use of gene profiling assays and reviewed the literature to provide levels of evidence and recommendations for the use of these assays for both prognostic and predictive (treatment selection) purposes for defined population and clinical scenarios (Table 1). Profiling tests differ in the technological platforms used for studying gene expression; in the number and specific genes that are being tested and in the patient populations used for their development, validation, and assessment of clinical utility. It is important to note that these guidelines were issued prior to publication of initial MINDACT results.

This review focuses on the development, methodology, validation, and most importantly, clinical utility of the assays listed and summarized on (Table 2). In the ASCO guidelines, similar levels of evidence are assigned to assays that are performed on samples.

Abstract
Standard clinical and pathological factors can estimate the risk of recurrence and mortality from early stage breast cancer and also predict the magnitude of benefit of classes of therapy (cytotoxic, hormonal, and HER2-directed biological). Multi-parametric analysis of several proteins or expressed genes can refine these estimates and allow for more precise estimation of risk/benefit calculations that can improve decision-making, particularly for hormone receptor-positive and HER2-negative cases. Recently, the American Society of Clinical Oncology (ASCO) developed guidelines and graded recommendations for commercially available gene profiling assays. This review places these recommendations in the context of the technology used, the clinical series and trials upon which these assays are based, and available data on the utility of these assays. Early results from prospective trials are starting to become available that can provide further support for the relative benefits and limitations of different assays. Ultimately, ongoing developments and refinements in technology as well as the maturation of numerous trials incorporating one or more gene profiling tests will further establish the accuracy and utility of gene expression profiling and other bioassays in decision-making for early stage breast cancer.
### TABLE 1. Summary of ASCO Guidelines on Biomarker Assays to Guide Decisions for Early Stage Invasive Breast Cancer*

**Key:**
- Assists in decisions on the use of adjuvant chemotherapy
- Prognostic, but should not be used for decisions on the use of adjuvant chemotherapy
- Should not be used for decision-making

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>ER/PR-positive, HER2-negative, Node-negative</th>
<th>ER/PR-positive, HER2-negative, Node-positive</th>
<th>HER2-positive or Triple Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Evidence Characteristics</strong></td>
<td>Type of Recommendation</td>
<td>Evidence Quality</td>
<td>Strength of Recommendation</td>
</tr>
<tr>
<td>Oncotype DX</td>
<td>Evidence</td>
<td>High</td>
<td>Strong</td>
</tr>
<tr>
<td>Prosigna (PAM50 ROR)</td>
<td>Evidence</td>
<td>High</td>
<td>Strong</td>
</tr>
<tr>
<td>EndoPredict</td>
<td>Evidence</td>
<td>Intermediate</td>
<td>Moderate</td>
</tr>
<tr>
<td>Mammaprint</td>
<td>Evidence</td>
<td>Intermediate</td>
<td>Moderate</td>
</tr>
<tr>
<td>Breast Cancer Index</td>
<td>Evidence</td>
<td>Intermediate</td>
<td>Moderate</td>
</tr>
<tr>
<td>IHC4</td>
<td>Evidence</td>
<td>Intermediate</td>
<td>Moderate</td>
</tr>
<tr>
<td>Mammostrat</td>
<td>Evidence</td>
<td>Intermediate</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

* Adapted from Harris L et al. (Ref 7)

### TABLE 2. Commercially† Available Gene Profiling Assays for Early Stage Breast Cancer

<table>
<thead>
<tr>
<th>Assay</th>
<th>Vendor</th>
<th>No. of Genes</th>
<th>Technology</th>
<th>Predictive*/ Prognostic</th>
<th>Eligible Patients</th>
<th>Measure/ Categories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncotype DX</td>
<td>Genomic Health</td>
<td>16 cancer 5 control</td>
<td>qRT-PCR</td>
<td>+/-</td>
<td>ER+ and HER2-, T1/2 0-3 nodes</td>
<td>RS: low (&lt;18), intermediate (18-31), high (&gt;31) risk RT-PCR assay for ER, PR and HER2</td>
</tr>
<tr>
<td>MammaPrint</td>
<td>Agendia</td>
<td>70</td>
<td>Microarray</td>
<td>-/+</td>
<td>Stage I and Stage II breast cancer</td>
<td>Good risk and poor risk Intrinsic Subtype</td>
</tr>
<tr>
<td>PAM50</td>
<td>Prosigna and Nanostring Technologies</td>
<td>50 cancer 22 control/ housekeeping</td>
<td>Digital bar-coded mRNA analysis</td>
<td>-/+</td>
<td>ER+, Stage I/II 0-3 nodes</td>
<td>ROR: Low (&lt;10), intermediate (10-20), high (&gt;20%) risk Intrinsic Subtype</td>
</tr>
<tr>
<td>Breast Cancer Index</td>
<td>bioTheranostics</td>
<td>MGI - 5 cell cycle genes H/I – Gene expression ratio</td>
<td>qRT-PCR</td>
<td>+/-</td>
<td>ER+</td>
<td>Low, intermediate and high risk</td>
</tr>
<tr>
<td>IHC4</td>
<td>None</td>
<td>4 (proteins)</td>
<td>IHC, semi-quantitative</td>
<td></td>
<td></td>
<td>Composite formula based on ER, PR, HER2, Ki67 semiquantitative expression</td>
</tr>
<tr>
<td>Genomic Grade Index</td>
<td>Ipsogen</td>
<td>70 6</td>
<td>Microarray qRT-PCR</td>
<td>-/+</td>
<td>ER+, intermediate grade</td>
<td>High or low grade</td>
</tr>
<tr>
<td>EndoPredict</td>
<td>Myriad/ Sividon Diagnostics</td>
<td>8 cancer 3 control</td>
<td>qRT-PCR</td>
<td>-/+</td>
<td>ER+, HER2-</td>
<td>The test result is composed of the &quot;molecular fingerprint&quot; of a tumor in combination with the established prognostic parameters nodal status and tumor size.</td>
</tr>
<tr>
<td>Mammostrat</td>
<td>Clarient</td>
<td>5 (proteins)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† All except IHC4 - not commercially available

* Based on analysis of tumor tissue from prospective randomized trial

ER indicates estrogen receptor; FFPE, formalin-fixed, paraffin-embedded; HER2, human epithelial receptor 2; IHC, immunohistochemistry; NC, not cleared or approved by the US Food and Drug Administration; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction; RS indicates recurrence score.
from randomized trials as are assigned to assays from non-randomized series. Therefore, an additional aim of this review is to provide further perspective on the methodology and utility of gene profiling assays for both prognosis and treatment decisions, especially in the framework of the level of evidence that takes into account study design upon which the assays are based including the more recently validated studies. The purpose of this review is:

• To discuss the available assays in the context of the underlying supportive data and the source of the data, such as large registries or randomized trials.

• To understand what each of these assays brings to clinical decision making.

• To relate and discuss the assays with respect to the early data emerging from prospective randomized clinical trials.

**Methodologies**

This section provides an overview of the general technologies used to develop an assay and the additional specifics are provided in each individual assay section. Early gene profiling assays required fresh tissue to perform RNA extraction, amplification, and labeling followed by hybridization to oligonucleotide arrays for detection and quantification. Adaptations to use formalin-fixed, paraffin embedded (FFPE) tissue using reverse transcriptase polymerase chain reaction (RT-PCR) have made these assays much more feasible and quantitative. One round of RT-PCR generates complementary DNA from RNA and this is followed by quantitative PCR (qPCR). NanoString’s nCounter technology is a modified version of the DNA microarray. It uses molecular “barcodes” and micro-imaging to detect and enumerate hundreds of unique transcripts in one hybridization reaction. Each color-coded barcode is linked to a single target-specific probe to a gene of interest. Profiling can also be made on the basis of several immunohistochemical assays that are processed and scored and integrated in a consistent and semiquantitative manner, although inter-observer variability remains a limiting factor.

**Validation and Utility**

Only commercially available assays are included in this review. For all assays, a threshold for the amount of invasive cancer is specified. The tissue should be representative of the tumor. It must be obtained and processed adequately to generate sufficiently high quality RNA for gene expression assays. Regulatory approval requires that a technique and readout be accurate and reproducible, with specific metrics for consistency on repeat testing. In addition, the association of the readout with outcome must be validated using independent datasets of appropriately described patients with specified follow-up, and maintaining constant cut points. In general, a reliably validated test will perform well across the intended population and over a range of variables included in the population such as receptor status, tumor grade, nodal status, and age.

The utility of a test describes its impact on decision-making and how it affects long-term outcomes. Establishing this utility requires the assay to be performed in a controlled (ideally randomized) trial that tests the treatment for which the assay is intended to help select. Verifying and measuring utility is the most challenging and rarely accomplished milestone for an assay to be prognostic or predictive. The most rigorous proof requires a randomized trial comparing the use of the assay to standard care to make a treatment decision and the demonstration that a clinically relevant outcome (eg, recurrence or quality of life) is improved with a quantification of the benefit and any counterbalancing harm. In the case of breast cancer, most of the data for predictive or prognostic factors is derived from retrospective studies or prospective observational studies, with ongoing large scale prospective trials of different designs ongoing described later in this review.

Therefore, the clinician must ask key questions prior to ordering a test:

• How reliable is the test? Does it accurately and reproducibly measure or estimate the index of interest (eg, risk of recurrence or expected degree of benefit from a given therapy)?

• Does the test provide information that is independent of patient and tumor factors?

• How useful is the test? Will it allow for a decision to maximize benefit and improve cancer-related outcomes, or to avoid a toxic treatment without sacrificing outcome?

This review focuses on the background, performance and utility of the various gene profiling tests available for early stage breast cancer. It provides additional perspective based on more recent supportive literature.

**Presently Available Gene Profiling Assays**

**Oncotype DX**

The Oncotype DX assay uses RT-PCR to measure gene expression of 16 cancer-related genes (identified in discovery cohorts and also chosen on biological rationale), and 5 housekeeping genes in breast cancer tissue samples. The test is performed on FFPE tumor samples in a Clinical Laboratory Improvement Amendments (CLIA) and College of American Pathologists (CAP)-regulated central laboratory. A Recurrence Score (RS) result (a continuous variable ranging between 0 and 100) is then calculated by an algorithm for each patient.

