HEPATOCELLULAR CARCINOMA
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RENAL CELL CARCINOMA
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MULTIPLE MYELOMA
Genomic Landscape and Mechanisms of Disease Evolution and Progression in Multiple Myeloma
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PROSTATE CANCER
An Integrative Approach for Sequencing Therapies in Metastatic Prostate Cancer
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LUNG CANCER
RET-Rearranged Lung Cancer
Fernando C. Santini, MD, and Artur Katz, MD

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HEPATOCELLULAR CARCINOMA

New Therapies and Potential Biomarkers for Hepatocellular Carcinoma
Merly Contratto, MD, and Jennifer Wu, MD
Despite its high mortality rate, hepatocellular carcinoma only has 1 first-line systemic treatment available—sorafenib. The authors anticipate combination strategies involving immunotherapy will provide better outcomes than are currently experienced.

RENAI CELL CARCINOMA

Clinical Commentary: The Use of Clinical Biomarkers to Inform Treatment Decisions in Advanced Renal Cell Carcinoma
Daniel J. George, MD
In this commentary, Dr George describes the impact the identification and validation of clinical biomarkers will have on the management of patients with advanced renal cell carcinoma (aRCC). He suggests that absent any validated predictive biomarkers in aRCC, monitoring adverse effects as surrogate markers of efficacy might aid in treatment planning for individual patients.

MULTIPLE MYELOMA

Genomic Landscape and Mechanisms of Disease Evolution and Progression in Multiple Myeloma
Malin Hultcrantz, MD, PhD, and Ola Landgren, MD, PhD
The researchers write that accurate and available technologies will further increase the collective knowledge of molecular driver events. This, in turn, is essential for development of early and targeted treatments to improve patient outcomes in multiple myeloma.

PROSTATE CANCER

An Integrative Approach for Sequencing Therapies in Metastatic Prostate Cancer
Yadi Li, BSc; Sindhu Malapati, MD; Yu Ting Lin, BSc; and Akash Patnaik, MD, PhD, MMSc
The researchers provide a comprehensive, evidence-based review of the selection and sequencing of different lines of therapy for metastatic castration-resistant prostate cancer.

LUNG CANCER

RET-Rearranged Lung Cancer
Fernando C. Santini, MD, and Artur Katz, MD
Specific RET inhibitors that have less off-target toxicity and more potency are currently undergoing investigation. The authors foresee that recent advances in diagnostics will help facilitate the identification of patients who will potentially benefit from novel therapies.

GASTROINTESTINAL CANCER CME

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Considerations and Advancements of Precision Medicine in GI Cancers
With Arturo Loaiza-Bonilla, MD, MSEd, FACP
Biomarkers and targeted therapies are changing the whole of oncology, and the way clinicians treat gastrointestinal cancers specifically. Arturo Loaiza-Bonilla, MD, MSEd, FACP, joins us for a discussion about the latest breakthroughs and how to incorporate precision oncology into your practice.

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Identifying the right patient to receive the right treatment at the right time is a situation often discussed by clinicians and administrators in the halls and conference rooms of cancer centers in the United States and internationally. It is also a common theme for a majority of the manuscripts in this issue of The American Journal of Hematology/Oncology.

In “Clinical Commentary: The Use of Clinical Biomarkers to Inform Treatment Decisions in Advanced Renal Cell Carcinoma,” Daniel J. George, MD, notes that although targeted therapies have vastly improved outcomes in patients with advanced renal cell carcinoma, identifying patients who respond to specific treatments will only grow in importance. Dr George comments that for VEGF-targeted therapies, specific treatment-emergent adverse events are believed to act as surrogate markers of the activity of the drug, with the most data available for hypertension. But more studies are needed.

Malin Hultcrantz, MD, PhD, and Ola Landgren, MD, PhD, provide an overview of the complex genetic landscape and the mechanisms of disease evolution and progression in multiple myeloma. Their review focuses on the genomic events of tumor cells, but also touches on the bone marrow microenvironment and the host immune system in their manuscript, “Genomic Landscape and Mechanisms of Disease Evolution and Progression in Multiple Myeloma.”

A better understanding of biomarkers in hepatocellular cancer will help identify patient populations who can benefit the most from such promising therapies as immunotherapies and targeted treatments, according to Merly Contratto, MD, and Jennifer Wu, MD, in “New Therapies and Potential Biomarkers for Hepatocellular Carcinoma.”

Over the past decade in the metastatic castration-resistant prostate cancer (mCRPC) arena, 6 agents have been approved by the FDA in the broad categories of androgen-directed therapies, immunotherapy, chemotherapy, and bone-targeting agents. However, there remains a lack of consensus on optimal sequencing of these therapies in mCRPC, write Yadi Li, BSc, and coauthors. In “An Integrative Approach for Sequencing Therapies in Metastatic Prostate Cancer,” they note a paucity of data regarding optimal therapy for patients with mCRPC who have progressed on androgen-directed therapy and chemotherapy. Genomic sequencing and enrollment in clinical trials is the way forward, write the researchers.

RET-rearranged lung cancers represent a small subset of lung cancer, most commonly observed in patients with adenocarcinoma and minimal or no exposure to tobacco. In “RET-Rearranged Lung Cancer,” Fernando C. Santini, MD, and Artur Katz, MD, review the main aspects of the biology of RET, the challenges of RET inhibition in lung cancer, and future perspectives.

This month’s continuing medical education features an interview with Arturo Loaiza-Bonilla, MD, MSEd, FACP, chief of medical oncology and medical director of research at the Cancer Treatment Centers of America at Eastern Regional Medical Center. Dr Loaiza-Bonilla discusses the current status of biomarkers and precision medicine in gastrointestinal cancers, detailing methods to target HER2, VEGF, PD-1, and more.

Michael J. Hennessy, Sr
Chairman and Chief Executive Officer
Prostate Cancer Standards—Are Trial Results Affecting Practice?

The title of this commentary may sound critical or impart a tone of disappointment. However, it is, in part, a result of more rapid output from clinical trials and the introduction of new drugs or older drugs in a different setting. In the review of new therapeutic paradigms for advanced prostate cancer by Li et al in this issue of *The American Journal of Hematology/Oncology*, this phenomenon is nicely illustrated. In the past few years, we have seen advances in all the therapeutic categories for prostate cancer—antiandrogen, cytotoxic, and bone-directed therapy, along with early-phase trials with PARP inhibitors and immunotherapy. The paradox of rapid progress is that successful trials of different designs leave uncertainty regarding which of several strategies is optimal. Additionally, as standards for first-line therapy change, interpretation of later-line trials becomes murky because the populations no longer reflect subjects from past studies in terms of prior treatments.

As pointed out in this article, the use of sequential androgen-targeting agents has become popular owing to fewer toxicities and the numerous new agents available in this category. However, there is little evidence demonstrating whether this strategy is effective and, if it is, which of the many permutations of sequence is best. While there is likely some degree of cross-resistance, this is not complete, yet the few available data suggest that the activity is modest. The timing of bone-targeted radium-223 that not only improves bone pain but has an overall antitumor effect, with improvement in survival and the role of specific concomitant androgen-directed therapies (and eventually in clinical trials with chemotherapy) also needs further investigation to be optimally used in the clinic.

The most recent set of advances have come in the initial treatment of metastatic prostate cancer. Three pivotal trials testing different partners with androgen deprivation therapy—namely docetaxel, enzalutamide, and abiraterone—have shown survival advantages that appear to be similar, although different follow-up times and endpoints make comparisons difficult. Of course, cross-trial comparisons must be viewed cautiously even when the populations and methods/parameters are comparable. There is considerable debate over whether there are specific factors that would favor the use of chemotherapy in the front line—so far, this has not been adopted extensively. All said, these important milestones are calling for even more trials so that effects on practice, such as therapy choices after progression, can be better determined. We are seeing this same challenge in other tumor types with the reporting of landmark studies. It may be time to design trials that also designate the next line of therapy—these likely would not be industry-supported trials, so cooperative groups and other consortia are beginning to discuss these strategies.
New Therapies and Potential Biomarkers for Hepatocellular Carcinoma

Merly Contratto, MD, and Jennifer Wu, MD

Abstract

Hepatocellular carcinoma (HCC) is the second leading cause of cancer mortality in the world. Sorafenib is the only first-line systemic therapy for HCC. HCC is an immunogenic cancer that might be treatable with immune checkpoint inhibitors as single agents or in combination; options could include anti–CTLA-4 agents (eg, tremelimumab), anti–PD-1 agents (eg, nivolumab, pembrolizumab), and anti–PD-L1 agents (eg, atezolizumab). Beyond immune checkpoint inhibitors, potentially useful treatments for HCC include immune modulator (lenalidomide), multikinase inhibitor (regorafenib), and monoclonal antibody (ramucirumab).

At least 3 biomarkers in HCC can predict prognosis and suggest potential benefits to therapy: AFP, C-MET, and PD-L1. At high values or when overexpressed, these biomarkers indicate poor prognosis of HCC, yet such markers also point to better response to specific therapies. For instance, AFP >400 may indicate a patient population that would especially benefit from anti-angiogenic agent ramucirumab.

AJHO. 2017;13(12):4-10

Introduction

Hepatocellular carcinoma (HCC) is the second leading cause of cancer mortality worldwide. In the United States, its mortality rate continues to increase, with 5-year survival rates of only 18% during 2005 to 2011. HCC most often results from chronic liver inflammation stemming from hepatitis B (HBV), hepatitis C (HCV), alcohol abuse, or nonalcoholic steatohepatitis. Curative treatments for HCC are surgery and liver transplantation. However, up to 80% of patients present at an incurable stage, eligible only for systemic therapy. As the first systemic therapy approved by the FDA for HCC, sorafenib offers a modest improvement in overall survival (OS) compared with placebo (10.7 months vs 7.9 months; \(P < .001\)). Sorafenib is a tyrosine kinase inhibitor that inhibits both RAF pathway in tumors and also angiogenesis such as vascular endothelial growth factor receptor-2 (VEGFR2).

To expand HCC treatment options, exploring such possibilities as immune checkpoint inhibitors and targeted therapies is essential. This brief review of HCC will discuss the most current information on promising new systemic therapies, including relevant biomarkers (eg, alpha-fetoprotein [AFP], C-MET, and PD-L1).

Immune Tolerance and Immune Activation in HCC

Mechanism of Action for Immune Checkpoint Inhibitors

PD-L1 produced by tumor cells (TCs) inhibits T-cell activation by binding to the PD-1 receptor. Interaction between PD-1 and PD-L1 inhibit T-cell receptor signaling for initiation of T-cell activation, causing T-cell apoptosis. CTLA-4 inhibits T-cell activation in 2 ways: (1) it induces regulatory T cells (Tregs) and competes with CD28 by binding to B7; and (2) it causes inhibition of the co-stimulatory signal for T-cell activation.

An immune checkpoint inhibitor (eg, an anti–PD-1, anti–PD-L1, or anti–CTLA-4 agent) increases antitumor activity by activating T cells via inhibition of the interaction between PD-1 and PD-L1, and between CTLA-4 and B7. Both PD-1 and CTLA-4 inhibitors can reduce T-cell suppression imposed by PD-1 and CTLA-4, leading to more T-cell activation.

When a foreign antigen such as HCC develops in a patient, 2 potential outcomes exist: immune tolerance and immune activation.

Immune Tolerance

Immune tolerance refers to immunosuppression by shifting T-cell balance toward Tregs, which suppresses T-cell activation (Figure 1). Meanwhile, cytotoxic CD4+ and CD8+ T cells, which promote T-cell activation, will be diminished, thereby creating an immunosuppressive environment to reduce T-cell activation. Immune tolerance can cause tumor growth and progression. There
are 2 ways to create immunosuppression. First, particular receptors expressed on T cells can lead to immunosuppression through binding with prospective ligands.\textsuperscript{10-12} For example, PD-1 is a receptor expressed on T cells, and when it binds to its ligand (PD-L1) expressed on antigen-presenting cells (APCs), T cells become deactivated.\textsuperscript{13-15} CTLA-4 is another receptor expressed on T cells, and its ligands are B7-1 or B7-2. Once CTLA-4 binds to either B7-1 or B7-2 on APCs, immunosuppression starts. Second, the immunosuppressive cytokines released from the tumor microenvironment can also contribute to immunosuppression. In HCC, several immunosuppressive cytokines have been identified, including interleukins 4, 5, 8, and 10.\textsuperscript{16}

**Immune Activation in Xenograft Study**

Immune activation leads to activity against HCC by activation of cytotoxic CD4\textsuperscript{+} and/or CD8\textsuperscript{+} T cells (Figure 2). When the CD28 receptor on T cells binds to its ligand (B7-1 or B7-2) in APCs, immune activation is initiated. In addition, immune-activating cytokines, such as tumor necrosis factors, interferon gamma (IFN-\gamma), and interleukin-1, can also contribute to cytotoxic T-cell activation.\textsuperscript{16} Immune activation can lead to tumor regression and contribute to longer OS in xenograft models of HCC.\textsuperscript{3,16,17}

Initiation of T-cell activation requires 2 steps that happen simultaneously:

1. T-cell receptor signal by major histocompatibility complex II that is bound to peptide antigen and digested by dendritic cells (DCs)
2. Co-stimulatory signal by interaction of CD28 receptor on T cells to B7-1 or B7-2 ligand on APCs

**Rationale of Immune Checkpoint Inhibitors in HCC**

Immune checkpoint inhibitors have achieved great success in melanoma and renal cell carcinoma. Both cancers are considered immunogenic, with reported
cases of spontaneous regressions of tumors after treatment with IFN. Because HCC has shown a similar response to IFN, it also is considered an immunogenic tumor, with the potential to respond to immune checkpoint inhibitors.

**Efficacy of Immune Checkpoint Inhibition as a Single Agent**

The FDA granted an accelerated approval to nivolumab (Opdivo) for the treatment of patients with HCC following prior treatment with sorafenib, regardless of PD-L1 status, in September 2017. Other trials involving checkpoint inhibitors include a phase II study that tested a single-agent anti-CTLA-4 antibody, tremelimumab, in 21 patients with HCC with Child-Pugh class B status. All patients had HCV and progressed on sorafenib. The results demonstrated an overall response rate (ORR) of 17.6%, disease control rate (DCR) of 76.4%, and median OS of 8.2 months. One patient also had decreased HCV viral load during tremelimumab treatment, indicating the potential of an anti-CTLA-4 antibody to treat HCV and HCC simultaneously.

The results of a phase I study using dose escalation of the PD-1 inhibitor nivolumab for second-line treatment in 48 patients with HCC with Child-Pugh score ≤7 (class A/B) have been reported. This study included 10 patients with HCV, 15 with HBV, and 23 without HBV or HCV. Three patients achieved complete response and 4 patients had a partial response (PR); DCR was 58%. About 83% of patients experienced adverse events (AEs) such as rash, pruritus, and elevations of aspartate aminotransferase, alanine aminotransferase, lipase, and amylase. Only 25% of patients had grade 3/4 AEs.

In the subsequent phase II dose-expansion cohort study, 214 patients with HCC were enrolled. This patient population included 50 patients with HCV, 51 with HBV, and 113 without HBV or HCV. Among the 113 patients, 56 patients were sorafenib-naïve and 57 patients had exposure to sorafenib. The primary endpoint, ORR, was 20% in all 214 patients with HCC. The ORR was 23% in sorafenib-naïve patients and 21% in those previously treated with sorafenib. The median duration of response was 9.9 months and the DCR was 64%, regardless of the degree of PD-L1 expression. The OS rate at 9 months was 74%.

An ongoing phase II clinical trial is randomizing patients to either pembrolizumab (an anti-PD-1 agent) or placebo in second-line treatment for unresectable HCC. All patients must be Child-Pugh class A, and have either failed or become intolerant to sorafenib. The primary endpoint is ORR.