Oncotype DX RS provides both prognostic and predictive information. The assay was developed as well as validated in patients with ER-positive early-stage invasive breast cancer who are treated with endocrine therapy and predicts the 10-year risk of distant recurrence in this group of patients. The score was found to predict in patients treated with tamoxifen and aromatase inhibitors. Also, the predictive value of RS with respect to the likelihood of benefit from chemotherapy has been demonstrated in two randomized trials. Paik and colleagues assessed 651 patients with node-negative ER+ breast cancer of which 227 were randomly assigned to tamoxifen and 424 to tamoxifen plus che-
motherapy. The study concluded that patients with high RS had a greater probability of significant benefit from cyclophosphamide/methotrexate/fluorouracil (CMF) chemotherapy as compared to those with a low RS score (P = .038). A study by Albain and colleagues assessed patients with node-positive breast cancer who were randomized to receive tamoxifen with or without prior cyclophosphamide, doxorubicin, and fluorouracil (CAF) chemotherapy. The tissue samples consisted of 40% of the 927 patients in the tamoxifen and CAF-T groups that had sufficient RNA for analysis (Total N = 367, tamoxifen, n = 148; CAF-T, n = 219). This study concluded that high RS was associated with substantial benefit from CAF, especially during the first 5 years as compared to those patients with low RS. This difference was statistically significant with a P-value of .029. One of the shortcomings of this study was the limited number of blocks available for analysis (40% of all patients). This, along with the lower number of events in patients with an intermediate RS in both studies, indicated a possibility that there may be some benefit of chemotherapy in this subgroup of patients.

Several large series have confirmed the ability of RS to provide prognosis independent of conventional risk assessment parameters using multivariable analyses, thereby fulfilling criteria for level I category B evidence for estimating the 10-year risk of distant recurrence and the likelihood of benefit from adjuvant chemotherapy. Consequently, Oncotype DX testing for these indications has been incorporated into international guidelines such as the European Society for Medical Oncology (ESMO), St. Gallen, NCCN (includes 1 to 3 positive nodes) and ASCO (node negative only), with node-positive cases excluded by ASCO but included by NCCN. More recently, larger population-based studies have shown less chemotherapy use and excellent short term outcomes in patients with low recurrence scores. NCCN includes 1-3 positive nodes and ASCO includes node negative only.

The potential benefit of chemotherapy in patients with an intermediate RS result is being addressed in the prospective Trial Assigning Individualized Options for Treatment (TAILORx), in which patients with hormone receptor-positive, HER2-negative, node-negative disease and RS of 11-25 were randomized to chemoendocrine or endocrine therapy alone. Patients with RS 10 or less received hormonal therapy alone and those with RS of 25 or higher were all treated with chemotherapy followed by hormonal therapy. Recently, outcomes from the low-risk cohort treatment only with hormonal therapy were reported, confirming a very low expected rate of distant recurrence of 0.7% at years at a median follow-up of 69 months.

To follow-up on the SWOG S8814 study, the recently closed prospective Rx for Positive Node, Endocrine Responsive breast cancer (RxPONDER) trial (or South West Oncology Group [SWOG] S1007 trial) was proposed to study non-inferiority of endocrine treatment in comparison with chemoendocrine treatment in patients with 1 to 3 positive nodes with RS of 25 or less. These two large prospective trials will validate and explore the utility of several aspects of the 21-gene assay such as:

1) Confirm that the low risk group of patients on Oncotype DX is truly at a low risk of recurrence without chemotherapy.
2) Assess the impact of chemotherapy in patients with node-negative intermediate RS and 1 to 3 positive nodes with RS <25.
3) Allow more robust analyses of the subgroups of patients based on tumor size, tumor stage, grade, age, etc.

MammaPrint

The MammaPrint assay studies the transcription of 70 genes associated with cell cycle, signal transduction, proliferation, invasion, angiogenesis, and metastasis. It uses a DNA microarray platform and is derived from a comparison of expression profile from tumors of patients who developed metastases within 5 years to those who did not within a node-negative cohort who received no systemic therapy. The US Food and Drug Administration cleared the MammaPrint assay for marketing as a prognostic test but not to select therapy or predict response to therapy. Subsequently, it has been validated by more studies. Hence, it was established that MammaPrint is significantly associated with prognosis in breast cancer patients 1 to 3 positive lymph nodes. Additionally, it was also shown by a pooled analysis of patients with tumors lesser than 2 cm that MammaPrint could identify a low-risk group independent of histologic grade, nodal status, treatment, HER2, and ER status.

The predictive ability of MammaPrint assay was assessed in a retrospective pooled series of 7 studies involving 541 patients who received chemo-endocrine or endocrine therapy alone. Although the analysis did show a significant benefit from chemotherapy in patients with a high risk profile, the hazard ratio for distant metastasis-free survival was similar in both the risk groups at 5 years and the P value was non-significant (0.45) indicating that the assay did not predict chemotherapy benefit.

The impact of MammaPrint assay on adjuvant treatment decisions was demonstrated by the RASTER observational study that showed that 81% of high-risk patients by MammaPrint received chemotherapy in comparison to only 15% of the low-risk patients by MammaPrint. Hence, based on the evidence supporting the prognostic utility of MammaPrint, it was included as a prognostic tool in 2 international guidelines (ESMO and St. Gallen) and in some national guidelines such as those issued by the Arbeitsgemeinschaft Gynäkologische Onkologie (AGO), where it is included as an option with level II category C evidence.

The Microarray In Node-negative and 1 to 3 positive lymph node Disease may Avoid ChemoTherapy (MINDACT) trial has included MammaPrint for risk assessment. The MINDACT trial obtained both clinical prognostic (Adjuvant! Online, a suite of online tools to aid health professionals and patients with early cancer discuss the risks and benefits of getting additional therapy.
after surgery), as well as gene profile scores, and assigned those who were low risk by both to no chemotherapy (N=2745) and those who were high risk by both (N=1806) to chemotherapy. Discordant cases (N=2142) were randomized to chemotherapy vs no chemotherapy. Hormonal therapy was given to hormone receptor positive cases. This trial also compared endocrine regimens of 2 years of tamoxifen followed by 5 years of letrozole vs 7 years of letrozole and chemotherapy regimens of FEC (fluorouracil, epirubicin, and cyclophosphamide) followed by docetaxel vs docetaxel-capecitabine treatment. Early results at 5 years of median follow-up confirm higher metastases, relapse, and death rates in high- compared with low-risk groups, and the discordant group exhibited an intermediate metastases-free survival rate of 95% with no difference based on chemotherapy assignment, but with a limited number of events to exclude a benefit, but showing that the assay can potentially reduce the number of patients who would otherwise be prescribed chemotherapy.31

The recently published 5 year follow-up shows that the low-risk group (N = 2745) had an excellent outcome with a distant disease-free survival of 97.6% without chemotherapy, whereas this value was 90.6% in the high-risk group (N = 1806) after receiving chemotherapy.32 In the discordant group (N = 2550, or 23.2% of the whole group), the overall distant disease-free survival was 94.7% (95% confidence interval 92.5% to 96.2%) with a non-significant 1.5% lower risk in the group randomized to chemotherapy. These data provide an estimate of potential reduction of patients who would otherwise be prescribed chemotherapy by 46%. More follow up will be needed to determine whether or not chemotherapy had a meaningful impact in the discordant group.32

Prosigna/PAM50

Prosigna is a gene profiling assay based on the 50-gene intrinsic subtype predictor set, PAM50.33 The nCounter Dx Analysis system (Nanostring Technologies, Inc., Seattle, WA) is used for the analysis of RNA obtained from FFPE breast tumor tissue in this assay, which measures the expression of 50 genes in the PAM50 panel along with 8 housekeeping genes (for normalization), 6 positive controls, and 8 negative controls by using a hybridization reaction with nucleic acid probes designed specifically for it. A Prosigna score (value between 0 and 100) also called Risk of Recurrence (ROR) Score is then assigned by the Prosigna algorithm. The Prosigna score along with the nodal status is used to determine risk categories (low, intermediate, or high) which represent the 10-year risk of distant recurrence for HR+ post-menopausal women with early-stage breast cancer. The assay is sensitive and uses 250 ng total RNA from FFPE tissue. It can be performed in a local pathology unit or molecular biology laboratory. Prosigna assay was cleared for marketing by the FDA in September 2013 as a prognostic test. However, it is not used to select therapy or to predict/detect response to therapy.

The archived samples from the Austrian Breast & Colorectal Cancer Study Group 8 (ABCSG-8) trial were used to validate the Prosigna assay. This trial randomly assigned post-menopausal women with HR+ early-stage breast cancer to receive 5 years of tamoxifen versus 2 years of tamoxifen, then 3 years of anastrozole. The cohort used to validate this assay consisted of 1,478 patients (node negative and node positive) and established that the Prosigna score provided significant prognostic information (10-year distant recurrence) and was better than the traditional clinicopathologic characteristics. These findings were applicable to the validation cohort as a whole as well as the node positive and node negative groups separately.33 The assay was further validated for patients with 1 to 3 positive nodes by 2 prospective trials (ABCSG-8 trial and the translational arm of the ATAC [anastrozole or tamoxifen alone or combined] trial [TransATAC]) using data from 2485 patients.35,36 Hence, Prosigna met the criteria of level I-II, category B evidence as a prognostic tool and has been included in AGO guidelines as a level II category B evidence.37,38 However, its ability to predict response to chemotherapy or impact treatment decisions has not been established by any studies. The use of intrinsic subtype beyond standard evaluation of HR and HER2 receptor status remains under study.

Breast Cancer Index (BCI)

Breast Cancer Index has 2 independent biomarker panels—Molecular Grade Index (MGI) and HoxB13/IL17BR (H/I).39 Both were derived from tumors from patients treated with or without tamoxifen and followed for outcomes, and identifying independently prognostic genes algorithmically. The MGI is prognostic of early and late recurrence and assesses tumor proliferation based on analysis of 5 cell cycle genes. The H/I is a gene expression ratio related to estrogen signaling. It is prognostic and predictive of the likelihood patient benefit from extended endocrine therapy. The BCI score (0-10) is a linear combination of MGI and H/I which together provide more accurate prognostic power. The BCI score serves as a continuous risk index for prognostication of early and late recurrence. The H/I gives a binary result, a “high versus low” BCI Score, which was validated in a retrospective analysis of 2 prospective trials. The population included 1340 patients with early stage estrogen receptor positive and lymph node negative breast cancer across three cohorts (TransATAC, Stockholm, Multi-Institutional).40 A large proportion of patients (55% to 65%) in all 3 cohorts were classified as low risk. These patients have continued to exhibit a low risk of recurrence beyond 5 years (<3.5% ROR).40 Therefore, the role of the H/I biomarker predicting benefit from extended hormone therapy was investigated in the MA.17 trial of extended hormonal therapy that compared letrozole to placebo after the completion of 5 years of adjuvant tamoxifen.40 From the 5157 patients in the overall trial, 249 tissue blocks were analyzed using a nested case-control design. High H/I was significantly associated with patient benefit from extended endocrine therapy with letrozole (P = .0061). Patient characterized as low H/I had no significant benefit. There was a significant association between treatment benefit and H/I (P = .03).
**IHC4**
The IHC4 assay is based on a multivariate model that uses semiquantitative scoring from immunohistochemistry for ER, PR, HER2, and Ki67. The assay uses FFPE tumor biopsy specimens and an algorithm calculates a risk score for recurrence.44-56 The validation cohort that was followed included 1125 patients from the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial who were estrogen receptor-positive (ER-positive) who did not receive adjuvant chemotherapy, had the Recurrence Score (RS) calculated, and had sufficient tissue for the IHC measurements of four parameters: estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2), and Ki67. The primary endpoint being distant recurrence was measured using proportional hazards model with sample splitting to control for overfitting. Additionally, a separate cohort of 786 patients was used to create and assess a prognostic model using the traditional variables and the four IHC markers (IHC4 score). These 4 IHC markers in the presence of classical variables provided independent prognostic information. In sample-splitting analyses, the information in the IHC4 score was found to be similar to that in the Oncotype RS, and little additional prognostic value was seen with the combined use of both scores.

The IHC 4 assay uses the information obtained from ER, PR, and Ki67 differently compared to the classical interpretation of these markers in daily practice. Unlike using these markers as binary categories, for example, ER-positive vs ER-negative, IHC4 uses a mathematical equation that combines the semi-quantitative expression values of these markers into a single risk score. This equation is available to the public however there is some inter-observer variability that is seen with the application of the equation to local pathology. The mathematical equation behind the IHC4 score is public; however, applying the formula to local pathology results could result in considerable inter-observer variability in the absence of standardized quantification of each of the four variables that would match the IHC assay sensitivity and the dynamic ranges used in the original analysis.42

**EndoPredict**
The EndoPredict test (Sividon Diagnostics GmbH, Koln, Germany), is a RT-PCR-based assay that classifies patients with ER-positive breast cancer being treated with adjuvant endocrine therapy alone into a low risk or a high risk of recurrence. This assay, measuring the expression of 8 cancer genes and 3 housekeeping genes, is available in Europe as a diagnostic kit and is performed by local laboratories. Additionally, a comprehensive risk score called EP is available in Europe as a diagnostic kit and is performed by local laboratories. Moreover, a comprehensive risk score called EP is available in Europe as a diagnostic kit and is performed by local laboratories.

**Genomic Grade Index**
The Genomic Grade Index (GGI) (MapQuant Dx, Ipsogen, France) assay measures the expression of 97 genes and designates a molecular grade by using microarray technique. It was developed by correlating gene expression profiles of histological grade I and grade III tumors.51 Also, a 6-gene version of this assay is available that uses RT-PCR technology and can be used for FFPE samples.52 The GGI can also stratify histologically intermediate-grade ER-positive breast cancers into high or low molecular grade with considerable difference in prognosis.52 Additionally, GGI could identify 2 clinically relevant ER+ subtypes with very distinct clinical outcomes in both systemically untreated and tamoxifen only treated BC patients.53

A cohort of 570 patients for which histological grade and relapse-free survival (RFS) was available was used to measure the prognostic information of GGI. The data set pooled from this cohort along with 3 publicly available datasets was used.54-56 A higher rate of relapse was observed in histological grade 3 tumors in comparison with histological grade 1 tumors (HR, 3.18; 95% CI, 2.1-4.8; P <.001). The histological grade 2 group (216 patients) was further subdivided into two categories: a grade 1-like gene profile and a grade 3-like gene profile. Here, a higher rate of relapse was observed in the grade 3-like gene profile subgroup in comparison to the grade 1-like subgroup (HR, 3.61; CI 2.25–5.78; P <.001). GGI divided the original cohort of 570 patients into two risk categories (high or low) with significant difference in RFS (HR, 2.83; CI 2.13–3.77; P <.001). It is important to note that only tumor size, lymph node status and GGI were statistically significant in multivariate analysis even though GGI histological grade, ER status, lymph node status, and tumor size were all associated with RFS in univariate analysis. In multivariate analysis, histologic grade was not significant (HR, 1.38; 95% CI 0.89–2.14; P =.11) whereas GGI showed significant prognostic information (HR, 1.99; 95% CI 1.43–2.78; P <.001). Hence, it was established that GGI can improve the precision of grading for prognostic purposes. The prognostic information of GGI was further validated in a large meta-analysis including almost 3000 patients.57

**Mammostrat**
Mammostrat is a 5-protein IHC that assesses 5 functional proteins - SLC7A5, which mediates nutrient transport; p53, a cell cycle checkpoint control; HTF9C, a cell cycle-dependent protein; NDRG1, a stress- and hypoxia-inducible gene product; and CEA-
CAM5, a carcinoembryonic differentiation antigen. This assay was validated in 2 node-negative ER+ trials, NSABP B14 and B20 (same used for Oncotype DX) including 711 cases, and gives low, moderate, and high risk readout, but still a rather high recurrence risk (85% recurrence-free at 10 year) in the low risk category, and did not out-predict the chemotherapy benefit identified by Oncotype DX 21-gene recurrence score.\(^{58}\) It was further evaluated in the Tamoxifen versus Exemestane Adjuvant Multicenter (TEAM) trial that included node-positive patients (47%) and those who received adjuvant chemotherapy (36%), with a total of 3837 cases analyzed, and showed an independent impact on 10-year distant recurrence-free survival over and above size, grade, nodal status and ER/PR/HER2 status.\(^{59}\) This assay is FDA-cleared although not recommended by either NCCN or ASCO.

Additional Caveats on Clinical Utility of Gene Profiling in Early Stage Breast Cancer

A prospective randomized trial testing the application of an assay compared to standard care, with a clinical relevant outcome as the primary endpoint is the ideal way to formally assess the utility of an assay. However, such trials have rarely been done in the past as the perception of utility from early studies led to rapid adoption, and neither CLIA certification nor FDA approval have required this level of proof. Retrospective analysis of tissues from a prospective trial using a uniform treatment and follow-up protocol can also be very helpful in validating prognostic value as long as there is not a chance of selection bias due to limited specimen availability. However, retrospective series may lead to bias, for example, the blocks available may be from the larger tumors so the smaller tumors are selectively depleted and this is an important component of standards that are being developed for the discovery and validation of prognostic factors and these criteria have been set forward as REMARK criteria.\(^{60}\) The optimal validation of predictive value is derived from a controlled randomized trial that is comparing the treatments whose magnitude is predicted by the assay in question - which until recently was best approximated retrospectively.\(^{10,11}\) However, we now finally are obtaining data from prospective randomized trials incorporating assays to stratify for treatment, no treatment or randomization to treatment or not.\(^{21,31}\) The FDA has signaled that diagnostic assays with therapeutic implications will require prospective trials demonstrating utility.

Another important aspect about most assays is that their prognostic and predictive ability may vary over time.\(^{61}\) Most statistical analyses assume constant hazard and odds ratios associated with a biomarker, yet studies with longer-term follow suggest that the impact of prognostic markers, including gene profiling scores are strongest in the first 5 years to beyond this time. Hence, the prognostic or predictive value of any assay is likely to be overestimating the effect if extrapolated to 10 years or beyond. In fact, data mining exercises and comparisons using large gene sets and patient samples tend to identify many fewer candidate genes and signatures that predict recurrences beyond 5 years, as shown with the BCI assay in a trial comparing 5 to 10 years of endocrine therapy.\(^{62,63}\) Likewise, the estrogen receptor and associated genes component of the Oncotype assay has been shown to predict late (>5 year) distant recurrence risk.\(^{64,65}\) EndoPredict also predicts late recurrence but it is not predictive of benefit from extended hormonal therapy like Breast Cancer Index.\(^{44}\) The lack of robust biomarkers for late recurrence may be a general biological phenomenon whereby mutational evolution and other factors introduce more chaos and unpredictability into the clinical trajectory, much like the tracking of weather or a storm becomes less definable at later time-points.

Additionally, intrinsic subtypes (eg, luminal A/B, basal and HER2-enriched) of breast cancer initially described based on unsupervised clustering of expression profiles are reported in some of the multi-gene assays, including PAM50 and Agenda’s BluePrint report.\(^{65}\) While there are growing data to suggest that intrinsic subtypes may exhibit specific biological characteristics, there is yet no role in reassigning patients to treatment that is not based on conventional assessment and interpretation of HR and HER2 receptor status.

For quantitative gene expression data, it is ideal to view point estimates with accompanying 95% confidence intervals, the width of which mainly depends on the sample size for the subset of interest. We have much more longitudinal outcome data linked to gene expression signatures since these technologies became available in mid 1990s whereas next generation sequencing (needed to accurately assess mutational status and burden) was not developed until early 2004-2005. Therefore, outcomes data with these technologies are less mature. The next generation of assays may relate to the actual sequence of specific genes or gene sets as opposed to quantitative gene expression.\(^{66}\) There are also emerging data that a higher mutational burden may predict a greater impact of chemotherapy. However, while mutational burden may predict short-term response to chemotherapy it may also be associated with worse longer term survival due to higher genomic diversity and emergence of therapeutic resistance.