A randomized phase III study is ongoing in 726 patients with HCC, investigating the anti-PD-1 agent nivolumab versus sorafenib in first-line treatment (CheckMate 459). Outcomes include OS, time to progression (TTP), progression-free survival, and ORR. The study will also evaluate the relationship between PD-L1 expression and the efficacy of nivolumab.

**Combination Therapies Including Immune-Checkpoint Inhibitors**

Results from a pilot study examining tremelimumab in combination with local therapies, including transcatheter arterial chemoembolization (TACE) or ablation, have been reported. In 32 patients, only 19 had evaluable disease. Results showed that the HCV and HBV viral loads decreased in patients infected with HCV and/or HBV. The median OS was 12.3 months. No dose-limiting toxicities or grade 3/4 toxicities were observed. The most common AE was pruritus, and only
I patient developed autoimmune pneumonitis. The rationale for this study was based on the mechanism of action for CTLA-4 inhibition. Tremelimumab, an CTLA-4 inhibitor, causes antitumor activity by inhibiting the immunosuppression environment. TACE/ablation induces cytotoxic CD8+ T cells, causing tumor cell death by decreasing vascular supply to HCC.28 When TACE/ablation and tremelimumab are combined, immune activation through T cells could be synergistically enhanced. The study results supported this rationale.

A phase I/II study of nivolumab-based therapy in the first line is still ongoing with 3 cohorts: nivolumab plus sorafenib, nivolumab plus ipilimumab, and nivolumab alone. All patients with HCC in this study are Child-Pugh class A and C in the unresectable HCC patient population. Therefore, this study provides an opportunity for immunotherapy to be tested in a broader patient population. Since sorafenib has single-agent activity in HCC and it works through a different pathway than the PD-1 pathway utilized by PD-1 inhibitors, the combination of nivolumab plus sorafenib may show synergy.

**Promising Second-Line Therapies Beyond Immunotherapy**

Lenalidomide, an immune modulator, is a potential therapy for patients with HCC after their exposure to sorafenib. It was tested in a phase II study of 40 patients with HCC who had progressed on or were intolerant to sorafenib: 19 were Child-Pugh class A, 16 B, and 5 C.29 The study results indicated a PR rate of 15% and OS of 7.6 months, suggesting that lenalidomide has a role in HCC treatment. Common grade 3/4 AEs were fatigue (7.5%), rash (10%), and neutropenia (2.5%).

Regorafenib has been approved by the FDA for second-line treatment of HCC since April 27, 2017. Additionally, a phase III double-blind study randomized patients to regorafenib versus placebo in a 2:1 ratio.30 The median OS in patients treated with regorafenib and placebo was 10.6 and 7.8 months, respectively (HR, 0.63; P < .0001). The most common AEs with regorafenib were hypertension (15%), hand-foot syndrome (13%), fatigue (9%), and diarrhea (3%).30 This is the first study that demonstrated OS benefit in second-line treatment for unresectable HCC. Median OS with first-line treatment (sorafenib) was 7.8 months, suggesting that regorafenib would be a good choice for patients who are intolerant to sorafenib. A phase III study of ramucirumab, an immunoglobulin G1 human monoclonal antibody, using 1:1 randomization between ramucirumab and placebo as second-line treatment in 565 patients with HCC.31 All patients were Child-Pugh class A and progressed on or were intolerant to sorafenib. There was no statistical difference in OS between the 2 arms. In a prespecified patient subgroup with AFP >400 ng/mL, ramucirumab demonstrated longer OS than placebo (7.8 vs 4.2 months). Common grade 3/4 AEs that were higher with ramucirumab than placebo were hypertension (12% vs 4%), ascites (5% vs 4%), asthenia (5% vs 2%), malignant neoplasm progression (6% vs 4%), and thrombocytopenia (5% vs 1%). This is the first phase III study in HCC in which AFP biomarker suggests benefits to therapy.31

**Biomarkers in HCC Predict Prognosis and Benefits to Therapy**

High expression of 3 biomarkers in HCC lead to poor prognosis13,14:

1. **AFP**
2. **C-MET** (proto-oncogene for receptor tyrosine kinase)
3. **PD-L1**

**AFP**

Alpha-fetoprotein, a protein, has been used to screen for individuals at risk of developing HCC. It cannot be used as a single diagnostic test due to its low sensitivity of 41% to 65% and specificity of 80% to 90%; however, up to 50% of patients with HCC have elevated AFP serum levels.16 In a single-institution prospective study, preoperative value of AFP >400 ng/mL in 108 patients with resectable HCC correlated with higher recurrence rates and lower survival rates in 2 years.13,14 In another retrospective study, in which 258 patients with HCC underwent surgical resection, those with AFP >400 ng/mL showed poorer prognosis, with a relative risk (RR) of 1.471 compared with patients with AFP <400 ng/mL.34,35

**C-MET**

C-MET, also called hepatocyte growth factor receptor, is a tyrosine protein kinase. It plays an important role in cellular proliferation, especially in TCGs such as HCC. C-MET is overexpressed in 50% of HCC cases, causes tumor growth and metastasis, and indicates poor prognosis.15 In a phase II second-line study, 107 patients with HCC who were Child-Pugh class A were randomized 2:1 to tivantinib (a C-MET inhibitor) and placebo. In patients with C-MET overexpression, TTP was 2.7 months in the tivantinib group versus 1.4 months in the placebo group. The most common grade 3/4 AEs with tivantinib compared with placebo were neutropenia (14% vs 0%) and anemia (11% vs 0%).33 A phase III study of second-line treatment of HCC patients with high C-MET expression, with tivantinib versus placebo with 2:1 randomization, has completed enrollment and preliminary data will be reported soon.
PD-L1
The ligand PD-L1 is expressed on APCs. It has been used as a potential biomarker to predict the efficacy of PD-1/PD-L1 inhibitors. Several assays to detect PD-L1 are currently available. In a phase I dose-escalation study of nivolumab in patients with HCC who failed sorafenib, RR was 19% versus 26% in patients with PD-L1 ≥1% and PD-L1 ≥1%, respectively. In a phase II dose expansion cohort study in patients with HCC, RR was 17.2% versus 32% in patients with PD-L1<1% and PD ≥1%, respectively.

Testing for PD-L1 Assay
Dako immunohistochemistry 28-8 (Dako IHC 28-8) is used to detect the activity of PD-L1 in TCs for responses to the PD-1 inhibitor, nivolumab. Higher expression of PD-L1 in TCs correlates with higher RR to PD-1 inhibitors. Results of the phase III CheckMate 057 trial, a retrospective analysis of PD-L1 expression in 231 patients with nonsquamous non–small cell lung cancer (NSCLC) who were treated with nivolumab (second-line), showed that 123 patients with PD-L1 ≥1% achieved RR of 31% versus 9% in 108 patients with PD-L1 <1%. In 95 patients with expression of PD-L1 ≥5%, RR was 36% versus 10% in 136 patients with <5% expression of PD-L1. On the other hand, 86 patients with PD-L1 ≥10% showed RR of 37% versus 11% in 145 patients with PD-L1 <10%. In all 3 groups, higher PD-L1 expression led to higher RR, and the difference was statistically significant.

Dako IHC 22C3 is used to measure the expression of PD-L1 in TCs for responses to the PD-1 inhibitor, pembrolizumab. In the phase II/III KEYNOTE-010 study, pembrolizumab was tested as second-line treatment in 442 patients with NSCLC with PD-L1 ≥50% expression on TCs. Patients were randomized 1:1:1 to arm A (pembrolizumab 2 mg/kg), arm B (pembrolizumab 10 mg/kg), or arm C (docetaxel 75 mg/m²). Results showed an OS of 14.9 months, 17.3 months, and 8.2 months in arms A, B, and C, respectively.

In the phase III KEYNOTE-024 study, 305 untreated patients with metastatic NSCLC whose TCs expressed ≥50% PD-L1 were randomized 1:1 to first-line treatment with either pembrolizumab or chemotherapy (platinum-based). At 6 months, OS was 80.2% versus 72.4% in the pembrolizumab versus chemotherapy group, respectively (P = .005). Pembrolizumab is the only immunotherapy that has proven OS benefits based on the result of PD-L1 assay; thus based on KEYNOTE-010 and KEYNOTE-024, pembrolizumab has been FDA approved as both first- and second-line, single-agent treatment for metastatic NSCLC in patients with PD-L1 ≥50%.

Ventana SP142 is used to detect the expression of PD-L1 in TCs and immune cells (ICs), such as macrophages and DCs, for response to atezolizumab (PD-L1 inhibitor). As second-line treatment, atezolizumab was compared with docetaxel in a phase III study (OAK), which enrolled 850 patients with NSCLC with PD-L1 ≥1% on TCs or ICs. It demonstrated OS of 13.8 months versus 9.6 months for atezolizumab versus docetaxel (HR, 0.73; P = .0003).

The phase II POPLAR study of atezolizumab as a second-line treatment compared it with docetaxel in 277 patients with NSCLC with TCs or ICs with PD-L1 at least ≥1%. Results showed an ORR of 38% in the atezolizumab group and 13% in the docetaxel group. Atezolizumab treatment demonstrated higher RR in patients with higher PD-L1 expression.

Ventana SP263 is used to detect PD-L1 in TCs with durvalumab (PD-L1 inhibitor). Durvalumab was studied as a third- or higher-line treatment in a phase II prospective analysis in 239 patients with NSCLC. The RR for durvalumab was 16.4% versus 7.5% in patients with PD-L1 expression ≥25% and <25%, respectively.

Conclusions
Hepatocellular carcinoma has a high mortality rate, yet the only FDA approved first-line systemic treatment is sorafenib. This treatment offers moderate benefits and has a high, but manageable, rate of AEs. Single-agent immunotherapy or targeted treatments seem promising in certain biomarker-selected patients. Combination strategies with novel modalities that involve immunotherapy will provide more opportunities to treat this grim disease. Better understanding of biomarkers in HCC will help identify patient populations who can benefit the most from such promising therapies as immunotherapies and targeted treatments.

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Financial disclosures: The authors report no relevant financial relationships.

References


Clinical Commentary: The Use of Clinical Biomarkers to Inform Treatment Decisions in Advanced Renal Cell Carcinoma

Daniel J. George, MD

Abstract
Recent advances in targeted therapies have provided physicians additional options for treating patients with advanced renal cell carcinoma (aRCC). However, identifying biomarkers that can help predict a patient’s response to a particular therapy remains elusive. Given that most targeted therapies have a relatively tight therapeutic index, and yet have recommended dosages that are the same for patients regardless of differences in height, weight, age, sex, race, comorbidities, drug target, or metabolic profiles, the use of predictive biomarkers would seem imperative to personalize up-front treatment for patients with aRCC and/or to adjust the therapeutic dosage. One readily available and potentially helpful approach is to comprehensively and longitudinally track treatment-emergent adverse events (teAEs) in individual patients as pharmacodynamic markers for dose optimization. With VEGF-targeted therapies, several drug-related AEs are believed to be directly or indirectly related to the effect of targeting VEGF in normal tissues; as such, these AEs may act as on-treatment indicators of the activity of the drug. These data suggest that early emergence of AEs related to VEGF-targeted therapy may be associated with tumor sensitivity to this class of agents and support the strategy of using teAEs as early clinical biomarkers to guide on-treatment management decisions.

AJHO. 2017;13(12):11-18

Introduction
The clinical development of targeted therapies has improved outcomes for many patients with advanced/metastatic renal cell carcinoma (aRCC) in the past 10 years, but not for all patients. VEGF-targeted therapies are the recommended and most commonly used first-line treatment option for the majority of patients with aRCC.1-2 Most patients demonstrate an initial clinical response to treatment. However, some patients exhibit no response to treatment because of primary resistance mechanisms, whereas others will eventually progress when being treated because of acquired resistance to VEGF-targeted therapies.3 Unlike many cytotoxic chemotherapies, which can have narrow therapeutic indices—and therefore are dose-adjusted according to body weight, body mass index, liver enzymes, or renal function—targeted therapy in patients with aRCC is generally given at the same dosage, or with a limited dosing range, for the entire population.

Unfortunately, many patients do not realize the full benefits of targeted therapy because of inadequate dosing or intolerable toxicity. Indeed, variability in patient responsiveness to treatment is evident, and personalized treatment using various targeted agents can improve outcomes in many patients with RCC.4-6 For example, a recent prospective, multicenter evaluation of more than 500 patients from the Canadian Kidney Cancer information system, who were treated between 2011 and 2015, demonstrated significantly improved overall survival (OS) when sunitinib was initiated at the standard dosing schedule, with subsequent schedule/dosage alterations based on toxicity, compared with standard first-line sunitinib or standard pazopanib dosing.4

In a related situation, a subgroup analysis of the COMPARZ noninferiority trial showed that patients treated with first-line sunitinib or pazopanib who underwent dosage reductions or interruptions achieved longer median progression-free survival (PFS), suggesting that individualized dosing due to toxicity may
not compromise effectiveness of either agent. Results from the ongoing randomized phase II/III STAR trial are expected to confirm the benefits of personalized treatment in RCC. The study is currently underway and evaluating patients with aRCC who receive physician-directed continuous or interrupted treatment with sunitinib or pazopanib; results are expected to be reported in 2018.

These and other study findings suggest that prospective trials investigating the influence of dose individualization on treatment outcome are warranted and that patient- or disease-specific factors may be responsible for differences in treatment responsiveness. Such personalized approaches to treatment suggest potential biomarkers that could be exploited to identify patients who are more likely to respond to treatment. The use of predictive biomarkers early in the treatment course can vastly improve our ability to personalize treatment for patients with aRCC by: 1) identifying patients who are likely to benefit from targeted treatments and 2) allowing for greater personalization of dosing to optimize the therapeutic index in individual patients before dose-limiting toxicities occur.

There are currently no validated predictive biomarkers to aid in personalization of medication for patients with aRCC. There are, however, validated prognostic models to predict survival of a patient with aRCC based on clinical and laboratory factors. The Memorial Sloan Kettering Cancer Center (MSKCC) model that was developed during the cytokine era is one of the older, but still utilized, prognostic scores available. Five risk factors in the MSKCC model predict shorter survival: Karnofsky Performance Scale Index score (KPS) <80%, time from diagnosis to treatment <1 year, lactate dehydrogenase >1.5x upper limit of normal (ULN), serum-corrected calcium >10 mg/dL, and serum hemoglobin <lower limit of normal (LLN). Patients with 0, 1-2, and ≥3 of these risk factors are designated as having favorable, intermediate, or poor risk status, respectively. Modified MSKCC scoring was used to select poor-risk patients in the pivotal trial for temsirolimus in aRCC. Patients were designated poor risk if they had ≥3 of 6 risk factors for survival, and treatment guidelines recommend temsirolimus for the first-line treatment of these poor-risk patients.

The International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) model is also widely used for prognostic scoring. Six factors in the IMDC model predict shorter survival in patients with mRCC treated with VEGF-targeted therapy: KPS <80%, time from diagnosis to treatment initiation <1 year, serum-corrected calcium >ULN; hemoglobin <LLN, absolute neutrophil count >ULN, and platelets >ULN. Patients with 0, 1-2, and ≥3 risk factors are classified as having favorable, intermediate, or poor risk, respectively. Both MSKCC and IMDC prognostic criteria have been used to stratify patients in recent randomized, controlled phase III trials in aRCC.