Conclusion and Summary

Gene profiling studies have been shown to be more reproducible than certain measure such as tumor grade and these can add further prognostic refinement over and above conventional clinical and pathologic features. Prospective validation has been carried out on all the commercially available assays, especially for shorter term recurrences and mortality. Only Oncotype Dx has shown to predict benefit of chemotherapy linked to randomized chemotherapy trials, but biological features that predict low recurrence risk determined by other validated assays may also predict less relative benefit from chemotherapy. Given the long natural history of breast cancer as well as time-dependent nature of both hazards of recurrence and the prognostic/predictive values of most assays, it will be critical to await additional data from prospective con-
trolled trials linked to gene profiles and other bioassays including mutational profiling to further optimize and personalize therapeutic decision-making for early stage breast cancer.

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REFERENCES


Targeting Bone Metastatic Castration-Resistant Prostate Cancer

Leah M. Cook, PhD, and Conor C. Lynch, PhD

Abstract

Metastatic castration-resistant prostate cancer (mCRPC) is incurable and typically manifests in the skeleton. In the bone microenvironment, prostate cancer cells survive by promoting bone remodeling, resulting in the release of factors that drive CRPC growth. These lesions contain areas of extensive bone destruction and formation that can lead to pathological fracture, thereby greatly contributing to patient morbidity and mortality. Therapies that can treat the disease and extend overall survival are an urgent clinical need. To this end, recent advances in our understanding of how mCRPC survives and grows in bone have contributed to the development of promising therapeutics. Here, we briefly review current standard of care therapies, ongoing trials, and novel therapies for the treatment of mCRPC.

Introduction

Metastasis is responsible for more than 90% of cancer-related deaths. Prostate cancer is no exception, with approximately 26,120 men expected to succumb to the disease due to complications of metastasis in 2016. Of these, it is expected that more than 90% will have evidence of skeletal lesions. The median survival time for patients with active metastatic castration-resistant prostate cancer (mCRPC) is approximately 3 years. Understanding how metastatic prostate cancer cells grow and interact with the surrounding tumor microenvironment can identify key circuits driving the progression of the disease. Research in this area has revealed targets for therapeutic intervention, the translation of which should enhance the overall survival (OS) of patients with mCRPC.

Androgen Deprivation Therapy (ADT) for Bone mCRPC

The National Comprehensive Cancer Network suggested guidelines for the treatment of men given a diagnosis with bone mCRPC are immunotherapy (sipuleucel-T) followed by androgen deprivation therapy (ADT; abiraterone acetate or enzalutamide), chemotherapy (docetaxel with prednisone), radiopharmaceutical therapy (radium 223), suggestion of a clinical trial, or a potential secondary hormone therapy such as ketoconazole.

The upregulation of pathways involved in androgen synthesis or mutations/amplification in the androgen receptor (AR) itself allows cancer cells to continue feeding on androgens despite the systemic depletion of the ligand. Further, androgen interaction with AR-expressing bone-building osteoblasts promotes differentiation and bone formation. Given the reliance of mCRPC cells on androgen for growth in bone, inhibitors that block androgen synthesis or the activity of mutant AR remain an intense area of investigation and clinical trial activity. For example, CYP17A1 is an important enzyme used by CRPC cells for the de novo synthesis of androgens, and this discovery led to the genesis of abiraterone, a small molecule inhibitor of CYP17A1 activity. Abiraterone given in combination with prednisone, a corticosteroid, was first shown to increase the median OS by 4.6 months, compared with placebo plus prednisone, in mCRPC patients who had previously received docetaxel. Median OS was increased to three years in chemotherapy-naïve patients, compared with placebo. Additionally, abiraterone was shown to also significantly delay the time to first skeletal-related event (SRE).

Enzalutamide, an AR antagonist, was first shown to increase median OS by 4.8 months in mCRPC patients who had previously received docetaxel, compared with the placebo group. The time-to-disease progression, measured by prostate-specific antigen (PSA) levels, was increased by 5.3 months and radiographic progression-free survival (PFS) increased by 5.4 months, compared with placebo. In a phase III randomized trial, enzalutamide increased the time to the first occurrence of an SRE, suggesting an impact on disease progression in bone. Given the success of these ADTs as single agents, they are now being investigated for their efficacy together or when combined with other therapies (Table).

Building on this approach, galeterone, a novel dual small molecule inhibitor of CYP17A1 and AR was compared with enzalutamide alone in clinical trials (ARMOR3-SV). The major endpoint for mCRPC patients was radiographic PFS but in July 2016, the trial was halted due to predicted failure to meet this goal. The drug, however, remains in phase II clinical trials examining safety and response of the patients that have progressive CRPC but have failed oral therapy (ARMOR2; NCT01709734). Other AR and CYP17A1 targeted inhibitors, such as apalutamide and seviteronel, remain the
### TABLE 1. An Overview of Ongoing Clinical Trials for Bone Metastatic CRPC (www.clinicaltrials.gov)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Class/Type</th>
<th>Target</th>
<th>Trial (Phase, Combination Drug)</th>
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<tr>
<td>Abiraterone acetate</td>
<td>Hormone therapy</td>
<td>CYP17A1</td>
<td>NCT02036060 (Phase 2, +/-Docetaxel); NCT01949377 (Phase 3, +/-Enzalutamide); NCT01972217 (Phase 2, +/- Olaparib); NCT01487983 (Phase 2, +/- Sipuleucel-T); NCT02415621 (Pilot-Adaptive Therapy)</td>
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<td>Hormone therapy</td>
<td>AR</td>
<td>NCT02116582 (Phase 4, post-abiraterone)</td>
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<td>NCT01709734 (Phase 2)</td>
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<td>EPI-506</td>
<td>Hormone therapy</td>
<td>N-term and AR</td>
<td>NCT02606123 (Phase 1/2)</td>
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<td>Seviteronel (VT-464)</td>
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<td>CYP17A1 and AR</td>
<td>NCT02445976 (Phase 2); NCT02130700 (Phase 2); NCT02012920 (Phase 1/2)</td>
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<td>Apalutamide (ARN-509)</td>
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<td>AR</td>
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<td>Dovitinib</td>
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<td>NCT01994590 (Phase 2, +Abiraterone/Prednisone)</td>
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<td>Bcr-Abl and SRC</td>
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<td>Cabozantinib</td>
<td>TKI</td>
<td>VEGFR2; cMET</td>
<td>NCT01630590 (Phase 2, +Androgen ablation); NCT01683994 (Phase 1/2, +/- Docetaxel/Prednisone)</td>
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<td>mTOR</td>
<td>NCT01174199 (Phase 1, +Vorinostat)</td>
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<tr>
<td>Cixutumumab</td>
<td>RTK effectors</td>
<td>IGF1R</td>
<td>NCT01026623 (Phase 1/2, +/- Temsirolimus)</td>
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ARV7, androgen-receptor splice variant 7; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; FGFR3, fibroblast growth factor receptor 3; GM-CSF, granulocyte-macrophage colony-stimulating factor; IGF1R, insulin-like growth factor 1 receptor; mTOR, mammalian target of rapamycin; OCL, osteoclast; PAP, prostatic acid phosphatase; PARP, Poly ADP ribose polymerase; PD-L1, programmed death-ligand 1; PSA, prostate-specific antigen; RTK, receptor tyrosine kinase; TKI, tyrosine-kinase inhibitor; and VEGFR2, vascular endothelial growth factor receptor 2.
focus of phase I and II clinical trials (Table 1).

While results for these inhibitors have been encouraging, a caveat has been the emergence of AR variants (AR-V) that, in some instances, lack ligand-binding domains but still drive the expression of AR-related genes. Recently, ARV7 has been linked to acquired resistance to enzalutamide and abiraterone. EPI-506 is a novel small molecule inhibitor that binds to the N-terminal domain of AR and therefore could be an effective treatment for mCRPC patients who have developed resistance to enzalutamide. The long-term safety of EPI-506 is currently being studied (NCT02606123).

Targeting Bone mCRPC From the Outside In

Although the emphasis has remained on ADT, understanding ligands, receptors, and signaling pathways that control CRPC has revealed critical circuits controlling cancer well in survival and growth. The mutation/amplification/upregulation of several receptor tyrosine kinases (RTKs) have been implicated in the development, growth, and progression of prostate cancer and are the focus of clinical trials. For example, dovitinib, a tyrosine kinase inhibitor (TKI) that binds fibroblast growth factor receptor 3 (FGFR3), is currently under investigation for efficacy in combination with abiraterone (NCT01994590), after being previously shown to improve bone scans and reduce SREs in 6 of 23 patients in a proof-of-principle study. FGFR signaling in bone stromal cells is an important regulator of bone formation, and it is possible dovitinib can impact prostate cancer cell growth and osteoblast behavior.

Overall, TKI trial results for the treatment of mCRPC have been varied. Dasatinib, an inhibitor of multiple TKIs including SRC family kinases, reduced disease progression in 57% and bone lesions in 30% of mCRPC patients in a phase I trial. However, in a recent phase 3 trial, the combination of dasatinib and docetaxel failed to provide a survival advantage compared with docetaxel and placebo. Interestingly, dasatinib has been shown to induce differentiation of mesenchymal stromal cells in bone-forming osteoblasts, which may exacerbate prostate tumor-induced osteogenesis. Combination therapy with an anti-androgen may circumvent this possibility. To that end, a combinational study of dasatinib and abiraterone/prednisone prior to chemotherapy is currently being investigated for impact on PFS as a primary outcome (NCT01685125).