Multiple molecular factors have been investigated for their potential prognostic and/or predictive power in RCC. Tissue-based biomarker expression has often been measured using immunohistochemistry (IHC), despite its drawbacks. Of the more extensively

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**FIGURE.** Proposed Treatment Algorithm for Patients with Advanced Renal Cell Carcinoma

<table>
<thead>
<tr>
<th>Disease Responsiveness</th>
<th>Anti-VEGF Sensitive</th>
<th>Anti-VEGF Refractory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-VEGF Tolerant</td>
<td>Continue anti-VEGF</td>
<td>Future combinations: anti-VEGF, other biologic targets, and immunotherapy</td>
</tr>
<tr>
<td>Anti-VEGF Intolerant</td>
<td>Continue anti-VEGF at modified dosage</td>
<td>Immunotherapy</td>
</tr>
</tbody>
</table>
studied tissue-based markers, meta-analyses suggest that low carbonic anhydrase IX expression, high PD-L1 expression, high Ki-67 expression, and high nuclear expression of hypoxia-inducible factor-1 alpha (HIF-1α; but not overall HIF-1α expression) measured by IHC in RCC tumors correlate with poor survival. Multiplatform analyses incorporating techniques that measure somatic DNA copy alterations, DNA methylation, mRNA expression, microRNA expression, and protein expression allow a more complete picture of the molecular alterations occurring in individual patients and the potential for identifying molecular subgroups of patients likely to respond to a particular therapy type. Although no single molecular marker has been validated as predictive in aRCC, a combination of multiple molecular biomarkers may eventually prove useful in the manner that multiple factors are used to arrive at an IMDC prognostic risk score. Various studies are currently underway to identify gene signatures that might be more or less predictive regarding treatment outcomes.

Despite significant attention to the molecular aspects of aRCC, identifying predictive biomarkers for the management of RCC is a challenge. With VEGF-targeted therapies, specific treatment-emergent adverse events (teAEs) are believed to act as surrogate markers of the activity of the drug. Therefore, the patient’s tolerance to VEGF-targeted therapy may be directly related to the individual’s sensitivity to VEGF-targeted therapy. This suggests that teAEs can be exploited as clinical biomarkers to be used to guide treatment decisions.

A number of potential clinical biomarkers have been identified that are commonly seen in patients treated with VEGF-targeted therapy as a class, and therefore may be viewed directly or indirectly as being related to the effects of systemic VEGF inhibition, including hypertension, hypothyroidism, hand-foot syndrome, and fatigue/asthenia. A summary of clinical studies reporting an association between on-treatment clinical biomarkers and efficacy with VEGF-targeted therapy in patients with mRCC is shown in the Table.

**Hypertension**

Treatment-induced hypertension is frequently reported in patients treated with agents that target VEGF (17%-40% in phase III trials of patients with mRCC). Although the pathophysiology underlying the relationship between VEGF-targeted agents and systolic blood pressure (BP) is not entirely known, it is associated with an increase in systemic vascular resistance resulting from a decrease in nitric oxide release in peripheral vascular beds, leading to vasoconstriction. A number of studies have shown that the development of treatment-related hypertension is associated with clinical benefit in patients treated with VEGF-targeted agents. Treatment with antihypertensive medication does not affect this improvement in clinical outcome; therefore, hypertension should be managed appropriately.

The majority of retrospectively analyzed clinical trial data in patients with mRCC treated with VEGF-targeted therapy, including VEGF receptor tyrosine kinase inhibitors (VEGFR-TKIs) and the anti-VEGF monoclonal antibody bevacizumab in combination with interferon-α, show a positive correlation between hypertension and OS. VEGF-TKI-induced hypertension is also predictive of prolonged PFS, OS, and improved objective response rate (ORR) in patients with mRCC in the community setting. Similarly, phase II dose titration of axitinib showed that patients with greater increases in diastolic BP had prolonged median PFS (16.6 vs 5.7 months, for ≥10 mm Hg increase vs <10 mm Hg increase; P < .001). However, there was only a weak correlation between steady-state axitinib exposure and diastolic BP change (R² = 0.225), and steady-state axitinib exposure was not strongly correlated with PFS. The results of this study suggest a complex relationship between the dosage of VEGF-targeted therapy, BP, and efficacy, and might suggest that BP should not be used exclusively to guide VEGFR-TKI dosing.

**Tumor Vascularity**

Primary RCC and its metastases are highly vascular. Therefore, imaging techniques that can identify changes in vascularity could be used as clinical biomarkers. Although the gold standard for assessing vascularity is histology, this method necessitates an invasive biopsy procedure, does not allow assessment of the entire tumor, and cannot account for tumor heterogeneity.

Functional in vivo imaging techniques that provide quantitative data regarding blood flow include dynamic contrast-enhanced MRI (DCE-MRI), DCE-CT, DCE-ultrasound (DCE-US), diffusion-weighted MRI, arterial spin label MRI (ASL-MRI), and fluorodeoxyglucose-PET (FDG-PET). Data from prospective clinical trials show initial evidence for DCE-CT, DCE-MRI, DCE-US, ASL-MRI, and FDG-PET in predicting response to VEGF-targeted agents (reviewed in Nathan and Vinayan and Bex and colleagues).

Evidence is best for DCE-US as a predictive marker of response to VEGF-targeted therapy in prospective trials that include patients with mRCC. Additionally, European guidelines for contrast-enhanced ultrasound recommend the use of DCE-US to monitor response to therapy in patients with mRCC, in dedicated centers with appropriate software. Advantages of DCE-US are its
### TABLE On-Treatment Clinical Biomarkers Associated With Efficacy During VEGFR TKI Therapy in Patients With a/mRCC

<table>
<thead>
<tr>
<th>Clinical Biomarker</th>
<th>Treatment</th>
<th>Main Findings</th>
<th>Study Type</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hypertension</strong></td>
<td>Sunitinib</td>
<td>Patients who developed hypertension (sBP ≥140 mm Hg) had improved PFS (HR, 0.24; P &lt; .001) and OS (HR, 0.28; P &lt; .001)*</td>
<td>Retrospective pooled analysis of 3 prospective clinical trials in patients with mRCC (N = 544)</td>
</tr>
<tr>
<td></td>
<td>Sunitinib</td>
<td>Association between hypertension and PFS remained significant in a combined AE multivariate model of patients who developed hypertension at any time (HR for PFS, 0.37; P &lt; .0001; HR for OS, 0.36; P &lt; .0001) and by the 12-week mark for OS (HR, 0.68; P = .036), but not PFS (HR, 0.81; P = .1305)*</td>
<td>Retrospective pooled analysis of 5 prospective clinical trials in patients with mRCC (N = 770)</td>
</tr>
<tr>
<td></td>
<td>Sunitinib</td>
<td>Patients who developed hypertension had a PFS (RR, 0.42; P &lt; .001) and OS (RR, 0.40; P &lt; .001) benefit vs patients with no hypertension*</td>
<td>Retrospective analysis of 1 hospital in Finland in patients with mRCC (N = 181)</td>
</tr>
<tr>
<td></td>
<td>VEGFR TKI (sorafenib or sunitinib) or IL-2–based immunotherapy</td>
<td>Patients who developed hypertension (sBP ≥140 mm Hg) within 4-12 weeks of treatment had improved OS (HR, 0.70; P = .0014)*</td>
<td>Retrospective analysis of Danish national cohort in patients with mRCC (N = 588)</td>
</tr>
<tr>
<td><strong>Axitinib</strong></td>
<td>Axitinib</td>
<td>Patients who developed hypertension (dBP ≥90 mm Hg) had significantly longer mPFS (16.5 vs 6.4 months; HR, 0.53; P = .019), and numerically longer mOS (25.8 vs 13.9 months; HR, 0.74; P = .228) vs patients with dBP&lt;90 mm Hg in an 8-week post hoc, exploratory, retrospective analysis</td>
<td>Post hoc, exploratory retrospective analysis of 2 phase II trials in patients with mRCC (N = 112)</td>
</tr>
<tr>
<td><strong>Bevacizumab + interferon-α</strong></td>
<td>Bevacizumab + interferon-α</td>
<td>Development of hypertension at 2 months was an independent predictor of OS (HR, 0.62; P = .046)*</td>
<td>Retrospective analysis of phase III trial in patients with mRCC (N = 366)</td>
</tr>
<tr>
<td><strong>Tumor vascularity (DCE-US)</strong></td>
<td>Antiangiogenic agents</td>
<td>A decrease of &gt;40% AUC correlated with OS (P = .05) and FFP (P = .005)</td>
<td>Prospective, multicenter study of patients with cancer of various solid tumor types (N = 539, including 157 with RCC)</td>
</tr>
<tr>
<td></td>
<td>Sunitinib</td>
<td>1 DCE-US parameter correlated with OS (time to peak intensity; P = .007)*</td>
<td>Prospective, single-center study in patients with mRCC (N = 38)</td>
</tr>
<tr>
<td></td>
<td>Sunitinib</td>
<td>2 DCE-US parameters correlated with DFS (time to peak intensity, P = .0002; slope of the wash-in, P = .02)</td>
<td>Prospective, single-center study in patients with mRCC (N = 38)</td>
</tr>
<tr>
<td><strong>Hypothyroidism</strong></td>
<td>Sunitinib or sorafenib</td>
<td>Patients who developed hypothyroidism with sunitinib (6 studies; N = 260) had no difference in PFS versus patients without hypothyroidism (HR, 0.82; P = .22)*</td>
<td>Meta-analysis of 11 mRCC studies (N = 500)</td>
</tr>
<tr>
<td></td>
<td>Sunitinib or sorafenib</td>
<td>Patients who developed hypothyroidism with sunitinib or sorafenib (3 studies; N = 205) had a PFS benefit versus patients without hypothyroidism (HR, 0.59; P = .003)</td>
<td>Prospective single-center study in patients with mRCC (N = 83)</td>
</tr>
<tr>
<td></td>
<td>Sunitinib or sorafenib</td>
<td>Patients who developed hypothyroidism with sunitinib (4 studies; N = 147) had an OS benefit over patients without hypothyroidism (HR, 0.52; P = .01)</td>
<td>Prospective exploratory study in patients with mRCC (N = 87)</td>
</tr>
<tr>
<td><strong>Sorafenib or sunitinib</strong></td>
<td>Sorafenib or sunitinib</td>
<td>Patients who developed hypothyroidism had longer PFS (HR, 0.34; P = .01)*</td>
<td>Prospective exploratory study in patients with mRCC (N = 83)</td>
</tr>
<tr>
<td><strong>Sorafenib or sunitinib</strong></td>
<td>Sorafenib or sunitinib</td>
<td>Development of subclinical hypothyroidism (TSH &gt;3.77 -M/mL with normal T3 and T4 levels) within the first 2 months of treatment was an independent predictor of OS (HR, 0.31; P = .014)*</td>
<td>Prospective exploratory study in patients with mRCC (N = 83)</td>
</tr>
<tr>
<td><strong>VEGFR TKI</strong></td>
<td>VEGFR TKI</td>
<td>Compared with patients with severe hypothyroidism, euthyroid patients had an increased risk for progression or death (HR for PFS, 3.15; P = .0093) and death (HR for OS, 9.51; P = .0159)*</td>
<td>Retrospective single-center analysis in patients with mRCC (N = 65)</td>
</tr>
<tr>
<td><strong>Sunitinib</strong></td>
<td>Sunitinib</td>
<td>Patients who developed hypothyroidism had longer mPFS (10 vs 17 mos; P = .001), mOS (39 vs 20 months; P = .019), and higher ORR (46.7% vs 13.7%) vs euthyroid patients</td>
<td>Retrospective analysis of patients with mRCC (N = 81)</td>
</tr>
<tr>
<td><strong>Sunitinib</strong></td>
<td>Sunitinib</td>
<td>Patients who developed grade 2 hypothyroidism had significantly longer mPFS (25.3 vs 9.9 months; HR, 0.40; P = .042) and numerically longer mOS (46.0 vs 22.1 months; HR, 0.54; P = .2052)</td>
<td>Retrospective single-center analysis in patients with mRCC (N = 41)</td>
</tr>
<tr>
<td><strong>Hand-foot syndrome</strong></td>
<td>Sunitinib</td>
<td>In a combined AE multivariate model, patients who developed hand-foot syndrome at any time (HR, 0.70; P = .0152) or by the 12-week mark (HR, 0.64; P = .218) had improved OS*</td>
<td>Retrospective pooled analysis of 5 prospective clinical trials in patients with mRCC (N = 770)</td>
</tr>
<tr>
<td><strong>Sunitinib</strong></td>
<td>Sunitinib</td>
<td>This association was not significant for PFS</td>
<td>Retrospective single-center analysis in patients with mRCC (N = 104)</td>
</tr>
<tr>
<td><strong>Sunitinib</strong></td>
<td>Sunitinib or pazopanib</td>
<td>Patients who experienced hand-foot syndrome had longer mPFS (27.6 vs 9.3 months; P &lt; .001) and mOS (69.0 vs 17.8 months; P &lt; .001) than patients not experiencing this toxicity</td>
<td>Retrospective single-center analysis in patients with mRCC (N = 104)</td>
</tr>
</tbody>
</table>
AE indicates adverse event; a/mRCC, advanced/metastatic renal cell carcinoma; AUC, area under the curve; dBP, diastolic blood pressure; DCE-US, dynamic contrast-enhanced ultrasound; DFS, disease-free survival; FFP, freedom from progression; IL-2, interleukin-2; mos; month; mOS, median overall survival; mPFS, median progression-free survival; mRCC, metastatic renal cell carcinoma; OS, overall survival; PFS, progression-free survival; RCC, renal cell carcinoma; RR, relative risk; sBP, systolic blood pressure; TSH, thyroid-stimulating hormone; TTP, time to tumor progression; VEGFR-TKI, VEGF receptor tyrosine kinase inhibitor.

*Multivariate analysis.

*Results are reported for patients treated on the standard sunitinib 4-weeks-on/2-weeks-off schedule.

*Independent of baseline International Metastatic Renal Cell Carcinoma Database Consortium risk group in time-dependent multivariate analyses stratified by TKI and IL-2–based immunotherapy.

Cost and the lack of any contrast agent. However, DCE-US has drawbacks: It is not a whole-body technique, and it is limited to only certain detectable lesions. Therefore, it might not detect new lesions and could result in mixed responses.³⁵

Clinical measures of tumor vascularity are not validated in mRCC. A number of ongoing prospective trials are assessing functional imaging changes with VEGF-targeted therapy in patients with mRCC.

**Hypothyroidism**

In small trials that routinely monitor thyroid hormone levels, hypothyroidism is reported to occur in 29% to 53% of patients with mRCC who receive VEGF-targeted therapy.³⁵-³⁷ In phase III trials, the incidence of hypothyroidism ranged from <1% to 19%³⁸; however, this number might be an underestimate because thyroid hormone levels were not routinely measured in the majority of early phase III trials.

The underlying mechanism is thought to be associated with destructive thyroiditis, resulting in follicular cell apoptosis, endothelial dysfunction, inhibition of iodine uptake, and reduced synthesis of thyroid hormone.³⁹ The results from a number of other studies suggest that VEGFR-TKI–induced thyroid dysfunction is associated with improved clinical outcomes in patients with mRCC.³⁵-³⁶

A meta-analysis of prospective and retrospective studies was intended to determine whether VEGFR-TKI–induced hypothyroidism was associated with improved clinical outcomes in mRCC.⁴² In studies of patients treated with sunitinib or sorafenib, PFS was improved with hypothyroidism (HR, 0.59; ⁴¹), and OS was prolonged only in patients treated with sunitinib (HR, 0.52; P = .01), relative to patients with a normal functioning thyroid. However, it was found that by assessing only patients treated with sunitinib, hypothyroidism was no longer predictive of PFS.³⁴ The small number of studies included in this meta-analysis (3-6 for each variable) and the clinical heterogeneity among studies (eg, the variation in the timing of hypothyroid detection) may have contributed to this discrepancy.

At this stage, the association is not believed to be sufficiently robust to qualify hypothyroidism as a biomarker. Although hypothyroidism can be treated with hormone replacement, there is some speculation that it is the thyroid dysfunction itself that might be beneficial. This was illustrated in a prospective study (N = 102) in which the median PFS was not significantly different between patients with mRCC with or without thyroid dysfunction who were treated with hormone replacement after 6 months of sunitinib treatment.³⁹

**Hand-Foot Syndrome**

Up to 51% of patients with mRCC treated with VEGF-targeted therapies developed hand-foot syndrome in phase III trials.²⁰ The underlying pathophysiology might be associated with dermal vessel alteration, endothelial cell apoptosis, or impaired vascular repair.²⁰ Several studies have shown that the patients treated with VEGF-targeted therapies in whom hand-foot syndrome developed had significantly improved clinical outcomes compared with those in whom hand-foot syndrome did not develop. In a pooled analysis of 770 patients with mRCC from 5 prospective trials of sunitinib, PFS and OS were significantly improved in those who experienced hand-foot syndrome in univariate analyses.²⁶ However, in a multivariate model examining...
the association between 5 different AEs and survival endpoints in patients on the 4-weeks-on/2-weeks-off dosing schedule, OS, but not PFS, was significantly improved for patients who experienced hand-foot syndrome at any time point, or prior to 12 weeks in a landmark analysis. Similarly, PFS and OS were significantly prolonged in VEGFR-TKI–treated patients with mRCC who experienced hand-foot syndrome (N = 104) in a retrospective analysis.

Despite these promising results, further prospective analyses with other VEGF-targeted agents are necessary, and the relationship between hand-foot syndrome and VEGF-targeted therapies should be treated with caution.

Fatigue and Asthenia
Fatigue is frequently reported in patients with mRCC treated with VEGF-targeted therapies. In a pooled analysis of 770 patients with mRCC from 5 randomized clinical trials of sunitinib, clinical outcomes (PFS) in a combined AE multivariate model of patients on schedule 4/2 were significantly improved in patients who experienced fatigue/asthenia at any time point (but not for patients who had fatigue/asthenia). However, the fatigue and asthenia could be related to other factors such as co-medications, hypothyroidism, anemia, hypogonadism, or mRCC itself. Fatigue/asthenia is frequently assessed too late in its development (when it is debilitating and less reversible). This AE is better managed when identified early in the treatment course, and addressed by dosage modifications before chronic deconditioning has set in.

Other Potential Clinical Biomarkers
Several other biomarkers have been associated with improved response to targeted therapies, including body weight, hypercholesterolemia, hyperglycemia, and hypertriglyceridemia. However, at this stage, the evidence for these biomarkers is cursory.

Clinical Implications and Proposed Treatment Algorithm
VEGF-targeted agents have been associated with AEs that might correlate with efficacy in patients with mRCC. A treatment algorithm has been proposed in which these patients are treated with an anti-VEGF agent in the first line, and treatment is continued until signs of intolerance or disease progression (Figure). Under this algorithm, patients should be monitored very closely during the first 2 months of treatment, ideally with clinical evaluations every 2 weeks, and with home monitoring and recording of teAEs in between. Patients who respond to anti-VEGF therapy but experience early signs of AEs should continue therapy with modified dosing. Patients who tolerate therapy initially and show evidence of disease response should continue on treatment with chronic monitoring for delayed toxicities. Patients who experience disease progression and intolerance to anti-VEGF therapy should switch treatment to targeted immunotherapy. Finally, for patients who tolerate anti-VEGF therapy but experience disease progression, there may be several options, including immunotherapy or a combination of targets including, but not limited to, VEGF inhibition.

No targeted immunotherapies have been approved for aRCC in the first-line setting. However, based on a phase III trial in which nivolumab improved OS compared with everolimus, nivolumab was recently approved for treatment of patients who were previously treated with a VEGFR TKI. In addition, several ongoing trials are assessing immunotherapy alone or in combination with VEGF-targeted agents as first-line treatment and in previously treated populations. The combination of targeted immunotherapy and anti-VEGF agents might be suitable for patients who tolerate VEGF-targeted therapy because there is evidence of synergy between these 2 agents.

Conclusions
With the development of targeted therapies that are capable of vastly improving clinical outcomes in patients with aRCC, the ability to identify patients who will respond to specific treatments becomes significantly more important, especially in light of the evolving alternative or combinatorial options with immunotherapy. Although insufficient data exist to consider whether there are similar markers for immunotherapeutic agents in RCC, teAEs in individual patients have proven informative with regard to understanding responsiveness to VEGF-targeted therapies. As highlighted, several teAEs are believed to be directly or indirectly related to the effect of targeting VEGF in normal tissues, with the most available data probably for hypertension. Currently, no predictive biomarkers for immunotherapy have proven clinically useful, as has been demonstrated for other tissue tumor types. However, ongoing studies may eventually identify patient and/or tumor characteristics that can guide physicians on the patients with aRCC who are most likely to achieve improved outcomes with immunotherapy-based treatment regimens. As much supporting data and interest currently exist around hypertension as a predictive pharmacodynamics biomarker for patients with aRCC, it would not be surprising if hypertension were to eventually become one of the first predictive biomarkers for aRCC. More studies will be telling.
Eventually, the identification and validation of clinical biomarkers that can be applied to the personalization of aRCC treatment will improve outcomes in patients, benefit the drug development process, and be economically efficient to the healthcare system. In the absence of any validated predictive biomarkers in aRCC, monitoring AEs as surrogate markers of efficacy might aid in treatment planning for individual patients.

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Financial disclosure: Dr George reports receiving consultant fees or payment for participation in advisory boards for Bayer, Dendreon, Exelisix, Medivation, Novartis, Pfizer, Sanofi, GSK, Astellas, Innotrin, BMS, Genentech, Janssen, Acceleron, Merck, and Myovant Sciences. He has received grants from Exelisix, Genentech, Janssen, Novartis, Pfizer, Astellas, BMS, Millennium, Acerta, Bayer, Dendreon, and Innotrin. He has received honoraria from Dendreon, Novartis, Sanofi, Bayer, Medication, Biopharm, and Axess Oncology. He has received lecture fees from Dendreon, Novartis, Sanofi, and Bayer.

References


Genomic Landscape and Mechanisms of Disease Evolution and Progression in Multiple Myeloma

Malin Hultcrantz, MD, PhD, and Ola Landgren, MD, PhD

Abstract

Multiple myeloma (MM) is a plasma cell malignancy, and although it is incurable in the majority of cases, survival has improved significantly with the introduction of novel agents in recent years. MM is consistently preceded by the precursor state of monoclonal gammopathy of undetermined significance, which can progress to smoldering MM, and later to MM requiring treatment. Malignant transformation of plasma cells occurs through a stepwise process involving multiple genetic hits, as well as close interaction with the bone marrow microenvironment, leading to deregulation and proliferation of plasma cells. The genomic landscape of MM is complex and involves various types of genetic aberrations and mutational processes. Early hits include immunoglobulin heavy chain translocations and hyperdiploidy, while secondary events include copy number variations and recurrent somatic mutations. With modern sequencing techniques, novel insights have been gained into the mutational landscape of MM. Studies using mainly whole-exome sequencing have identified a number of frequently mutated genes with oncogenic potential and mutations within certain signaling pathways. These studies have also revealed clonal heterogeneity, where competition among subclones contributes to tumor progression. The prognostic implication of these mutations and chromosomal aberrations is currently being redefined as more and more effective treatment regimens are used for patients with MM. In this review, we focus on the complex genetic landscape and the mechanisms of disease evolution and progression in MM.

AJHO. 2017;13(12):19-25

Introduction

Multiple myeloma (MM) is characterized by proliferation of bone marrow plasma cells and secretion of monoclonal paraprotein or light chains in blood and/or urine. It is an incurable disease, but novel medications introduced during recent years have led to a significant increase in progression-free survival and overall survival (OS). MM is consistently preceded by the precursor state of monoclonal gammopathy of undetermined significance (MGUS), which can progress to smoldering MM (SMM) and to MM requiring treatment. MGUS exists in 2.4% of the population older than 50 years, and it is more common in African Americans compared with Caucasian whites and Mexican Americans. The onset of MGUS starts between age 30 to 40 years, and starts an average 10 years earlier in blacks. Approximately 0.5% to 1% of patients with MGUS and 10% of patients with SMM progress to MM each year. The risk of progression is higher in individuals who have a higher M-protein, non-IgG MGUS and a skewed free light chain ratio. Current risk scoring systems rely mainly on the overall burden of disease, and there is a lack of well-defined biological features that hold prognostic information.

The pathogenesis of MM includes multiple genetic aberrations as well as changes in the bone marrow microenvironment that allow evolution and proliferation of malignant plasma cells. The genetic landscape in MM is complex and includes translocations, copy number variations (CNVs), and somatic mutations that affect several molecular pathways and cellular functions (Table). Additionally, the disease consists of a number of subclones that may vary in size and number throughout the disease course. In this review, we focus on the current knowledge of genomic complexity and disease evolution in MM.

Plasma Cell Development

Plasma cells originate from B lymphocytes produced
in the bone marrow. After initial rearrangement of the immunoglobulin heavy chain and light chain genes, the naïve B cells leave the bone marrow expressing immature immunoglobulins on their surfaces. After encountering an antigen, the B cells migrate to the germinal center of the lymph node for further maturation. The maturation process includes somatic hypermutation, which induces point mutations in the variable region of immunoglobulin heavy chain (IGH), resulting in highly specific immunoglobulins and class switch recombination, which enhances the affinity and specificity of the immunoglobulins. These processes require genetic editing through DNA double-strand breaks in IGH mediated by activation-induced cytidine deaminase (AID), an enzyme belonging to the apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) family. The maturation process results in production of plasmablasts, which are initial antibody-producing effector cells, and memory B cells. Terminal differentiation of plasmablasts results in long-lived plasma cells with highly specific antibodies. The maturation process occurs in production of plasmablasts, which are initial antibody-producing effector cells, and memory B cells. Terminal differentiation of plasmablasts results in long-lived plasma cells with highly specific antibodies.

Neoplastic development of plasma cells in MM occurs through a multistage process involving acquisition of genetic events and deregulation of plasma cells. The microenvironment plays an important role in promoting the expansion of the premalignant and, later, malignant clones. As the disease evolves, the neoplastic plasma cells and the subsequent overproduction of immunoglobulin heavy and light chains eventually lead to end-organ damage defined by the CRAB criteria (hyperCalcemia, Renal failure, Anemia, Bone lesions)

### Genetic Landscape

The genetic landscape in MM is complex and involves multiple types of genetic aberrations, such as translocations, CNVs, and somatic mutations. Initially, chromosomal aberrations were assessed using metaphase cytogenetics, a technique that requires cell division in vitro, which may be challenging since plasma cells are slow-dividing cells. In addition, some of the more common translocations are cryptic and not captured by conventional cytogenetics. In clinical practice, fluorescence in situ hybridization (FISH) is widely used to capture translocations and CNVs. This technique is labor-intensive, and findings are limited to the specific targeted probes used in each patient. With the development of massive parallel sequencing, novel insights into the genomic landscape of MM have been gained.

### Chromosomal Translocations and Copy Number Variations

The cytogenetic changes in MM can be broadly divided into 2 groups: IGH translocations and hyperdiploidy. IGH translocations occur during the genetic editing in the germinal center of the lymph node, where occasionally the double-strand DNA is aberrantly rejoined. The IGH breakpoints tend to fall within certain preferred loci, and these translocations result in the juxtaposition of IGH and an oncogene. The oncogene is placed under the strong IGH enhancer and is overexpressed. The most common IGH translocations are t(4;14), t(6;14), t(11;14), t(14;16), and t(14;20). Translocation 4;14 results in MMSET and FGFR3 being overexpressed in 100% and 70% to 75% of cases.

<table>
<thead>
<tr>
<th>Translocations and Involved Genes</th>
<th>Cellular Function</th>
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<tr>
<td>t(4;14)(p16;q32) MMSET and FGFR3/IGH</td>
<td>MAPK pathway</td>
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<tr>
<td>t(14;16)(q32;q23) IGH/MAF</td>
<td>Cyclin D upregulation and promotion of cell cycling</td>
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<tr>
<td>t(14;20)(q32;q11) IGH/MAFB</td>
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<tr>
<td>t(6;14)(p21;q32) CCND3/IGH</td>
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<tr>
<td>t(11;14)(q13;32) CCND1/IGH</td>
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<tr>
<th>Somatic Mutations</th>
<th>Cellular Function</th>
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<tbody>
<tr>
<td>KRAS, NRAS, BRAF</td>
<td>MAPK pathway</td>
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<tr>
<td>CCND1, CCND3</td>
<td>Cyclin D upregulation</td>
</tr>
<tr>
<td>TP53, ATM, ATR</td>
<td>DNA repair</td>
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<tr>
<td>TRAF3, CYLD, LTB, IKK β, BIRC2, BIRC3, CARD11, TRAF3IP1</td>
<td>NFKB pathway</td>
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<tr>
<td>FAM44C, DIS3</td>
<td>RNA editing and regulation</td>
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<tr>
<td>PRDM1, IRF4, LTB, SP140</td>
<td>B-cell maturation</td>
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MAPK indicates mitogen-activated protein kinase; NFKB, nuclear factor kappa B.
respectively. Upregulation of MMSET alters epigenetic gene regulation, and FGFR3 encodes for a tyrosine kinase receptor with oncogenic potential when up-regulated or activated.\textsuperscript{15,17-19} Translocations between chromosome 14 and chromosomes 11 and 6, affecting the partner genes CCND1 and CCND3, respectively, result in upregulation of cyclin D proteins and promotion of cell cycling.\textsuperscript{19,20} Furthermore, t(14;16) and t(14;20) affect MAF and MAFB, respectively, in which the downstream effects include upregulated expression of a number of genes, including CCND2.\textsuperscript{19,21-23}

Translocations t(4;14), t(14;16), and t(14;20) are considered high-risk aberrations and associated with adverse prognosis.\textsuperscript{21,24,25} Translocation 11;14 was previously considered to have an overall neutral effect; however, emerging data on t(11;14) implies that it may confer a worse-than-standard-risk prognosis.\textsuperscript{26}

Yet it is important to emphasize that these and other genetic subtypes have been designated as high risk in terms of survival among patients with these genetic markers (vs those without). Because clinical outcomes are highly dependent on the given treatment, in the future, many patients with genetic features that previously were considered to confer high risk will likely have the same outcome as standard-risk patients. Thus, with better therapies the proportion of patients with poor outcomes will become smaller.\textsuperscript{27}

Approximately 45\% of patients with MM harbor IGH translocations, while approximately 40\% have trisomies of the odd-numbered chromosomes 3, 5, 7, 9, 11, 15, 19, and 21. The mechanism behind hyperdiploidy is less clear, but the acquisition of extra chromosomes is hypothesized to happen during one catastrophic mitosis rather than a step-wise gain of chromosomes.\textsuperscript{19} IGH translocations and hyperdiploidy are early hits and are both found already at the MGUS stage (Figure), and they are therefore not considered by themselves to be sufficient for development of MM.\textsuperscript{28,29}

Secondary chromosomal events include 17p deletion, gain of 1q, deletion of 1p, and deletion 13q, of which the majority are associated with adverse OS.\textsuperscript{30,31} Many of the secondary CNVs are subclonal, indicating that they are acquired during the disease course rather than being founder events, in contrast to IGH translocations and hyperdiploidy.\textsuperscript{32}

Approximately 3\% to 4\% of patients with MGUS and SMM harbor MYC translocations, whereas MYC translocations are found in up to 15\% to 20\% of patients with newly diagnosed MM.\textsuperscript{30,31} When including patients who have gains of the MYC locus, up to 30\% of patients have events involving MYC, making this one of the most common aberrations in MM.\textsuperscript{32}