Constitutive activation of multiple signaling pathways via different RTKs can provide a significant survival advantage for tumors; thus dual targeting of TKIs may be beneficial for impacting tumor growth. Cabozantinib, for example, is a dual TKI of VEGFR2, the receptor for angiogenic factor vascular endothelial growth factor (VEGF), and c-MET, a receptor for hepatocyte growth factor (HGF). In a phase II randomized trial, daily administration of cabozantinib improved bone scans in 68% of mCRPC patients (with complete resolution in 12%), reduced soft tissue lesions, and improved PFS. However, cabozantinib failed to reach the primary endpoint of increasing OS, compared with prednisone alone, in a phase III randomized trial of mCRPC patients who had previously received docetaxel and abiraterone. Trials examining cabozantinib in combination with androgen ablation (NCT01630590) or chemotherapies such as docetaxel (NCT01683994) are ongoing and recruiting. RTKs mediate their effects via cell signaling circuitry and inhibitors of RTK effectors — such as mTOR for example — are also being explored clinically (NCT01174199 and NCT01026623).

Under selective pressures induced by therapeutic regimens, prostate cancer cells often acquire resistance to programmed cell death. For example, upregulation of DNA repair mechanisms is a common way for prostate cancer cells to avoid apoptosis induced by environmental stress. Currently, inhibitors of poly ADP ribose polymerases (PARPs) that repair DNA “nicks” are being investigated. PARP-1 is a nuclear enzyme that detects single- and double-strand DNA breaks and initiates repair mechanisms. Further, PARP-1 can bind and regulate AR transcriptional function. PARP-1 has also been shown to play a critical role in mesenchymal stem cell-driven osteogenesis, making it a promising target for treating bone mCRPC. Olaparib (Lynparza), a PARP-1 inhibitor, was included in a phase II trial of 50 mCRPC patients, 16 of which had mutational defects in DNA-repair genes, in bone and visceral organ metastasis biopsies measured before and after treatment. Olaparib produced a PSA response (decline of 50% or more) in 22% of the patients and reduced the numbers of circulating tumor cells in 29%. Eighty-eight percent of patients with defects in DNA-repair genes (including BRCA1, ATM, CHEK2, and HDAC) showed a positive response to olaparib, suggesting that mutations in DNA repair genes may serve as a biomarker for mCRPC response to PARP inhibition. A current trial is examining the efficacy, safety, and tolerance of olaparib given in combination with abiraterone and will be compared with placebo with abiraterone (NCT01972217).

Bone Microenvironment Targeted Therapies for mCRPC Treatment

The surrounding bone microenvironment is a key driver of CRPC growth, and as such, presents therapeutic opportunities. Although a hallmark of mCRPC is bone formation, the lesions also contain areas of extensive osteolysis and osteoclast activity. The monoclonal antibody denosumab binds to the receptor-activator of nuclear factor kappa B-ligand (RANKL), a key regulator of osteoclast formation. By preventing interaction with its cognate receptor RANK, denosumab effectively inhibits osteoclast formation and activation. Denosumab has been proven to significantly increase the median time to 1 SRE by 18% (20.7 months vs 17.1 months) compared with bisphosphonates. Despite these results, no impact on OS of the patients was noted compared with the control arm. Because denosumab is well tolerated, it is currently being explored in combination with other therapies such as abiraterone (NCT02758132).

Another class of bone-targeted inhibitors commonly used for the treatment of mCRPC is bisphosphonates. Bisphosphonates specifically target normal and pathological bone formation by binding to calcium in newly-formed bone, and upon resorption, they are taken up by osteoclasts, inducing their apoptosis. Compared with placebo, bisphosphonates such as zoledronate significantly increased me-
TARGETING BONE METASTATIC CASTRATE RESISTANT PROSTATE CANCER

dian time to a SRE (488 days vs 321 days for placebo treatment) and reduced the frequency of SREs (39% vs 49%). Similar to denosumab, zoledronic acid does not enhance OS of men with mCRPC.32-33 Bisphosphonates are also well tolerated in patients and thus provide an advantageous strategy for delivering therapies to the bone tissue and, specifically, areas undergoing remodeling. Guanidine, for example, is a potent chemotherapy but noted side effects make applying the treatment to patients difficult.34 Osteodec is a novel therapy that grafts guanidine onto a bisphosphonic foundation with the goal of specifically targeting bone metastases and avoiding dose-limiting toxicities via bone-specific delivery of guanidine. Osteodec is currently in phase II trials that are investigating time to SRE (NCT02825628).

A recent breakthrough has been the FDA approval of radium-223 dichloride. The radium isotope is similar in nature to calcium and is preferentially absorbed by bone tissue where it emits high-energy alpha particles, killing cancer cells within a short range (less than 100 microns). Radium-223 was found to improve median OS by 3.6 months compared with placebo.35 Because of its success, clinical trials are investigating the efficacy of radium-223 with other therapies such as abiraterone (NCT02043678) and docetaxel (NCT01106352).

Significant advances have been made in the past decade with the development of immune-targeted therapies aimed at activating anti-tumor immunity and inhibiting pro-tumoral immunity. The immunostimulant sipuleucel-T and immune vaccine, PROSTVAC, activate the immune system against 2 well-defined prostate antigens, prostate acid phosphatase (PAP) and PSA, respectively. Sipuleucel-T is a personalized treatment involving ex vivo culture of patient-derived antigen-presenting cells (APCs) with a fusion protein (PA2024) of recombinant PAP and granulocyte-macrophage colony-stimulating factor (GM-CSF), an immune stimulating factor. APC-expressing PA2024 cells are then transfused back into the patient where they induce immune activation against cancer-derived PAP. Compared with placebo, sipuleucel-T proved to be most beneficial for mCRPC patients with low disease burden, improving median OS by 4 months and 3-year survival, but has not been as successful for patients with more advanced disease (>20 detected bone lesions), demonstrating a need for greater understanding of mCRPC immunogenicity in bone.36,37

PROSTVAC utilizes 2 recombinant poxviruses: vaccinia (PROSTVACv), which primes the immune system; and fowlpox (PROSTVAC-F), an immune system booster. Each vector has been transduced to express 4 human genes: PSA and 3 costimulatory molecules that enhance T-cell activation (leukocyte function-associated antigen-3, [LFA3]; intercellular adhesion molecule-1, [ICAM1]; and B7-1).38 In a phase II trial, PROSTVAC significantly improved median OS in mCRPC patients.39 These findings contributed to the initiation of an ongoing phase III trial investigating the impact of PROSTVAC alone or in combination with GM-CSF on overall survival in symptomatic mCRPC patients (NCT01322490).

A new wave of immunotherapies has arisen in recent years that specifically targets checkpoint inhibition, a mechanism of tumor immune evasion that prevents cytotoxic T-cell lymphocyte (CTL) activation. Although the percentage of T cells in the bone marrow is relatively low, CD4+ and CD8+ CTLs have been shown to exert anti-tumor effects in bone metastases of other cancers, such as breast and melanoma.40-41 Ipilimumab, a monoclonal antibody against receptor cytotoxic T-lymphocyte antigen-4 (CTLA-4), a negative regulator of T-cell activation, inhibits regulatory T-cell function and activates cytotoxic T-cells. Although ipilimumab has been highly successful for the treatment of metastatic melanoma,42,43 it has not proved efficacious for the treatment of mCRPC. In a recent phase III trial, ipilimumab failed to improve overall survival in comparison to placebo, yet reduced PSA, and improved 3-month progression-free survival.44

Several studies have demonstrated adverse effects that ended initial clinical trials but of note, a single patient had a dramatic response with a reduction in the number of bone lesions and disease-free survival at 6 years.45 Defining markers predictive of patient response to checkpoint inhibitors will be critical for their clinical application. Nevertheless, clinical trials are investigating the combination of ipilimumab with ADT on disease progression (NCT014988978) and the safety of using ipilimumab in combination with GM-CSF (NCT00064129). Another checkpoint inhibitor drug, nivolumab, a monoclonal antibody that targets PD-1/PD-L1, has shown little promise, as there has been no clear indication that CRPC tumors express PD-L1.46 Targeting T-cell activation through 2 different mechanisms, APC-mediated activation and immune checkpoint inhibition, such as combinational PROSTVAC with ipilimumab treatment, may enhance drug efficacy over the individual compounds. Clinical trials are studying the efficacy of the checkpoint inhibitors combined (NCT02601014) or when added to ADT (NCT014988978).

Upcoming Opportunities and Threats for Bone mCRPC Treatment

Significant progress has been made in the development of therapies that target mCRPC growth in the bone microenvironment. Moving forward, the upfront application of therapies in combination — such as ADT with radium-223 — for example, may prove more effective than sequential treatments in extending OS. Molecular profiling of individual mCRPC patients and the identification of response predictors will clearly be beneficial for the smart application of targeted therapies, such as TKIs and immune checkpoint inhibitors, in order to achieve maximal responses. A major challenge for the medical oncologists is mCRPC heterogeneity and the emergence of resistant disease.47-49 Adaptive therapy aims to prevent the emergence of resistant subpopulations by maintaining therapy-sensitive populations.50 The application of therapies, as needed, to stabilize disease progression, rather than continuously, is currently being explored in the clinic for abiraterone in mCRPC (NCT02415621). Further, novel computational modeling approaches to define optimal therapeutic strategies for heterogeneous bone metastatic prostate cancer are under investigation.51,52

In conclusion, a greater understanding of the molecular underpinnings of bone metastasis has contributed to an expansion of potential therapies for mCRPC. Defining the optimal sequence and combina-
tions needed for these therapies, identifying key characteristics of the tumor that could determine which patients would benefit most, and controlling tumor evolution in the bone microenvironment will no doubt improve the efficacy of current therapies and significantly extend the OS of men with mCRPC.

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Stereotactic Body Radiotherapy for Oligometastases: An Opportunity for Cure?

Greg Kauffmann, MD; Jeffrey Lemons, MD; and Steven J. Chmura, MD, PhD

Abstract

Advances in radiation oncology have enabled the delivery of ablative doses of radiotherapy (RT) to a variety of anatomic sites with increased precision and minimal toxicity. Stereotactic body radiotherapy (SBRT) has emerged as an attractive alternative to surgical resection. In this review, we will discuss the following: the proposed state of limited metastatic disease (commonly referred to as oligometastases) and the growing role of SBRT in the management of these patients; potential challenges in selecting patients with limited metastases who are most likely to benefit from aggressive local interventions; some of the key nonrandomized studies that have demonstrated the feasibility and safety of SBRT to treat multiple metastatic sites; important questions including the safety of SBRT in combination with systemic therapies; and the existing randomized data to support treatment of limited metastases and the multiple ongoing randomized trials. Lastly, we will examine the interaction between SBRT and the immune system, and explore future applications that include combining SBRT with immunotherapy.