Somatic Mutations
The introduction of massive parallel sequencing has allowed high-resolution sequencing of large cohorts yielding important new insights into the genetic landscape of MM. During the past 5 years, mainly through whole-exome sequencing, a number of frequently mutated genes have been reported. The most common mutations are found within the mitogen-activated protein kinase (MAPK) pathway: KRAS, NRAS, and BRAF are mutated in approximately 50\% of patients with MM.\textsuperscript{34,35} Mutations in KRAS and NRAS are mutually exclusive in the majority of cases, but they do coexist in 2\% of patients.\textsuperscript{36}

Additional genes that are frequently mutated in MM are FAM46C, TP53, DIS3, IFR4, TRAF3, CYLD, RBI, SPI40, LTB, MAX, EGR1, FGFR3 ATM, ATR, and more.\textsuperscript{15,36,38} Of these, KRAS, NRAS, TP53, BRAF, FAM46C, and DIS3 are generally considered to be driver mutations.\textsuperscript{10} These mutations affect various cell functions, such as MAPK and nuclear factor kappa B signaling, DNA repair mechanisms, and RNA editing.\textsuperscript{19,35,36,38}

The genetic complexity and mutational burden increase as the disease progresses, but there is no genetic profile specific to MGUS, SMM, or MM.\textsuperscript{10,28,30,38} Disease evolution may occur either through gains of additional mutations or expansion of clones that are already present at an early disease stage, but that initially fall below the level of detection.\textsuperscript{30,40} Interestingly, Mailankody et al\textsuperscript{41} found that the overall number of somatic mutations was similar between SMM and MM, but the pattern of mutations was different between the disease stages. In patients with MM, there were more mutations in genes that have been reported as frequently mutated and in driver genes, compared with patients with SMM. Furthermore, patients who had a good response to treatment had fewer mutations in these frequently mutated genes compared with those with a poorer response when treated with the modern combination treatment of carfilzomib/lenalidomide/dexamethasone.\textsuperscript{41}

Mutational Processes and Altered Pathway Activity
The overall mutation rate in MM is higher than in other hematologic malignancies, but lower than in many solid tumors.\textsuperscript{38,42} Several mutational processes and signatures are present in MM, with the most prominent mutational signatures being 1, 2, 5, and 13, which are commonly signatures of aging and AID/APOBEC activity.\textsuperscript{43} Kataegis, a process of regional clustering of mutations close to translocation breakpoints, is also present in MM, both in IGH and MYC translocations.\textsuperscript{40} In 11\% to 25\% of patients, the partner gene on der(14) in an IGH translocation—CCND1, FGFR3, MAF, or MAFB—is hypermutated.\textsuperscript{32,38}

Thus, cellular pathways and functions can be altered
through different mechanisms, which may have additive effects. As an example, TP53 located on 17p may be inactivated through chromosomal arm deletion or inactivated though TP53 mutations. Another example may be the MAPK pathway, which is activated through mutations in KRAS/NRAS/BRAF, but can also be activated through translocation involving t(4;14) or FGFR3, leading to increased activity of the tyrosine kinase and downstream upregulation of the MAPK pathway.

There is also evidence of co-occurrence of gene–gene and gene–CNV aberrations where biallelic events (eg, 17p deletion and TP53 mutation as well as 1p deletion and FAM46C mutation) are associated with a worse prognosis than if only 1 allele is affected.

Clonal Evolution
In MM, there are, on average, 5 heterogeneous subclones, and the disease is thought to progress through Darwinian evolution driven by competing subclones. As with CNVs, mutations can be clonal or subclonal, and the subclones vary in size and distribution over the disease course in response to clonal competition and treatment. Bolli et al analyzed longitudinal samples in a subset of patients and described different patterns of progression: linear progression with the same clone present at relapse, branching progression with a new subclone appearing at relapse, or branching progression with a different dominant clone at relapse, with or without the initial dominant clone still present. Moreover, certain high-risk events (eg, 17p deletion, and mutations conferring treatment resistance, such as CRBN) are more common in relapse samples.

Clinical Implications
The clinical staging system is currently based on laboratory findings (beta-2 microglobulin, lactate dehydrogenase, and albumin levels) and presence of high-risk IGH translocations and CNVs, t(4;14), t(14;16), or deletion 17p. Presence of these aberrations can impact the clinical decision making in favor of more aggressive treatment. As more and more targeted drugs are being developed, the mutational landscape will be increasingly important to assess in patients with MM. For instance, the BCL2 inhibitor venetoclax has effect in relapsed/refractory MM harboring t(11;14), and ongoing studies with BRAF and MEK inhibitors are targeting the MAPK pathway for
patients with mutations in $BRAF$, $KRAS$, and $NRAS^{53,54}$. Furthermore, it seems logical to propose that future prognostic markers/models—across hematologic malignancies—likely will focus more on the genetic landscape of residual tumor cells posttherapy. Indeed, such observations have already been made in patients with acute myeloid leukemia (AML). Clearance of adverse genetic aberrations 30 days posttherapy has been proposed to be one of the strongest favorable prognostic factors in AML. $^{33}$ We and others are currently conducting such studies in plasma cell disorders to better define these dynamics in MM.

**Summary and Future Perspectives**

Detailed interrogation of the genetic landscape of MM and its precursor disease using modern sequencing techniques has revealed a complex genetic landscape, as well as interpatient and intrapatient spatial and temporal heterogeneity. Multiple genetic editing and mutational processes are in place, as well as Darwinian evolution through competing subclones. In this review, we focused on the genomic events of the tumor cells. Nonetheless, the bone marrow microenvironment and the host immune system likely play a large role in the pathogenesis of MM. The understanding of interactions among the microenvironment and the tumor cells is, however, less developed and goes beyond the scope of this review.

Despite the recent advances in genomic events in MM, areas of interest still exist where information is currently limited. First, the majority of sequencing studies have been cross-sectional and included heterogeneous patient populations. Thus, longitudinal studies with serial samples are needed to increase our knowledge on temporal relationships and clonal evolution, both in early disease as well as at relapse. Second, through assessment of gene–gene, gene–CNV, and gene–treatment interactions in the era of modern combination treatments, we will be able to identify distinct molecular profiles and optimize treatment prediction models. As the availability and accuracy of the sequencing techniques and bioinformatic analyses increase, sequencing will be used to identify translocations, CNVs, and mutations and to monitor minimal residual disease, and it will eventually replace conventional cytogenetics and FISH.$^{34,53}$ With additional high-resolution methods such as circulating tumor cells and cell-free DNA, the possibilities of assessing genomic profiles of plasma cell disorders throughout the disease trajectory will be increasingly accessible.$^{55,56}$ In summary, accurate and available technologies will further increase our knowledge of molecular driver events. This, in turn, is essential for development of early and targeted treatments to improve patient outcomes in MM.

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**Financial disclosures:** The Swedish Research Council (2015-00564) (Hultcrantz), Multiple Myeloma Research Foundation (2015 Research Fellow Award) (Hultcrantz), The Swedish Cancer Society (CAN 2014/1329) (Hultcrantz), and MSKCC Core grant (P30 CA008748) (Hultcrantz, Landgren).

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An Integrative Approach for Sequencing Therapies in Metastatic Prostate Cancer

Yadi Li, BSc; Sindhu Malapati, MD; Yu Ting Lin, BSc; and Akash Patnaik, MD, PhD, MMSc

Introduction
Prostate cancer is the most common cancer and the second leading cause of cancer-related death among men in the United States.1 The majority of patients with prostate cancer present with localized disease at the time of diagnosis.2 However, metastatic castration-resistant prostate cancer (mCRPC) was responsible for an estimated 26,120 US deaths in 2016.3 The current standard of care for patients with metastatic prostate cancer is androgen-deprivation therapy (ADT). Most patients initially respond well to ADT in the form of surgical or chemical castration, which lasts for a median duration of 18 months to 2 years, following which the disease becomes castration resistant.

With a multitude of FDA-approved treatment options for mCRPC, which include abiraterone acetate (Zytiga), enzalutamide (Xtandi), docetaxel (Taxotere), cabazitaxel (Jevtana), sipuleucel-T (Provenge), denosumab (Xgeva), and radium-223 (Xofigo), the optimal treatment sequence for these therapies remains a conundrum in the field. In addition, there are limited data to guide sequencing of later lines of therapy and the utility of combining existing therapies. Given the recent practice-changing data demonstrating a significant overall survival (OS) improvement with docetaxel and abiraterone use in frontline therapy for hormone-sensitive, locally advanced, and metastatic prostate cancer, the future of these agents following mCRPC progression remains to be determined.4,5 The main objective of this article is to provide a comprehensive, evidence-based review of the selection and sequencing of different lines of therapy for mCRPC.

Current Landscape
Although several new agents have become available to treat mCRPC in the past decade (Figure 1), limited evidence provides guidance for sequencing these treatments in routine clinical practice. In 2004, the chemotherapeutic agent docetaxel became the first agent to receive

AJHO. 2017;13(12):26-31
FDA approval in mCRPC. This approval was based on 2 clinical trials, TAX 327 and SWOG 99-16. Both studies showed that docetaxel improved median survival relative to mitoxantrone. Cabazitaxel is another taxane-based chemotherapy that was approved by the FDA, in 2010, in patients with mCRPC previously treated with docetaxel. Both docetaxel and cabazitaxel work by disrupting cellular microtubule dynamics that are critical for mitosis and cell division. Study findings suggest that cabazitaxel has a better pharmacokinetic profile than docetaxel, but the former is more myelosuppressive, resulting in a higher incidence of febrile neutropenia.

In the FIRSTANA trial, which studied cabazitaxel versus docetaxel as first-line therapy in chemotherapy-naïve mCRPC, different dosages of cabazitaxel did not show superiority over docetaxel, with each agent having different toxicity profiles but overall less toxicity with lower-dose cabazitaxel. Cabazitaxel is used mostly in cases of progression on docetaxel since it was designed to overcome the resistance mechanisms to docetaxel, and this remains the only indication for which it is FDA approved. Given their potential toxicities and the established efficacy of less toxic alternatives, such as androgen-directed therapies (see below), taxane-based chemotherapy agents are generally reserved for use as second- or third-line therapies.

From 2011 to 2012, 2 androgen-directed therapies, abiraterone acetate and enzalutamide, were approved by the FDA for patients with mCRPC, initially in the postchemotherapy setting. Abiraterone inhibits testosterone production in the adrenal glands, testes, and prostate via inhibition of CYP17A1. Enzalutamide antagonizes the androgen receptor (AR) with higher binding affinity relative to prior AR antagonists, such as flutamide, nilutamide, and bicalutamide.

The approval of abiraterone was based on a multinational phase III trial, COU-AA-301, which showed a 4-month improvement in OS, whereas enzalutamide’s approval was based on the AFFIRM trial, which found a 4.8-month improvement in median OS. Following these initial registration studies, COU-AA-302 and MDV3100-03 demonstrated efficacy of abiraterone and enzalutamide in the prechemotherapy setting. Both studies improved radiographic progression-free survival (rPFS) and OS in asymptomatic and minimally symptomatic chemotherapy-naïve patients with mCRPC. Specifically, the COU-AA-302 trial randomized 1088 asymptomatic to mildly symptomatic, chemotherapy-naïve patients with mCRPC to either abiraterone plus prednisone or placebo plus prednisone. Compared with the placebo group, abiraterone showed significant improvement in median OS (34.7 vs 30.3 months; HR, 0.81; \(P = .0033\)). Similarly, in the MDV3100-03 trial, 1717 asymptomatic-to-mildly symptomatic, chemo-naïve patients with mCRPC were randomized to either enzalutamide or placebo daily. Enzalutamide demonstrated improvement in both OS (HR, 0.71; 32.4 vs 30.2 months; \(P < .0001\)) and median rPFS (HR, 0.17; not reached vs 3.7 months; \(P < .0001\)) relative to placebo. These results led to the approval of both abiraterone and enzalutamide in the prechemotherapy space in 2012 and 2014, respectively. Given their more favorable adverse

![FIGURE 1. Several New Agents Approved Since 2004](image)
event (AE) profile and relative ease of administration, AR-directed agents have replaced taxane chemotherapy as first- and/or second-line treatments for mCRPC.

Cross-resistance is commonly observed between abiraterone and enzalutamide when used sequentially for the treatment of mCRPC (ie, the use of one AR-directed therapy typically results in a decreased duration of response and blunted response to the next AR-targeted therapy). Thus, several studies have explored the optimal sequencing of abiraterone and enzalutamide in an attempt to maximize clinical efficacy.

A recent report presented at the 2017 American Society of Clinical Oncology Annual Meeting by the Kyoto-Baltimore Collaboration suggested that abiraterone as first-line treatment before enzalutamide prolonged combined prostate-specific antigen (PSA) PFS (HR, 0.56; P < .001), but not OS, relative to enzalutamide as first-line treatment before abiraterone. However, the opposite trend was observed in another study, where enzalutamide as first-line treatment resulted in more patients experiencing >50% PSA reduction than with abiraterone (73% vs 53%; P = .004), but with no difference in time to PSA progression (TTTP). In this study, baseline pathogenic circulating tumor DNA (ctDNA) alterations in AR, TP53, RB1, and DNA repair (BRCA2, ATM) genes were associated with a shorter TTTP.

In addition, a retrospective analysis of a real-world mCRPC database showed that treatment effect persistence was significantly longer in chemotherapy-naïve patients treated with enzalutamide relative to abiraterone (HR, 0.86; P = .02). Despite the data presented here, there have been insufficient definitive evidence on the optimal sequencing of the 2 AR-directed agents, and a prospective, randomized clinical trial is needed in order to draw a definitive conclusion. Currently, the pattern for sequencing AR-targeted therapies is individualized, and it is dependent on clinical context, with considerations that include AE profile and baseline medical co-morbidities. In the context of predictive biomarkers for AR-directed therapies, recent studies have shown that the detection of AR splice variant 7 on circulating tumor cells predicts for resistance to both AR-directed agents.

Several recent trials have suggested improved survival with up-front utilization of docetaxel or abiraterone in metastatic castration-sensitive prostate cancer (mCSPC), when combined with ADT. The CHAARTED trial of docetaxel enrolled 790 patients with mCSPC to ADT plus docetaxel or ADT alone. It found that ADT plus docetaxel prolonged OS by 13.6 months compared with ADT alone (57.6 vs 44.0 months; HR, 0.61; P < .001). The median time to biochemical, symptomatic, or rPFS was 20.2 months in the combination group compared with 11.7 months in the ADT-alone group (HR, 0.61; 95% CI, 0.51 to 0.72; P < .001).

Recent studies have also established abiraterone plus ADT as a new standard of care for mCSPC. The STAMPEDE trial in CSPC demonstrated a 3-year OS of 83% versus 76% (HR, 0.63; P < .001) and failure-free survival of 75% versus 45% (HR, 0.29; P < .001) for ADT plus abiraterone versus ADT alone. In addition, the double-blind, randomized, phase III LATITUDE trial reported results similar to those of the STAMPEDE trial. The LATITUDE trial studied 1199 men with mCSPC receiving either ADT plus abiraterone plus prednisone or ADT with dual placebos. It found that the treatment group had significantly longer median OS (not reached vs 34.7 months; HR, 0.62; P < .001) as well as rPFS (33 months vs 14.8 months; HR, 0.47; P < .001).

In addition, an open-label, single-arm, phase II study evaluated the efficacy of enzalutamide in hormone-sensitive prostate cancer as a single agent without ADT. At week 97 post treatment, 45 of a total 67 patients (67%) were still on enzalutamide, and all 45 had a PSA response (100%; 95% CI, 92%-100%). Of 26 patients who originally presented with metastases, 13 achieved a complete response (50%) and 4 (15.4%) demonstrated a partial response. Taken together, these results highlight the positive clinical impact of using chemotherapy and AR-directed agent therapy for the upfront treatment of mCSPC, which will likely alter disease biology, clinical course, and sequencing of these agents in the mCRPC setting.

Prostate cancer most commonly metastasizes to the bone, resulting in significant morbidity due to pain and decreased quality of life. For patients with symptomatic bone metastases but no visceral disease, the radionuclide radium-223 was FDA approved, based on data from the ALSYMPCA trial, which showed a median OS benefit (HR, 0.7; 14.9 vs 11.3 months; P < .001) in 921 men with symptomatic bone metastasis, regardless of previous chemotherapy status. Due to its chemical structure and calcium-mimetic properties, radium-223 is preferentially taken up in areas of increased bone turnover, such as bone metastases. Following bone uptake, radium-223 emits cytotoxic alpha radiation, which has a shorter range of action than that of beta and gamma particles. Therefore, the effect is more localized and targeted, leading to decreased bone marrow toxicity. While there are preliminary data showing clinical benefits of radium-223 as a first-line agent, it is typically used as second- or third-line therapy to palliate symptomatic bone metastases on an as-needed basis.
combination could be used for treatment of symptomatic bone metastases. Radium-223 can be safely combined with abiraterone or enzalutamide, with these findings extending to patients who were asymptomatic at baseline. Median OS was longer in patients who received radium-223 plus abiraterone, enzalutamide, or both relative to radium-223 without concomitant use of these agents (median NA [not available]; 95% CI, 16 months–NA vs median 13 months, 12-16 in radium-223 alone) and in patients who received radium-223 plus the RANK ligand inhibitor, denosumab (median NA, 15 months–NA), relative to patients who received radium-223 without denosumab (median, 13 months, 12 months-NA). The findings of improved survival with concomitant treatment require confirmation in randomized trials.

In 2010, the FDA approved sipuleucel-T, the first and only immunotherapy to receive FDA approval in mCRPC. Sipuleucel-T is a customized vaccine composed of a patient’s own antigen-presenting cells (APCs), which are cultured ex vivo with a fusion protein of prostatic acid phosphatase (PAP) and granulocyte-macrophage colony-stimulating factor. The APCs are then re-infused into the patient to initiate PAP-directed T-cell antitumor response. In the phase III IMPACT trial, sipuleucel-T showed a median survival of 25.8 months versus 21.7 months in the placebo arm (n = 512; P = .03) in men with asymptomatic mCRPC. However, the feasibility of using sipuleucel-T in routine clinical practice has been controversial, given the inability to use PSA as a biomarker for treatment response, and given the vaccine’s high cost and cumbersome preparation process. Clinical trial data exploring combinations of sipuleucel-T with other immunomodulatory agents in mCRPC are awaited.

Neuroendocrine prostate cancer (NEPC) is a rare but lethal subtype of advanced prostate cancer. It develops in a subset of patients with mCRPC after ADT, and has increased in emergence with the advent of AR-directed therapies. About 10% to 15% of patients with NEPC present de novo with the typical phenotype of small-cell lung carcinoma (SCLC). This small-cell “AR-indifferent” subtype is treated with platinum- and etoposide-based chemotherapy, similar to treatment of SCLC, and it does not fit the standard treatment sequence paradigm for mCRPC.

Sequencing of mCRPC Treatment

Based on limited data, we propose the following algorithm for sequencing therapies in mCRPC (Figure 2). For asymptomatic-to-mildly symptomatic patients with mCRPC, sipuleucel-T may be used as first-line therapy for asymptomatic, chemotherapy-naive patients without any visceral disease, followed by AR-directed agents for the second line, and chemo agents as third line. For symptomatic patients, AR-directed agents are used in the
first line, followed by docetaxel for the second line and cabazitaxel in the third line once the patient has become resistant to docetaxel. There are no known differences in clinical outcomes regarding the use of taxanes or androgen-directed therapies as first-line treatments, but the latter are generally used in the first line due to their favorable AE profile and more convenient oral dosing schemes. In clinical practice, taxanes are commonly used in the first-line setting for symptomatic patients with rapidly progressive visceral/bone metastases, but evidence to support this strategy is lacking.

Alternatively, radium-223 can be used as a palliative therapy in patients who are presenting with symptomatic bone metastases, regardless of previous chemotherapy status. It is safe in combination with androgen-directed therapies, but the finding of improved OS needs confirmation in randomized trials. There are limited data on mCRPC treatment beyond the third line, leaving it to the discretion and experience of the treating physician to decide upon the optimal treatment plan. Notably, emerging data from tumor genomics suggest that tumor and germline sequencing may be invaluable in guiding future treatment choices, particularly as sequencing relates to defects in the DNA repair pathways that can be targeted with DNA-damaging agents, such as PARP inhibitors and platinum-based chemotherapy.

In a multicenter analysis of 692 patients with metastatic prostate cancer, 11.8% of patients were found to carry germline mutations in DNA-repair pathways.41 In the phase II TOPARP trial of olaparib, a PARP inhibitor, 16 of 50 patients with mCRPC responded, and the majority of responders (88%) carried somatic alterations in homologous recombination-associated DNA-repair genes, such as BRCA2 and ATM.42,43 Finally, immunotherapy clinical trials with immune checkpoint blockade therapies are revolutionizing cancer treatment, and they are beginning to show signs of clinical benefit in a subset of patients with mCRPC.44 The future looks promising for the treatment of mCRPC.

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Financial disclosures: The authors report no financial disclosures relevant to this publication.

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**RET-Rearranged Lung Cancer**

Fernando C. Santini, MD, and Artur Katz, MD

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**Abstract**

In recent years, we have witnessed the discovery of several oncogenic driver mutations as well as the emergence of specific inhibitors with high response rates and few treatment-related adverse events. RET-rearranged lung cancers represent a small subset of lung cancer, most commonly encountered in patients with adenocarcinoma and minimal or no exposure to tobacco. Several multikinase inhibitors have been tested with high “off-target” toxicity and low RET inhibition activity. Early-phase clinical trials with more selective inhibitors are awaited. Here, we review the main aspects of the biology of RET, the challenges of RET inhibition in lung cancer, and some future perspectives.

*AJHO. 2017;13(12):32-37*

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**Introduction**

Genomic analysis of lung cancer has shown that these tumors contain distinct genetic alterations. The discovery of the EGFR mutation and its sensitivity to EGFR tyrosine kinase inhibitors (TKIs) revolutionized the treatment of lung cancers. At that time, we were mainly focused on small genetic modifications. Notwithstanding, in 2007, a chromosomal rearrangement involving ALK was discovered, which was followed by publication of the activity of the ALK inhibitor crizotinib, with high response rates. The evolution of genomic analysis led to the discovery of novel oncogenic fusion genes such as ROSI and RET.

In 1985, Takahashi and colleagues first described a new transforming gene that appeared to be activated by the recombination of 2 unlinked human DNA segments, possibly by co-integration during transfection of NIH 3T3 cells with human lymphoma DNA, which was designated RET (rearranged during transfection).

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**The Biology of RET**

RET is a proto-oncogene localized in the pericentromeric region of chromosome 10q11.2, which encodes the protein RET, a receptor tyrosine kinase (RTK). RET undergoes alternative splicing of 3′ exons to generate 3 protein isoforms: RET9, RET32, and RET51, which differ at their carboxy terminal amino acids number. RET has 3 domains: a large extracellular domain, a transmembrane region, and an intracellular kinase domain. It is the only RTK with 4 cadherin-like domains in its extracellular region. RET is the signaling receptor for the glial cell-derived neurotrophic factor (GDNF) family of ligands (GFLs): GDNF, neurturin, persephin, and artemin. Unlike other RTKs, downstream signaling requires co-receptors that are tethered at the lipid rafts (cholesterol-rich membrane subdomains). Although there can be some crosstalk, each GFL interacts primarily through its specific co-receptor, represented by 4 GDNF family receptor-alpha (GFR-α) 1-4. Upon binding of GFLs to GFR-α1-4 complex, RET dimerization and autophosphorylation stimulate multiple downstream pathways, including RAS-MAPK, PI3K-AKT, and STAT3. These signs play a key role in kidney and nervous system development, neuronal survival and differentiation, and maintenance of spermatogonial stem cells.

RET receptor is expressed in several neural and neuroendocrine cell lineages, such as the thyroid C cells and adrenal chromaffin cells. RET loss-of-function mutations give rise to Hirschsprung disease and congenital abnormalities of the kidney and urinary tract, while RET gain-of-function mutations result in aberrant activation of the receptor; they are pathognomonic in patients with multiple endocrine neoplasia type 2 (MEN2). Both germline and somatic RET mutations represent an important step of medullary thyroid carcinoma oncogenesis. At the same time, somatically occurring RET rearrangements occur in 20% to 40% of papillary thyroid carcinoma. The increasing use of new techniques,
such as genomic sequencing and transcriptome analysis, has led to the identification of chromosomal rearrangements in other cancers.

Chromosomal rearrangements involving RET are frequently found in irradiation-induced papillary thyroid carcinoma.5

RET and Lung Cancers
In 2012, Ju and colleagues9 first reported on a 33-year-old never-smoker patient with lung adenocarcinoma with a novel fusion gene between KIF5B and the RET proto-oncogene caused by a pericentric inversion of 10p11.22 – q11.21. KIF5B contains a coiled-coil domain functioning as a dimerization unit, which activates the oncogenic tyrosine kinase domain of RET by autophosphorylation after homodimerization. The RET kinase domain portion is preserved in all kinase fusions, despite the breakpoint leaving downstream intracelluar kinase activity intact.

The transformation potential of RET fusions has been reported in Ba/F3 cells and LC-2/ad (human adenocarcinoma cell-line), while anchorage-independent cell proliferation has also been shown in NIH3T3 cells.10 The mutually exclusive nature of the RET fusions and other oncogenic alterations suggests that the KIF5B-RET fusion is a driver mutation. However, Kim and colleagues11 reported the co-occurrence of EGFR or KRAS mutations in KIF5B-RET rearranged lung adenocarcinoma, and RET rearrangement was also reported in patients with EGFR-mutated lung adenocarcinoma who had progressed on TKI therapy.12

A variety of breakage points have been identified within the KIF5B locus, which is the most common fusion partner gene. More importantly, several other RET fusion partner genes have been identified: CCDC6 (coiled-coil domain containing 6), CUX1 (cutlike-homobox 1), TRIM33 (tripartite-motif containing 33), NCOA4 (nuclear-receptor coactivator 4), KIAA1468, KIAA1217, CLIP1 (CAP-Gly domain containing linker protein 1), ERC1 (ELKS/Rab6-interacting/CAST family member 1), and MYOS5 (myosin 5C), among others. Importantly, all of these fusion partners contain coiled-coil domains that are believed to mediate ligand-independent dimerization and constitutive activation of RET.10,13-16

To date, several cancer genome sequencing studies have discovered RET fusions in 1% to 2% of unselected lung cancers, which might be higher in the pan-negative population (negative for all known oncogenic driver mutations).14,17 Several studies have tried to elucidate the clinicopathological characteristics of RET-rearranged lung cancers. Most of the tumors are adenocarcinoma, but some cases involve other histological types, such as adenosquamous carcinoma. The tumors were significantly more common in younger patients and tended to occur in never-smokers and light smokers. The RET-rearranged lung adenocarcinomas are mostly well or moderately differentiated cancers and are thyroid transcription factor 1 (TTF-1) positive; the predominant growth pattern is very heterogeneous.18,21 Interestingly, Lee and colleagues22 reported that the mucinous cribriform pattern was more frequent with CCD6-RET-positive tumors (4/5, 80%), whereas the solid signet-ring cell pattern was present in 3 of 6 (50%) of the KIF5B-RET-positive tumors.

Takeuchi and colleagues13 showed results similar to the aforementioned ones: that the frequency of mucinous cribriform carcinoma was significantly higher in the kinase-fusion–positive group (ALK, ROSI and RET) of tumors than in the fusion-negative adenocarcinomas. Conversely, the mucinous cribriform pattern was infrequently observed (13.6%) in a Japanese cohort of 22 cases selected from resected specimens at the National Cancer Center, Tokyo.23 Unlike in non–small cell lung cancer (NSCLC), there are some reports of RET gain-of-function point mutations in small cell lung cancer (SCLC). Dabir and colleagues24 identified an activating M918T RET somatic mutation in a metastatic small–cell lung cancer (SCLC) tumor specimen, which is among the most highly transforming RET mutations in vitro and leads to a severe clinical MEN2B phenotype.

It is interesting that RET rearrangements develop with a large prevalence in radiation-induced thyroid cancers. Furthermore, exposure to radon is a major risk factor for developing lung cancers. Thus, RET fusions may represent a genetic mechanism of radiation-induced lung adenocarcinoma, but further studies are needed.25 There is no gold-standard technique to detect RET gene fusions, and most studies use multiple techniques, such as whole-genome and transcriptome sequencing, RNA sequencing, reverse transcription polymerase chain reaction (RT-PCR), fluorescence in situ hybridization (FISH), and immunohistochemistry (IHC). Although normal lung tissue shows low RET expression, IHC is not a reliable method to detect overexpressed RET because staining can vary and the immunoreactivity of available antibodies is weak. Overall, a combined strategy of RT-PCR and FISH, with dual color break-apart probe, is an effective tool for detection of RET chromosomal rearrangements. Reverse transcription polymerase chain reaction alone is usually insufficient to detect new partners or isoforms; therefore, FISH may be better in terms of sensitivity.26 More recently, broad hybrid, capture-based, next-generation
sequencing (NGS) was able to identify genomic alterations in 65% of tumors from never- or light-smokers with lung cancers that had previously been deemed free of genomic alterations by the aforementioned types of non-NGS testing. Therefore, NGS should be considered, if feasible.27

**Targeting RET**

Several commercially available multikinase inhibitors, such as vandetanib (Caprelsa), cabozantinib (Cabometyx), sorafenib (Nexavar), sunitinib (Sutent), lenvatinib (Lenvima), ponatinib (Iclusig), dovitinib (TKI-258), and alectinib (Alecensa), have activity against the RET kinase. In 2013, Drilon et al27 first reported the response to a RET inhibitor, cabozantinib, in patients on a prospective, molecularly enriched trial for RET-positive lung cancers, and in 2016, they published the first stage of a phase II study with 25 cases29 (Table). The most common grade 3 treatment-related adverse events (TRAEs) were lipase elevation, increased levels of alanine aminotransferase and aspartate aminotransferase, decreased platelet count, and hypophosphatemia. Seventy-three percent of patients required cabozantinib dose reduction, most commonly due to palmar-plantar erythrodysesthesia, fatigue, and diarrhea.

Subsequent reports from 2 phase II trials testing the effect of vandetanib on RET-positive lung cancers showed discordant results, which may be explained by differences in patient selection and choice of assay30,31 (Table). The most common AEs with vandetanib were hypertension, diarrhea, rash acneiform, dry skin, prolonged QT corrected interval, anorexia, and increased creatinine.

Twenty-one percent of patients required vandetanib discontinuation, most commonly due to rash and pneumonitis, and 81% required dose reductions due to rash and hypertension.

Lenvatinib showed clinical benefits in patients with RET-rearranged lung adenocarcinomas, with a disease control rate of 76%, according to a phase II study presented at the 2016 European Society for Medical Oncology Congress. The most commonly reported trAEs were hypertension, nausea, anorexia, diarrhea, and proteinuria.