Introduction

Technical advances in radiotherapy (RT) oncology have enabled the delivery of highly conformal, ablative doses of RT to multiple extracranial sites, referred to as stereotactic body radiotherapy (SBRT). In contrast to conventionally fractionated RT, which often involves daily doses of 1.8 to 2.0 Gy delivered over 6 to 8 weeks, SBRT utilizes higher doses per treatment (6-30 Gy) delivered over a shorter time frame (typically 1-5 fractions over 1-2 weeks). Advances in RT treatment planning and image guidance have enabled delivery of SBRT with increased accuracy and precision to limit radiation exposure to surrounding normal tissues. As a result, delivering ablative RT to limited metastases has become an attractive and increasingly utilized treatment paradigm for patients with good performance status. Questions remain regarding the benefits in treating limited metastases with ablative RT, how to identify optimal candidates, and the safety of incorporating newer RT techniques with novel systemic therapies. Herein, we describe the state of limited metastatic presentation commonly referred to as oligometastases. We explore the growing role of SBRT in the management of patients with limited metastases.

Oligometastases: Definitions and Patient Selection

The concept of oligometastases was first proposed by Hellman and Weichselbaum in 1995, who described it as an intermediate state of cancer pathogenesis between purely localized disease and widespread metastases. Although no consensus definition exists, the oligometastatic state is defined as 5 or fewer clinically detectable metastatic lesions. As a consequence, it has been hypothesized that patients with a low number of metastases may benefit from metastasis-directed local therapies in addition to standard systemic therapies. It has been shown that long-term survival can be achieved after metastasectomy for well-selected patients with limited hepatic or pulmonary metastases. Such studies are often cited as evidence of the oligometastatic state; however, it is unclear whether these favorable results should be attributed to aggressive interventions or indolent tumor biology.

Since the initial description of oligometastases by Hellman and Weichselbaum, additional terms have been introduced to help explain the range of clinical behavior observed in distinct metastatic settings. Oligorecurrence describes limited metastases in the setting of a controlled primary tumor, and oligoprogression describes the growth of only a limited number of metastases while other sites are controlled by or responding to systemic therapy. The incidence and natural history of oligometastatic disease for different tumor histologies is still being defined. For example, in one study of patients with metastatic non–small cell lung cancer (NSCLC), 50% had 3 or fewer metastatic sites. Similar reports have identified subsets of patients with limited metastases in other common malignancies, such as prostate, breast, and colorectal cancer.

Currently, classifying patients as oligometastatic relies on the ability of diagnostic imaging to accurately identify the number of metastatic sites. Advanced imaging modalities such as PET/CT and MRI have improved the ability to evaluate patients for metastatic disease. In addition, novel prognostic biomarkers, such as
circulating tumor cells and microRNA expression profiles, may improve the ability to select patients who have more indolent tumors, and who are perhaps most likely to benefit from aggressive local therapies.11,12

In a prospective study of metastasis-directed SBRT that included patients with 1 to 5 metastases, the estimated 5-year overall survival was 32%, demonstrating that long-term survival may be achieved in a subset of oligometastatic patients after metastasis-directed SBRT.12 In general, several clinical factors appear to be associated with prolonged survival, such as primary tumor histology (ie, breast), fewer number of metastases, prolonged time from diagnosis to development of metastases, and stable or controlled disease prior to SBRT.2 Despite efforts to stratify by clinical factors, patient selection remains a major challenge, and many patients considered oligometastatic may harbor subclinical micrometastases that will progress despite metastasis-directed ablative therapies.

**SBRT: Applications, Efficacy, and Safety**

Early applications of stereotactic RT techniques focused on ablative treatments for intracranial metastases. The development of technologies to allow image guidance and real-time assessment of tumor motion have facilitated the application of SBRT to complex extracranial targets. A growing body of evidence suggests that SBRT is technically feasible for multiple extracranial sites with acceptable toxicity, including lung, liver, spine, and many others.13,14 SBRT has potential advantages compared with surgical resection since SBRT is generally less invasive, can target anatomic locations not accessible by surgery, and can be administered with minimal interruptions in systemic therapy.

Thus far, the bulk of evidence supporting SBRT to treat oligometastases comes from single-institution retrospective experiences or single-arm dose-escalation trials. The Table summarizes results of select studies of SBRT for oligometastases with longer-term follow-up. Treated metastasis control after SBRT appears to be comparable to metastasectomy, ranging from 70% to 90%.10 Interestingly, ablative RT doses also appear to be equally effective in controlling metastases from historically radio-resistant histologies, such as sarcoma, melanoma, and renal cell carcinoma.15,16 These findings are consistent with the notion that SBRT works through a different mechanism than conventional RT, such as endothelial cell damage.20 Furthermore, RT dose is important for achieving local control. In an SBRT dose-escalation study from the University of Chicago, treated metastasis control was 100% in the highest-dose cohort (48 Gy in 3 fractions) compared with only 45.7% for the lowest-dose cohort (24 Gy in 3 fractions).

Several studies have evaluated the safety of SBRT as applied to specific anatomic sites. In general, rates of grade 3+ pulmonary toxicity are relatively low (<10%) after lung SBRT.21 However, serious and even fatal complications have been reported after SBRT for central lung tumors.21 In a multi-institutional study of SBRT for liver metastases, rates of grade 2 and grade 3 toxicities were 1.9% and 3.2%, respectively.22 In a large, multi-institutional study, there was a 6% rate of fracture after spine SBRT.21 The ongoing NRG-BR001 trial will provide additional insight regarding the safety and treating multiple metastases with SBRT and the optimal dose-fractionation scheme (NCT02206334).

Aside from the ultimate goal of prolonging survival, SBRT for oligometastases might have other clinically meaningful benefits. One such benefit could be the use of SBRT as a means to delay the start of systemic therapy or allow for prolonged chemotherapy breaks. Furthermore, in the setting of oligoprogression, SBRT might enable the continuation of an otherwise effective targeted therapy. For example, in a study of patients with ALK-positive NSCLC and oligoprogressive disease, prolonged crizotinib use was seen in those who received ablative local therapy to all metastases compared with those who did not (median duration, 28 months vs 10.1 months).24 In addition, it is possible that SBRT will result in more durable palliation and local disease control compared

<table>
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<th>TABLE 1. Select Studies of SBRT</th>
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<tr>
<td>Pulmonary Multi-institutional (US)</td>
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<tr>
<td>Hepatic Multi-institutional (US)</td>
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<td>Spine MD Anderson Cancer Center</td>
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RT, radiotherapy
with conventionally fractionated RT. The RTOG 0631 study will evaluate whether spine SBRT improves pain control compared with conventional “palliative-dose” RT (NCT00922974).

Randomized Data and Ongoing Trials
Level I evidence showing a survival benefit to metastasis-directed ablative therapy is limited to surgical resection or radiosurgery for limited brain metastasis.\(^2,26\) Although level I data are lacking, the use of SBRT for oligometastases has increased in the United States and internationally. According to an international survey of over 1000 radiation oncologists published in 2015, 61% of respondents reported using SBRT to treat extracranial oligometastases, with the majority of nonusers planning to start within the next 1 to 3 years.\(^27\) More recently, results of a multi-institutional phase II randomized trial demonstrated improved progression-free survival (PFS) with the addition of consolidative local therapy with surgery or SBRT in patients with oligometastatic NSCLC and no disease progression following induction systemic therapy (median PFS, 14.4 months vs 3.9 months).\(^28\)

Multiple clinical trials evaluating the role of ablative therapies for oligometastases are ongoing, although accrual has been challenging for some. In the United States, NRG-BR002 (Figure) is a randomized trial comparing ablation of all metastases versus standard-of-care systemic therapy for patients with oligometastatic breast cancer (NCT02364557). Internationally, a number of studies are accruing patients, including SABR-COMET (NCT01446744), CORE (NCT02417662), and STOMP (NCT01558427) trials. Ideally, ongoing and future studies incorporating blood and tissue samples will add to our knowledge of prognostic and predictive biomarkers to aid in patient selection.

Future Directions: SBRT and the Immune Response
An intriguing application of SBRT is the potential to enhance tumor-specific immunity, and thus “prime” the immune system to immunotherapy. Beyond DNA damage and direct cell death, the therapeutic effects of SBRT appear to be mediated via CD8+ T cells.\(^29\) Furthermore, in addition to tumor debulking, preclinical models suggest a synergistic antitumor effect when RT is combined with immunotherapy.\(^30,31\) A number of mechanisms have been proposed to support this phenomenon, such as increased exposure to tumor antigens, enhanced T-cell function, and downregulation of immunosuppressive cell populations.\(^32\)

With the recent emergence of cancer immunotherapy as a standard treatment for many solid tumors, there is growing interest in combining immunotherapy and SBRT as a means to improve response rates. However, the optimal RT dose, fractionation schedule, and timing of therapies is unknown. Numerous ongoing clinical trials combining RT with immunotherapy will hopefully shed light on these important questions.\(^33\)

Conclusion
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Assessing Current and Emerging Data Sets to Optimize Sequencing Considerations in ALK-Positive NSCLC

Learning Objectives
After participating in this CME/CE activity, learners should be better prepared to:
- Review current sequencing options in patients with ALK-positive NSCLC
- Assess the potential impact of ongoing clinical trials/emerging data with respect to treatment sequencing in patients with ALK-positive lung cancer
- Discuss the impact on clinical decision making that central nervous system metastases have on treatment and sequencing choices in ALK-positive NSCLC

Overview
This activity is designed to inform physicians about current and emerging data sets in the context of optimizing sequencing considerations in patients with ALK-positive non-small cell lung cancer (NSCLC).