All of the aforementioned drugs are multikinase inhibitors with activity against advanced RET-rearranged lung cancers. The objective response rates (ORRs) were modest, but greater than with single-drug chemotherapy or single-drug immunotherapy, after progression on initial platinum doublet treatment in unselected patients with advanced NSCLC. Although clinically meaningful benefit was seen (Table), their activity was lower than that shown with EGFR and ALK inhibitors. These multikinase inhibitors are much more effective at inhibiting VEGFR, EGFR, and KIT than RET, which explains the high rate of off-target dose-limiting toxicities leading to frequent dose reductions and drug discontinuations. Hypertension and proteinuria, both commonly reported, can be related to VEGFR inhibition, while rash acneiform and diarrhea can be due to EGFR inhibition, and skin hypopigmentation and marrow suppression are related to KIT inhibition.

Alectinib, a known inhibitor of ALK, was shown to inhibit RET kinase activity (IC₅₀ = 4.8 nmol/L) and the growth of RET fusion–positive cells by suppressing RET phosphorylation.32 In addition, alectinib showed kinase inhibitory activity against RET gatekeeper mutations (RET V804L and V804M). Lin and colleagues33 described 4 patients with advanced RET-rearranged lung cancers who were treated with alectinib. In total, 2 of 4 patients had overall responses, with durations of therapy of 6 months and more than 5 months. Given its more favorable safety profile, alectinib may be dosed more effectively to target RET, and it can represent an alternative to multikinase inhibitors.

More-specific RET inhibitors, with improved potency

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**TABLE. Phase II Trials of Multikinase Inhibitors for Advanced RET-Rearranged Lung Cancers**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Kinase inhibition</th>
<th>Author</th>
<th>N</th>
<th>ORR (95% CI)</th>
<th>mPFS (95% CI)</th>
<th>mOS (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cabozantinib</td>
<td>RET, ROS1, MET, VEGFR2, AXL, TIE2, KIT</td>
<td>Drilon et al28</td>
<td>26</td>
<td>28% (95% CI, 12%-49%)</td>
<td>5.5 mos (95% CI, 3.8-8.4)</td>
<td>9.9 mos (95% CI, 8.1-NR)</td>
</tr>
<tr>
<td>Vandetanib</td>
<td>RET, EGFR, HER2, VEGFR</td>
<td>Yoh et al (LURET)24</td>
<td>17</td>
<td>53% (95% CI, 28%-77%)</td>
<td>4.7 mos (95% CI, 2.8-8.5)</td>
<td>11.1 mos (95% CI, 9.4-NR)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lee et al31</td>
<td>18</td>
<td>18%</td>
<td>4.5 mos</td>
<td>11.6 mos</td>
</tr>
<tr>
<td>Lenvatinib</td>
<td>RET, VEGFR, FGFR, PDGFR, KIT</td>
<td>Velcheli et al32</td>
<td>25</td>
<td>16%</td>
<td>7.3 mos (95% CI, 3.6-10.2)</td>
<td>NR (95% CI, 5.8-NR)</td>
</tr>
</tbody>
</table>

mOS indicates median overall survival; m, months; mPFS, median progression-free survival; NR, not reached; ORR, objective response rate.
and reduced toxicity, are currently being investigated in the clinical and preclinical settings. Early-phase clinical trials of RXDX-105, a RET and BRAF inhibitor, which spares VEGFR2/KDR and VEGFR1/FLT, have been launched. A patient with advanced RET-rearranged lung cancer had a rapid and sustained response to RXDX-105 in both intracranial and extracranial disease.24 Other RET-specific inhibitors in development include LOXO-292 and BLU-667, which are both potent VEGFR-sparing RET inhibitors with specificity for RET and predicted resistant mutants. Of note, different sensitivities to RET inhibitors among different RET fusion forms are still unknown and need further study.

As in the case of other oncogene-driven lung cancers, resistance to RET inhibition is likely to emerge. We speculate that resistance to RET inhibition from the available multikinase inhibitors may be mediated more frequently by bypass signaling mechanisms than by RET-resistant mutations, because lower activity against RET exerts less selective pressure over the RET pathway. Also, RET-rearranged lung cancers might rely on alternative signaling pathways, and combination treatment may represent an alternative in the future.35,36

As with ALK- and ROSI-rearranged lung cancers, durable benefits with pemetrexed-based therapies in RET-rearranged lung cancers were seen. Drilon and colleagues37 retrospectively evaluated 104 patients with RET-rearranged lung cancers who received treatment with pemetrexed alone or in combination. Patients had a median PFS of 19 months (95% CI, 12-not reached) and an ORR of 45%. One might expect lower response rates to immunotherapy in RET-positive lung cancers, in accordance with other oncogene-driven lung cancers. A recent meta-analysis to assess the role of immunogenic target. Also, RET-rearranged lung cancers might rely on alternative signaling pathways, and combination treatment may represent an alternative in the future.35,36

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Conclusions
RET-rearranged lung cancers represent a small subset of lung adenocarcinomas with clinicopathological features similar to those of other rearrangement-driven lung cancers. Given the low frequency of these cancers, collaboration among various international research centers can generate meaningful knowledge about them. A global, multicenter network of thoracic oncologists (RET registry) identified 165 patients with RET-rearranged lung cancers and has recently published the resultant data.39

Several multikinase inhibitors have shown activity and clinical benefit with RET-rearranged lung adenocarcinomas, which raises the question of whether this activity might be related to VEGF inhibition solely, as these drugs have shown increased response rates with unselected lung cancers after platinum-based chemotherapy.40,41 Dose reductions, likely related to off-target toxicities due to concomitant inhibition of non-RET kinases, prevent the delivery of optimal dosage. In addition, RET-rearranged lung cancer may also harbor concomitant genetic alterations that can decrease the likelihood of response to available RET inhibitors.

We eagerly await the new specific RET inhibitors, which, encouragingly, have less off-target toxicity and more potency. Recent advances in diagnostics should facilitate the identification of patients who will potentially benefit. Unbiased approaches using next-generation sequencing, including whole-genome sequencing, sequencing after capture of selected regions of RNA or DNA encompassing the relevant breakpoints in RET, or transcriptome sequencing of RNA, may be the best methodologies for the detection of RET chromosomal rearrangements in lung adenocarcinoma.42 This approach supports the conduct of “basket trials,” early-phase studies of novel targeted therapies specifically in patients whose tumors harbor the putative oncogenic target.

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Financial disclosures: The authors report that they have no relevant financial relationships to disclose.

References


Precision Medicine and Targeted Therapies for Gastric Cancer and Other GI Malignancies

Arturo Loaiza-Bonilla, MD, MSEd, FACP

Learning Objectives:
After completing this CME/CE activity, participants should be better prepared to:
• Describe the biomarker-based rationale behind GI cancer classifications
• Explain the development history leading to the approval of targeted therapies in GC and other GI cancers
• Discuss emerging targeted strategies and new indications for precision medicine in GI cancers

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This activity is designed to inform physicians about the current availability and use of targeted therapies and precision medicine in gastric cancer (GC) and other gastrointestinal (GI) cancers.

Target Audience:
This activity is directed toward medical oncologists, primary care physicians, nurses, and nurse practitioners who treat and/or manage patients with GC or other GI cancers. Surgical oncologists, radiation oncologists, pathologists, internists, fellows, physician assistants, and other health-care providers are also invited to participate.

Dates of certification: December 31, 2017, to December 31, 2018
Medium: Print with online posttest, evaluation, and request for credit

The American Journal of Hematology/Oncology® Editorial Board
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Disclosure: Grant/Research Support: Genentech/Roche, Pfizer, Puma Biotechnology Inc, and Novartis (clinical trial support contracted to the University of Southern California and MD Anderson Cancer Center); Consultant: Eisai, OncoPlex Diagnostics, Merck, and Novartis.

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Introduction
Gastric cancer (GC) is among the most common malignancies in the United States. In 2017, it is estimated that 28,000 new cases of GC will be diagnosed, accounting for 1.7% of all new cancers. It is further estimated that nearly 11,000 people will die of GC in the United States alone in 2017, accounting for nearly 2% of cancer-related deaths. The incidence of and death from GC has steadily decreased over the past half-century, decreasing from the most common cancer in the United States to the 15th most common. GC occurs most often in the elderly population, with a median age of diagnosis of 68 years. Although survival has increased, the percentage of patients surviving more than 5 years remains low, at just 30.6%. On a global scale, approximately 990,000 people are diagnosed with GC each year, of whom about 738,000 die from this disease, making GC the fourth most common cancer by incidence and the second most common cause by death.

Gastric cancers are solid tumors with complex genetic and environmental interactions that contribute to their initiation and progression. Most GCs (90%) are adenocarcinomas. Traditionally, GCs are divided into 2 main subtypes on the historical basis of the Laurén classification: intestinal and diffuse. The World Health Organization (WHO) also has a classification system that divides GC into papillary, tubular, mucinous, and poorly cohesive carcinomas.

In the era of precision medicine and next-generation sequencing (NGS), a solely histological classification of GC is insufficient to detail the complexity of disease. A comprehensive and biomarker-based classification system lends itself to better patient care. Both the Laurén and WHO classification systems allow for a better understanding of the biology of GC, but have limited clinical utility in guiding patient therapy due to the complex molecular heterogeneity of the disease.

A Genetic-Based Classification
Recently, several comprehensive studies have attempted to provide new approaches to subdividing GCs. Two systems, based on molecular markers, have been developed to complement currently used histological classifications.

One comprehensive analysis from The Cancer Genome Atlas (TCGA) evaluated 295 GC tumors, primarily from the United States and Western Europe. The TCGA analysis included somatic copy-number alterations (SCNAs), whole-exome sequences, RNA sequencing (including both messenger and microRNA), and DNA methylation analysis. A total of 4 molecular subgroups were identified. The first group, which accounted for 8.8% of GCs, was positive for Epstein-Barr virus (EBV) and had several other molecular commonalities. The second group, which accounted for 21.7% of GCs, was microsatellite instability–high (MSI-H). The third group of patients, accounting for 19.7% of disease, had a low level of SCNAs and was considered genomically stable (GS). The final group, accounting for 49.8% of disease, was characterized by a high levels of SCNAs and chromosomal instability (CIN).

The EBV subtype, as identified by TCGA, regularly displayed recurrent PIK3CA mutations, DNA hypermethylation, high levels of PD-L1 and PD-L2 expression, and amplification of JAK2 proteins, as well as rare TP53 mutations. The MSI subgroup, besides displaying high levels of MSI, is often hypermutated, including oncogenic driver genes such as KRAS or NRAS. The GS subtype, more common in younger patients, is characterized by mutations in the RHOA gene. Finally, the CIN subtype displays high levels of aneuploidy as well as receptor tyrosine kinase (RTK) activation, including EGFR, VEGFR, and MET.

A second GC classification was performed by the Asian Cancer Research Group (ACRG), which studied GCs in a Korean population. While ultimately similar to TCGA, ACRG did not identify a distinct EBV-positive subtype, but rather noted a group of GCs defined by an expression signature of epithelial-to-mesenchymal transition (EMT). The ACRG identified 4 groups of GCs: First, the microsatellite-stable (MSS)/EMT subgroup accounted for 15.3% of GCs; second, a MSS/TP53-mutation–positive group accounted for 35.7% of GCs; third, an MSS/TP53-mutation–negative group accounted for 26.35% of GCs; and fourth, an MSI group accounted for 22.7% of GCs. A summary of alternative molecular marker divisions and their incidence in patients is presented in the Table.

<table>
<thead>
<tr>
<th>Subtype</th>
<th>% of GCs</th>
<th>Subtype</th>
<th>% of GCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBV</td>
<td>8.8</td>
<td>MSS/TP53</td>
<td>35.7</td>
</tr>
<tr>
<td>GS</td>
<td>19.7</td>
<td>MSS/EMT</td>
<td>15.3</td>
</tr>
<tr>
<td>MSI</td>
<td>21.7</td>
<td>MSI</td>
<td>22.7</td>
</tr>
<tr>
<td>CIN</td>
<td>49.8</td>
<td>MSS/TP53</td>
<td>26.3</td>
</tr>
</tbody>
</table>

ACRG indicates Asian Cancer Research Group; CIN, chromosomal instability; EBV, Epstein-Barr virus; EMT, epithelial-to-mesenchymal transition; GC, gastric cancer; GS, genomically stable; MSI, microsatellite instability; MSS, microsatellite-stable; TCGA, The Cancer Genome Atlas.
Targeted Therapies in Gastric Cancer

Trastuzumab

Trastuzumab is a humanized monoclonal antibody (mAb) that interferes with human epidermal growth factor receptor type 2 (HER2). Trastuzumab has become a staple of HER2-positive breast cancer care, and its use has expanded to GCs.\(^9\)

The Trastuzumab for Gastric Cancer (ToGA) trial (NCT01041404) was an open-label, placebo-controlled, phase III trial that randomized patients with HER2-positive GC or gastroesophageal junction (GEJ) cancer to receive a chemotherapy regimen of capecitabine/cisplatin or fluorouracil (5-FU)/cisplatin with or without trastuzumab. A total of 594 patients were randomly assigned between the 2 groups.\(^10\)

The median overall survival (OS) was 13.8 months (95% CI, 12-16) in patients who received trastuzumab in combination with chemotherapy compared with 11.1 months (95% CI, 10-13) in patients who received chemotherapy alone (HR, 0.74; 95% CI, 0.60-0.91; \(P = .0046\)). Median progression-free survival (PFS) for patients receiving trastuzumab was 6.7 months (95% CI, 6-8) compared with 5.5 months (95% CI, 5-6) for patients receiving chemotherapy alone (HR, 0.71; 95% CI, 0.59-0.85; \(P = .0002\)). The objective response rate (ORR) for patients receiving the trastuzumab combination was 47% compared with 35% for those receiving chemotherapy alone.\(^10\)

The most common adverse events (AEs) of any grade for patients receiving trastuzumab were nausea (67%), vomiting (50%), and neutropenia (53%), and did not differ significantly from patients receiving chemotherapy alone. Across either treatment group, 68% of patients experienced grade 3/4 AEs. The most common grade 3/4 AEs for patients receiving the trastuzumab combination were neutropenia (27%), anemia (12%), diarrhea (9%), and nausea (7%).\(^10\)

The results of this trial led to the 2010 approval of trastuzumab in combination with cisplatin and either capecitabine or 5-FU for patients with HER2-positive, metastatic GC or GEJ cancer who have not received prior treatment for metastatic disease.\(^11\)

Trastuzumab is being investigated in other disease types, including colorectal cancer (CRC). Early in 2017, a biosimilar, trastuzumab-dkst, was approved under the same indication as the reference product.\(^12\)

Ramucirumab

Another target in GC is VEGFR2, which plays a critical role in the pathogenesis and progression of disease. VEGFR2 is a transmembrane RTK that binds to other VEGF proteins, causing increased cell proliferation, migration, and inflammation. Approximately 50% of GCs express VEGF, with VEGFA and VEGFD overexpression being associated with a poor prognosis. Ramucirumab is a mAb VEGFR-2 antagonist shown to be efficacious in GC cancers.\(^13\)

The first phase III trial establishing ramucirumab in GC was the REGARD trial (NCT00917384), in which 355 patients with advanced GC or GEJ cancer that had progressed on first-line platinum-based or fluoropyrimidine-containing chemotherapy were randomized 2:1 to receive best supportive care plus ramucirumab monotherapy or placebo.\(^14\)

Although the best ORR was low for patients receiving ramucirumab (4%), the rate of stable disease was 45% compared with 21% for patients receiving placebo; the disease control rate (DCR) was 49% and 23%, respectively.\(^14\) Patients receiving ramucirumab had a median OS of 5.2 months compared with 3.8 months in the placebo arm (HR, 0.776; 95% CI, 0.603-0.998; \(P = .047\)). The survival benefit of ramucirumab was reported to have remained unchanged after multivariable adjustment for other prognostic factors. Six-month PFS also was improved for patients receiving ramucirumab, to 42% versus 32%.\(^14\) Rates of hypertension were higher for patients in the ramucirumab group (16%) compared with the placebo group (8%), whereas rates of other AEs were primarily similar between groups, 95% and 88%, respectively.\(^14\)