Target Audience
This activity is directed toward medical oncologists, pulmonary care specialists, primary care physicians, nurses, and nurse practitioners who treat and/or manage patients with lung cancer. Surgical oncologists, radiation oncologists, pathologists, internists, fellows, physician assistants, and other healthcare providers interested in the treatment of lung cancer are also invited to participate.

Instructions for Participation/How to Receive Credit:
1. Read the article in its entirety.
2. Use the QR code or type http://bit.ly/2bzFZ1Q into your Web browser to access the posttest.
3. Complete and pass the posttest with a score of 70% or higher.
4. Complete the evaluation and request for credit.

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Lung cancer is the leading cause of cancer deaths in the United States and is the second most commonly diagnosed cancer; an estimated 224,390 new cases of lung cancer are expected to be diagnosed this year. Most cases present with advanced disease, and the treatment of advanced non-small cell lung cancer (NSCLC) has gone through a substantial paradigm shift in recent years as our understanding of “targetable” driver oncogenes continues to grow. The presence of key oncogenic alterations, such as activating mutations and chromosomal rearrangements, can now help physicians predict responsiveness to specific targeted therapies. For instance, most epidermal growth factor receptor (EGFR) mutations seen at initial presentation are associated with sensitivity to the EGFR tyrosine kinase inhibitors (TKIs) such as erlotinib, gefitinib, or afatinib. Gene rearrangements activating the anaplastic lymphoma kinase (ALK) have also been identified as an additional targetable oncogene in NSCLC. The most common 5’ fusion partner in these rearrangements is EML4, but other partners can, and do, exist. ALK rearrangements possess potent oncogenic activity both in vitro and in vivo, and are prevalent in 2% to 7% of patients with NSCLC. Published data indicate that many patients with ALK-rearranged NSCLC share some of the same clinical characteristics associated with EGFR-mutations; for instance, adenocarcinoma histology and being a never or light smoker. But just as with EGFR-mutant disease, exceptions to these clinical “stereotypes” occur.

According to the National Comprehensive Cancer Network (NCCN) guidelines, testing for ALK gene rearrangements is a category 1 recommendation. Once the presence of ALK rearrangement is confirmed, small-molecule tyrosine kinase inhibitors (TKIs) targeting ALK are recommended. Crizotinib, an inhibitor of ALK, ROS1, and MET tyrosine kinases is approved by the FDA for patients with locally advanced or metastatic NSCLC with ALK-positive disease. Data from a randomized phase 3 trial comparing crizotinib with either pemetrexed or docetaxel in the second-line setting demonstrated superior survival of the targeted approach (PROFILE 1007). Later, a phase 3 trial compared crizotinib with first-line platinum-pemetrexed therapy without continued maintenance pemetrexed (PROFILE 1014) and demonstrated improved progression-free survival (PFS), response rate (74% vs 45%; P < .001), lung cancer symptoms, and quality of life with crizotinib.

Based on this data, the NCCN 2016 guidelines recommend crizotinib as category 1 for first-line therapy in patients with ALK-positive NSCLC, assuming the molecular information is available in time to make a first-line treatment decision. Unfortunately, in whatever line the crizotinib is used, patients eventually develop resistance, and for those patients, the next-generation ALK inhibitors—ceritinib and alec tinib—are options that can be utilized. Ceritinib is recommended by the NCCN guidelines for patients with ALK positive metastatic NSCLC who have progressed on crizotinib or who are intolerant to crizotinib. Alec tinib is another oral TKI of ALK that is recommended by the NCCN guidelines as a category 2A option for patients with ALK-positive metastatic NSCLC who have progressed on crizotinib or are intolerant to crizotinib. Several other agents—including X396, brigatinib, and lorlatinib—are also currently being investigated for the same indication.

These next-generation ALK inhibitors can regain disease control post crizotinib in two main ways. Firstly, they have activity against a range of different ALK mutations that can develop during crizotinib treatment that alter the resulting fusion protein’s sensitivity to the drug. Secondly, they have greater potency against ALK-positive disease within the central nervous system (CNS) than crizotinib. Up to 40% to 50% of patients on crizotinib may develop brain metastases in the setting of otherwise controlled extracranial disease likely due to poor CNS penetration of crizotinib. ALK-positive patients developing brain metastases can be treated with CNS-directed radiotherapy for local control of brain metastases while continuing with their crizotinib treatment. However, with the introduction of next-generation TKIs, the paradigm is set to shift for the treatment of brain metastases in ALK-positive patients. Clinical trial data have shown that next-generation TKIs have increased CNS penetration and activity. Treatment with next-generation TKIs, such as alectinib, can potentially help delay or avoid the need for whole brain radiation therapy (WBRT) in select patients and may help convert patients from WBRT to stereotactic radiosurgery (SRS) along with drug therapy.

Data from a phase I/II study of alectinib in crizotinib-refractory ALK-positive NSCLC that included patients with brain metastases demonstrated a complete response in 29% of the patients. The efficacy of alectinib in brain metastases was also demonstrated in another phase II trial that enrolled crizotinib-refractory disease. In this study, treatment with alectinib demonstrated a complete response (CR) in 43% of patients with baseline brain metastases and no prior radiation. Similarly, intracranial overall response rates (ORRs) of 36% and 63% in brain metastases have been reported with and without exposure to prior ALK-inhibitor therapy, respectively, in ALK-positive patients treated with ceritinib. Similarly, treatment with brigatinib has shown an intracranial ORR of 50% in a phase I/II trial. As these next-generation agents are changing the ALK-positive NSCLC treatment options post crizotinib, one issue that physicians currently face in the potential setting of multiple post crizotinib approvals is understanding which next-generation ALK inhibitor should be chosen for patients that have progressed on crizotinib and whether whichever one is not used should then be used as a third-line ALK TKI, (ie, is there a certain sequence that always works best when it comes to choosing ceritinib or alectinib or does the choice depend on the specific molecular situation at the time?). Case reports support the sequential use of alectinib after ceritinib in the setting of progressive CNS disease or in patients who have developed the ceritinib resistance mutation F1174V. Similarly, ceritinib may be active after alectinib in patients who become resistant due to the alectinib resistance mutation I1171T.

Given their excellent efficacy post crizotinib, another emerging question is the potential role of next-generation ALK inhibitors in the first-line setting instead of crizotinib. In 2016, results from the phase III J-ALEX study were presented for the first time. In this trial, conducted
only in Japan, patients with TKI-naive advanced ALK-positive NSCLC were randomized to receive either crizotinib or alectinib. Alectinib showed superior PFS. The PFS hazard ratio (HR) of the alectinib arm to the crizotinib arm was 0.34 (99.6826% CI: 0.17-0.70; P <.0001). At the time of the analysis, median PFS was not reached (95% CI, 20.3-not estimated) in the alectinib arm while it was 10.2 months (95% CI, 8.2-12.0) in the crizotinib arm. A similar study of alectinib versus crizotinib (ALEX) has yet to read out, and similar first-line comparisons to crizotinib with both brigatinib and X396 are currently enrolling. With the potential of multiple next-generation ALK TKIs eventually being available across multiple lines of therapy, understanding the differences between these agents will be key in optimally tailoring treatment for each patient.

Ross Camidge, MD is the director of Thoracic Oncology at the University of Colorado. Dr Camidge provided his insights and point of view on the current and emerging data sets to optimize sequencing considerations in ALK-positive NSCLC.

Moderator: What are some of the current unmet needs or challenges in the treatment of patients with ALK-positive NSCLC?

Dr Camidge: The first problem that could occur, from the perspective of an individual patient, would be intolerance of some of the side effects of some of the ALK inhibitors then later on progression, (ie, acquired resistance to a given ALK TKI either in the body and/or in the brain).

Moderator: Most patients with ALK-positive NSCLC who begin treatment with an ALK-targeted agent develop resistance. How do you currently sequence treatment in your patients with ALK-positive NSCLC?

Dr Camidge: Assuming somebody has advanced ALK-positive NSCLC and they’re treatment-naïve, well, if we edit out the brain for the purposes of this initial discussion, there is currently only one FDA-licensed, first-line ALK inhibitor, and that’s crizotinib. So, I start them on crizotinib. For the patients who eventually do develop resistance in the body to crizotinib, there are three broad drug options. There are two licensed next-generation ALK inhibitors in the United States, ceritinib, and alectinib. There is platinum/pemetrexed chemotherapy and then there are other ALK inhibitors within clinical trials such as brigatinib or lorlatinib.

Moderator: What are some of the factors that impact your sequencing decisions in patients with ALK-positive NSCLC?

Dr Camidge: I think most people would move from crizotinib to one of the two licensed next-generation inhibitors, aiming to keep cytotoxic chemotherapy in reserve and then obviously, if they don’t have access to clinical trials, their only two options are ceritinib and alectinib. In terms of choosing between those two drugs, although the efficacy signals that we’ve seen in the body look similar, there are two major differences between alectinib and ceritinib.

The first is the tolerability. So, approximately 60% of people starting ceritinib at the standard dose of 750 mg require a dose reduction. The rate for dose reduction for alectinib is much closer to 20%. So, ceritinib is relatively poorly tolerated, mostly because of gastrointestinal side effects, nausea, stomach cramps, diarrhea. Alectinib has issues, in terms of peripheral edema and a photosensitive skin rash, but, in general, alectinib tends to be better tolerated than ceritinib. So, that’s the one I would reach for first after crizotinib—all other things being equal.

The other difference between the two, and it’s hard to get a direct head-to-head comparison, is the activity of these in the brain. So, we know that ALK-positive disease has a certain propensity to go to the brain and we also know that crizotinib has a relative Achilles’ heel, in terms of its ability to penetrate and have activity within the brain. As this became apparent while studies of many of these other drugs were ongoing, the ability to generate data on the activity of these drugs in the brain was really captured retrospectively. So, there’s not perfect data sets and I think [that] revealed how relatively poor we’ve been in the past at capturing that kind of information. But from what we can see, the response rate and duration of benefit in the brain probably are better with alectinib than it is with ceritinib—both in terms of any fundamental differences between the drugs, plus any effect of the higher dose reduction rate with ceritinib. Dose reduction will obviously reduce the CNS exposure, and if ceritinib has a 60% dose reduction rate, that would certainly further lessen its activity within the brain when you reduce the dose. So, when choosing between alectinib and ceritinib, tolerability is the number one decision maker and then the second is activity within the central nervous system.