Next, the phase III RAINBOW study (NCT01170663) of 665 patients with advanced GC or GEJ cancer who had progressed on or within 4 months of first-line chemotherapy randomized them 1:1 to receive paclitaxel in combination with ramucirumab or paclitaxel with placebo.\(^15\) Median OS was reported to be 9.6 months (95% CI, 8.5-10.8) in the paclitaxel-plus-ramucirumab arm versus 7.4 months (95% CI, 6.3-8.4 months) in patients receiving the placebo (HR, 0.807; 95% CI, 0.678-0.962; \(P = .017\)). Median PFS also was improved for patients receiving the ramucirumab combination, 4.4 months compared with 2.9 months for patients receiving placebo (HR, 0.635; 95% CI, 0.536-0.752; \(P < .0001\)). The ORR for patients receiving ramucirumab was 28% versus 16% for those receiving placebo. In addition, the DCR was 80% and 64% for ramucirumab and placebo, respectively.\(^15\)

Common grade \(\geq 3\) AEs for patients receiving the ramucirumab/paclitaxel combination included neutropenia (41%), leukopenia (17%), hypertension (14%), fatigue (12%), anemia (9%), and abdominal pain (6%). All listed AEs, except anemia, were significantly higher in patients receiving ramucirumab than those receiving placebo.\(^15\)

Based on the results from the REGARD trial, the US FDA approved ramucirumab as a single agent for patients with GC or GEJ cancer in April 2014.\(^16\) Then, in November 2014, following the results of the RAINBOW study, ramucirumab was approved in combination with paclitaxel for the treatment of GC and GEJ cancer following failure of first-line treatment.\(^16,17\) The European Medicines
Agency (EMA) also approved ramucirumab as monotherap- or in combination with paclitaxel for this patient population in September 2014.13

**Emerging Targets**

Inhibition of checkpoint proteins, specifically PD-1 and its ligand PD-L1, have been an increasing focus of immunotherapy strategies across tumor types. The PD-1 / PD-L1 axis works primarily to suppress an overresponse of effector T cells as a part of the immune system’s defense against self-cannibalism.18

In May 2017, the FDA approved pembrolizumab for all patients with metastatic or unresectable MSI-H or mismatch repair-deficient solid tumors, the FDA’s first tissue- or site-agnostic approval.19 Checkpoint inhibitors increasingly have become an option in GC.

Results of the phase II SWOG 1406 (NCT02164916) trial were presented at the American Society of Clinical Oncology (ASCO) Gastrointestinal Cancers Symposium in January 2017. For patients with metastatic CRC who have mutations in BRAF V600, the addition of the BRAF inhibitor vemurafenib to cetuximab and irinotecan significantly improved PFS. The trial met its primary endpoint, improving median PFS from 2.0 months with cetuximab/irinotecan to 4.4 months with the addition of vemurafenib. Grade 3/4 AEs were significantly higher in the experimental arm and included neutropenia (28%), anemia (13%), and nausea (15%).20

Other targeted therapies under investigation include binimetinib, a MEK inhibitor, in combination with BRAF and EGFR antibodies; cobimetinib, another MEK inhibitor, in combination with PD-L1 inhibitor atezolizumab; and claudiximab, which targets claudin 18.2.13

For more information on precision medicine in GC and GI cancers, as well as insights into the future of targeted therapies, please see our interview with Dr Loaiza-Bonilla below.

**What is “precision medicine,” and what role can it have in treating patients with gastrointestinal [GI] cancer?**

**Dr Loaiza-Bonilla:** Precision medicine and precision oncology are basically the implementation of a high level of evidence—disease-specific and biomarker-driven evidence—to inform either diagnostic or treatment recommendations for improved and optimized cancer care. The purpose of precision medicine in the field of oncology is to find the right options at the right time for the right patient. I think throughout the general field of oncology, the use of personalized approaches can improve outcomes, lead physicians to the best diagnosis, and result in the identification of the right biomarkers, which, when targeted, lead to better responses. Certainly, GI cancers are not an exception to this approach.

**What is the role of next-generation sequencing [NGS] in precision medicine? What do physicians need to know about NGS?**

When we’re looking at diagnostics in oncology and talking about precision medicine, it’s important to recognize that, in the past, pharmaceutical companies and clinical trials did not limit patient populations based on molecular alterations. Their approach focused mainly on histology and a “one size fits all” approach. Over time, we found that there was a change in the treatment paradigm from the phenotype to the genotype, where we’re looking at the specific biomarkers on tumors to determine treatment. The best way to find these biomarkers is by the use of NGS techniques.

With the advent of targeted therapies and clinical trials run by cooperative groups and pharmaceutical companies, we have now begun to focus on niche subgroups of patients who carry a specific molecular alteration. They use these basket trials in which specific biomarkers determine participation, regardless of histology. Next-generation sequencing plays a key role in that. The type of vendors available for NGS depends on the institution. For example, large cancer centers often have their own panels for the identification and validation of markers for their own targeted therapies being researched. For community-based clinics, there are also commercially available companies that perform NGS, either through liquid biopsies or traditional tissue testing.

Commercially available tumor-profiling services can complement this local tumor testing, and help to find the right treatment options for patients for whom no clinical trial options could be found. Hopefully, these technologies continue to become more available, affordable, and reimbursable, once we show their value in large subsets of patients. Something of utmost importance is to develop a system that assists clinicians in ordering these tests when the time is right, and then guide them in using and operationalizing these results to the benefit of the cancer research field, patients, and public health.

**In 2014, The Cancer Genome Atlas identified 4 subtypes of GI cancer. Can you discuss the importance of this study and the main takeaways?**

The Cancer Genome Atlas (TCGA) is a widespread effort that is done across multiple types of malignancies, aiming to elucidate any specific biomarkers that characterize different tumors. In GI cancers, particularly in gastric cancer [GC], there was a finding that not all GCs are equal. In the past, we had a classification system called the Laurén classification, which grouped GCs by diffuse type and intestinal type. Now, with the work of TCGA, we have a new and useful classification that can help explore thera-
pies in specific patient subpopulations whose tumors have determined biomarker abnormalities.

TCGA identified 4 distinct groups of GI cancer: Group 1, about 9% of patients, are positive for Epstein-Barr virus; Group 2, about 22% of patients, are microsatellite instability–high [MSI-H], with a tendency to accumulate mutations in multiple sequences of DNA; Group 3, about 20% of patients, have a low level of copy number alterations and are considered genomically stable [GS]; and Group 4, about 50% of patients, are chromosomally unstable, which may correlate with tumor mutation burden [TMB].

In 2015, the Asian Cancer Research Group [ACRG] proposed a separate classification system. Can you discuss this system and the differences between the 2 systems? Is there 1 that we should use over the other?

Certainly I’ll discuss it. I believe that both efforts, the TCGA and the ACRG classifications, are useful—equally useful—and there is a significant level of clinical correlation between them. The 2 systems were just used in different populations during different periods of time, but, in the end, many of those groups overlapped. For example, the ACRG had a subtype called the “microsatellite stable with markers of epithelial-to-mesenchymal transition group,” which is similar to the third group of TCGA, the GS group. More than 80% of cases of this subtype were stage III/IV with diffuse type histology by Laurén classification. So, putting it into perspective, it’s important to differentiate the molecular subtypes in GC, which may tailor treatment based on specific alterations to improve outcomes in this difficult-to-treat cancer. Either classification is useful, and gives us insights into the different subgroups of GC that we should try to tackle.

Trastuzumab, a monoclonal antibody [mAb], is approved to treat patients with HER2-positive metastatic GC or gastro-esophageal junction cancer. How do you use trastuzumab in your patients, and what does this indication tell us about the future of precision medicine in GI cancer?

Based on the results of the ToGA trial, trastuzumab is now routinely added to first-line chemotherapy in patients with advanced or metastatic GC with HER2 overexpression by immunohistochemistry, or in some cases, by ERBB2 gene amplification detected by NGS. The chemotherapy backbone in the pivotal trial was cisplatin and capecitabine or fluorouracil (5-FU). However, the addition of trastuzumab to other combination chemotherapy regimens such as EOX [epirubicin, oxaliplatin, capecitabine], DCF [docetaxel, cisplatin, and 5-fluorouracil], and FOLFOX [folinic acid, fluorouracil, and oxaliplatin] that are accepted as alternative standards of care has not been studied in a prospective, randomized fashion.

Findings from the German noninterventional observational study HERMES studied trastuzumab in combination with cisplatin and 5-FU or capecitabine, as well as other regimens such as oxaliplatin and docetaxel. Although most patients did not receive the regimen described in the ToGA trial, the median progression-free survival (PFS) was comparable at 6.8 months.

Further studies of trastuzumab in combination with other regimens are ongoing, and this was 1 of the first targeted therapies to use in GI malignancies, prompting the search for additional biomarkers, and to understand further the drivers of resistance (escape pathways) and how to overcome resistance.

For example, there are some compelling data from the combination of trastuzumab plus lapatinib, which achieved positive results in patients with heavily pretreated, HER2-positive, metastatic colorectal cancer [CRC], according to the final results of the phase II HERACLES-A trial. The HERACLES-B trial is evaluating pertuzumab and ado-trastuzumab [T-DM1], and HERACLES-RESCUE is looking at T-DM1 monotherapy in metastatic CRC that has progressed on lapatinib and trastuzumab in HERACLES-A. It should be very interesting to learn how these trials perform in light of other biomarkers and therapies, such as MSI status and immunotherapy.

Ramucirumab, another mAb, targets VEGFR2. How has this precision medicine had an impact on how you treat patients with GI cancer?

Following the success that we just discussed about the ToGA trial and trastuzumab, there was a lot of hype looking for new biomarkers. Ultimately, that led to agents targeting the VEGF pathway. There were initial efforts with bevacizumab in the AVAGAST trial that unfortunately hampered initial enthusiasm, but also led to finding a subset of patients who might respond. The analysis also suggested that the difference when assessing overall survival data in GC could be explained by the higher use of third-line therapy following study discontinuation in Asian patients compared with non-Asian patients—nearly 70% vs nearly 40%.

Ramucirumab, by specifically binding to VEGF2, prevents all known VEGFs from binding to VEGF2, and therefore could lead to more complete inhibition of angiogenesis than agents directly binding to a single VEGF, demonstrating a significant survival benefit in the second-line setting. This was reported in the phase III REGARD trial, which investigated the agent as monotherapy, and the phase III RAINBOW trial, which investigated ramucirumab in combination with paclitaxel. The FDA and European Medicines Agency approval of this anti-angiogenic agent has led to its incorporation in the vast majority of second-line therapy for my patients.

What important considerations should be made in terms of combinations or sequencing for patients with GI cancer?
First, it’s important to understand which mutations are the driving mutations. What biomarkers are we targeting in the precision medicine field? Second, we need to make rational decisions. A combination is either aimed at improving the previously detected signal when we use a single agent, or it’s aimed at overcoming a resistance that was found in a specific drug. It’s important to understand that some biomarkers may change over time; a patient’s HER2 status may change, as well as many other biomarkers.

So, for example, a patient with CRC may show up with a RAS wild-type phenotype, and after you expose the patient to EGFR inhibitors, they may develop a resistance that is driven by a secondary mutation. In that area, then, we need to use agents that overcome that mutation resistance and potentially re sensitize the tumor. Those are the key questions that we need to formulate in the future for the management of these patients.

An exciting advancement, in multiple cancer types, is inhibition of the PD-1/PD-L1 pathway. What role does checkpoint inhibition have in GI cancers?

Immune checkpoint inhibitors are the “new kid on the block.” So, following very exciting successes in melanoma and lung cancer, basket trials based on PD-L1 positivity demonstrated that some of these patients had significant responses in GI cancers. When they looked further into why this response was happening, they noted that patients with a history of either Lynch syndrome or MSI, as well as those with high TMB (tumor mutational burden), are able to produce large amounts of epitopes that the immune system detects, and where potential biomarkers are predictive of response to PD-1 and PD-L1 inhibitors.

Recently, the FDA granted accelerated approval to pembrolizumab for adult and pediatric patients with unresectable or metastatic MSI-H or mismatch repair-deficient (dMMR) solid tumors that have progressed following prior treatment, and who have no satisfactory alternative treatment options, or with MSI-H or dMMR CRC that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan.

In addition, the FDA granted accelerated approval to nivolumab for the treatment of patients 12 years and older with dMMR and MSI-H metastatic CRC that has progressed following treatment with fluoropyrimidine, oxaliplatin, and irinotecan.

Interestingly enough, compared with other cancers, such as lung cancer and melanoma, where the PD-L1 expression may predict response to treatment, in GI malignancies that has not been the case. We’ve seen multiple clinical trials of combination agents, actually sometimes using CTLA4 inhibitors plus immune checkpoint inhibitors or monotherapy with immune checkpoint inhibitors, where PD-L1 overexpression has not been a predictive biomarker of response. So, if these patients are going to respond, they will respond across the board. Something that will be interesting to find out is whether TMB has anything to do with the responses these patients showed regardless of PD-L1 overexpression. Additional studies will reveal those answers.

Other future potential targets in precision medicine include receptor tyrosine kinases, RAS, and PI3 kinase. Can you talk about the early-stage development of these targets in GI cancer so far?

Overall, the results of multiple phase II clinical trials targeting alterations of MET, EGFR, PI3 kinase, and the always elusive RAS mutations, have been quite disappointing in showing survival advantage in GI cancer. However, there are some promising results in certain subsets of patients. So, quite simply, BRAF V600-mutated CRC seems to be the most recent bearer of good news. It represents about 7% to 15% of CRCs that are a difficult-to-treat subtype. But there are some encouraging emerging data out from a phase II study presented by SWOG at the 2017 ASCO GI Cancers Symposium, which showed that patients who had the combination of vemurafenib, a BRAF inhibitor, and cetuximab plus irinotecan had improved PFS. That trial met its primary endpoint, and we will likely follow vemurafenib on further studies.

More recently, at the European Society for Medical Oncology [ESMO] 2017 annual meeting, the phase III BEACON CRC study showed that binimetinib (a MEK inhibitor) plus encorafenib (a BRAF inhibitor) and cetuximab in patients with BRAF-mutated disease and at least 2 prior regimens showed significant improvement in response rate, with good tolerability and good outcomes.

Personally, I have had success in the management of BRAF V600E-mutated cholangiocarcinoma using a combination of BRAF/MEK inhibitors. Isocitrate dehydrogenase 1 and 2 (IDH1/2) may also be targetable with specific inhibitors or with cyclin inhibitors. Results from large basket studies, such as the TAPUR study and NCI-MATCH, will be crucial to identify which patients may be the most likely to respond to the currently available targeted therapies.

How will oncologists stratify treatment strategies based on the subtypes discussed earlier?

Given the ever-increasing number of biomarkers and therapies, it’s important to make sure that we follow guidance from emerging and strong-evidence data. Consensus guidelines from well-established groups such as ASCO, ESMO, and the National Comprehensive Cancer Network will be very valuable and important. Personally, I have always been an advocate of institutional or virtual genomic tumor boards, because those efforts have proven to help allocate patients who undergo biomarker testing and NGS, and improve utilization of drugs and outcomes.
patients in clinical trials will help us to find predictive signals of response, and will be essential in how we allocate these patients further. Continuous education and following guidelines and expertise is always essential, and aids collaboration between us physicians and pathologists.

References
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