Moderator: Primary results from the J-ALEX study that compares alectinib with crizotinib in the front-line setting were presented recently at this year’s ASCO. What potential impact can this data have on...
treatment sequencing in patients with ALK-positive NSCLC?

Dr Camidge: I think we have to wait for it to really impact us in the United States and the rest of the world outside of Japan. Currently, alectinib does not have a first-line license outside of Japan. I think we would have to wait for the results of the ALEX trial to see if alectinib does get a first-line treatment option in the United States and the rest of the world. The ALEX and J-ALEX studies are similar and what J-ALEX did was raise the hypothesis that initially going on a drug with a broader spectrum of coverage of some of the mechanisms of resistance to crizotinib—both penetration into the brain to edit out CNS progression and also activity against some of the known acquired resistance mutations to crizotinib—would be absolutely better than just going on alectinib after crizotinib.

What we’ve seen is that when you go onto that drug first, compared to crizotinib, the J-ALEX studies seem to suggest that the median progression-free survival could be longer than you might imagine if you were to use crizotinib followed by alectinib (Figure 1).9 Clearly, it’s a positive study, but it’s not just asking ‘is the PFS longer than crizotinib,’ because that’s not the question, in and of itself, because you can still use these drugs sequentially. The question starts to become, ‘is starting with alectinib just exactly the same as you would get if you did crizotinib then alectinib?’ So, is it just the sum of sequential therapy or does it somehow change the natural history of the disease and going on the next-generation drug first will actually have a longer median PFS than if you were to use these drugs sequentially.

The J-ALEX study was stopped early and so the data are not mature, but one might imagine that the median progression-free survival is usually about 10 months on crizotinib. The median progression-free survival for alectinib post crizotinib is running about seven or eight months. So, anything that is looking like the median progression-free survival on first-line alectinib is sort of north of 18 months makes you think that you might actually be changing the natural history of the disease, by preventing a bulge in dividing cells at the time of the initial acquired resistance on crizotinib—bulge being where more diversity and more mechanisms of resistance can be bred (Figure 2).7 And the lower limit of the confidence intervals for the alectinib arm in the J-ALEX study was 20.3 months, which certainly suggests that may be going on.

Now, before we all leap to prescribing alectinib off-label in the first-line setting, one should point out that the J-ALEX study differs from the ALEX study in three main ways. First of all, the J-ALEX study was only conducted in Japan with Japanese patients, and they may have somewhat different biology than the rest of the world. Secondly, it uses patients who are allowed to have had prior first-line chemotherapy, whereas the ALEX study is in a true treatment-naïve population. And perhaps most importantly, the J-ALEX study was actually done with a different dose than the dose of alectinib that is currently licensed

![FIGURE 2A. Suggested Impact of Sequential Suppression on Acquired Resistance (AR).](image1)

Hypothetically, even if new Rx active against defined resistance mechanism shorter systemic PFS likely in AR setting

![FIGURE 2B. Suggested Impact of Sequential Suppression on Upfront Suppression.](image2)

Giving drug with activity against BOTH baseline (A) and AR (B) forms upfront: PFS A+B or greater than the sum?

![Dividing cells](image3)

Resistant cells continually arise. If total burden of dividing cells is lower for longer may take more time for next resistant form to arise.

### References


with the FDA. So, they use actually a lower dose, 300 mg twice a day, whereas in the rest of the world, it’s 600 mg twice a day. Now, that might make you think that ALEX, if anything, would be even better, but I would suggest we have to wait for those results.

**Moderator:** How does CNS metastases impact your treatment and sequencing choice?

**Dr Camidge:** I think you have to think about CNS metastases in two broad categories. One is people who have brain metastases when they’re first diagnosed, and then the second is people who develop brain metastases while still on crizotinib. So, let’s do the first of those first. So, if you have brain metastases at baseline, your real decision that you’re trying to make—again, recognizing that there are no next-generation drugs which are currently licensed in the first-line setting—is between going on crizotinib and keeping an eye on the brain and giving radiotherapy, as needed, later or treating the brain first with radiation/surgery and then going on the crizotinib.

Now, the data for crizotinib in the central nervous system reveal that it is certainly less effective in the brain than it is in the rest of the body but it is not ineffective. So, the response rate in the brain for crizotinib is about 18%, whereas it’s closer to about 60% in the rest of the body. And certainly other metrics of efficacy, be they PFS, duration of response, are lower in the brain than they are in the rest of the body. The way I actually tend to do it is based on if the patient is asymptomatic. If so, I think it’s reasonable to try the crizotinib in case they’re in the 18% and keep a close eye on the brain, keeping radiotherapy in reserve. I think if they’re symptomatic, I would be much more inclined to give a local therapy, be it radiotherapy or neurosurgery first, and then go onto the crizotinib.

Now, the one area I’d like to expand on is it depends on whether the number of lesions in the symptomatic cases are enough that you might be considering whole brain radiotherapy as opposed to stereotactic radiosurgery. And, in that situation, and probably that situation only, because whole brain radiotherapy has longer-term cognitive side effects and these patients live long enough to manifest them, I might try any way I could to try and avoid the whole brain radiotherapy, and I would probably, therefore, actively go looking for either a clinical trial or off-label use of a next-generation ALK inhibitor first line.

**Moderator:** Some patients can develop highly-resistant mutations, such as the ALK G1202R for which alectinib or ceritinib are ineffective. Do we have any data on brigatinib for this mutation?

**Dr Camidge:** Brigatinib has shown activity against G1202R. However, it depends on the dose of brigatinib that you’re giving. These mutations, they’re not all or none. You can have a higher IC50, but it depends on whether your exposure of the drug is above that IC50 and that’s the key thing to bear in mind. So, G1202R certainly has a higher IC50 than most other mutations. So, most of these other drugs, and it certainly looks at the licensed doses of ceritinib and alectinib, you can get G1202R emerging, suggesting that you’re not suppressing it at standard doses. But I think what we saw this year at ASCO is there was a case of somebody treated with 240 mg of brigatinib who had G1202R who responded. However, that is higher than the planned recommended dose of 180 mg.

**Moderator:** Based on the recent data release at ASCO, treatment with lorlatinib demonstrated durable clinical responses including intracranial responses in ALK-positive and ROS1-positive NSCLC patients. Similarly, good efficacy was also reported for brigatinib in ALK-positive NSCLC. How, in your opinion, would approval of these agents impact your treatment choice?

**Dr Camidge:** Well, I think both of these drugs, brigatinib and lorlatinib have wider spectrums of coverage against the known resistance mechanisms than either ceritinib or alectinib. They both also have good activity within the brain.

I think it’s a challenge to look at them head-to-head because they’re being done in different studies. The median progression-free survival with brigatinib that we saw at ASCO is now over a year. And although, with all the caveats of comparing between studies, that does appear to be longer than alectinib or ceritinib in the immediate post-crizotinib setting. So, that’s actually very exciting.

For lorlatinib, one of the more interesting thing is they’re showing activity after more than two prior ALK inhibitors, so crizotinib and, presumably, some next-generation ALK inhibitor. However, it’s hard to pull apart the lorlatinib data, partly because there are lots of different next-generation ALK inhibitors and also because, in the way in that particular study, they counted re-challenge with crizotinib as another line of ALK inhibitor.

Both of the drugs have got interesting side-effect profiles. Brigatinib has got about a 20% dose reduction rate, so similar to alectinib. In about 3% of people within about 48 hours of starting the drug, so very early on, you can get what they call early onset pulmonary events, which appears to be some kind of pneumonitis. But it seems to be different from the pneumonitis we see as a late event with other TKIs since it seems to actually disappear in some people if you can stay on the drug. And they found that by starting at a lower dose and then seven days later going up on the dose, they could really bring that number down to the very small percentage of patients it is now reported in.

For lorlatinib, as we have seen data from the phase I trial presented, it is important to try and assess the side effects not across all of the doses, but at the planned phase II dose, because the ones at the lower doses will have a lower rate of side effects and dilute out the real safety signal perhaps. However, it’s been hard to pull that apart so far. We can certainly see that hypertriglyceridemia, hypercholesterolemia, some confusion, and possible peripheral edema may be its associated side effects.

With brigatinib, we’re starting to see a fairly mature data set in the proposed 90 mg escalating to 180 mg planned dosing and we’re fairly confident how well that’s tolerated. Lorlatinib, I don’t think we’ve seen that mature data. And so trying to get a feel for whether these are always going to remain salvage drugs or whether any of them are going to be well tolerated enough to go into that first-line setting like alectinib, remains to be seen.
**Moderator:** Does the combination of ALK TKIs with checkpoint inhibition have a potential in patients with ALK-positive NSCLC? If so, in what setting would it work?

**Dr Camidge:** So, certainly this is something that is currently being explored; for example by combining lorlatinib with avelumab, a PD-L1 antagonist. As far as I can tell, there is zero data to support the combination. And, in general, ALK-positive disease has a relatively low mutation burden. It often occurs in never-smokers and is probably not particularly immunogenic. So, I think the combination is being done because people are combining PD-L1 inhibitors with everything. I am not optimistic that it is going to show true synergy in most cases, although an occasional patient may benefit.

**Moderator:** How important is clinical trial enrollment for patients with ALK-positive NSCLC? Do you have any recommendations for when practitioners can refer their ALK-positive NSCLC patients to clinical trials?

**Dr Camidge:** On the one hand, a community practitioner has easy access to, now, three different ALK inhibitors, one in the first-line setting, two in the second-line setting. Yet, we must recognize that the emerging data on some of the later runners, brigatinib and lorlatinib, may be looking even more promising. And access to these drugs may be limited by line of therapy in specific trials. So, for a patient who is savvy, who wants to potentially have access to the best treatment, I would consider a clinical trial from the moment they’re diagnosed.

**REFERENCES**


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