Immunotherapy for Gynecologic Malignancies: The Way Forward
Christine H. Kim, MD, Russell J. Schilder, MD

The Role of Regional Perfusion for Locoregionally Metastatic Melanoma
Jonathan S. Zager, MD, FACS

Current and Future Roles of Molecular Profiling in Colorectal Cancer
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Case Study in Del(17p) Chronic Lymphocytic Leukemia

Challenges in the Treatment of Elderly Patients With Diffuse Large B-Cell Lymphoma

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Table of Contents

GYNECOLOGIC MALIGNANCIES

3 Immunotherapy for Gynecologic Malignancies: The Way Forward
  Christine H. Kim, MD, Russell J. Schilder, MD

Further understanding of tumor immune escape mechanisms has allowed targeting of specific immunosuppressive pathways that are ubiquitous among different tumor types, thus allowing the treatment of gynecologic malignancies to benefit from basic science and clinical research established in other solid tumors. Discovery of novel inhibitors targeting tryptophan metabolism, various immune checkpoint T cell receptors and their corresponding ligands, as well as other immunomodulatory agents using viral proteins have created exciting new treatment possibilities that harness a patient's own immune system to better recognize tumor cells.

MELANOMA

13 The Role of Regional Perfusion for Locoregionally Metastatic Melanoma
  Jonathan S. Zager, MD, FACS

Locoregional recurrence of melanoma is a spectrum of recurrent disease encompassing a true local recurrence which is in or near a scar from a previous melanoma wide excision, as well as macro satellite and in-transit metastases. Treatment options for locoregionally recurrent melanoma include surgical resection, local intratumoral injections, hyperthermic isolated limb perfusions (HILP), isolated limb infusions (ILI), topical therapies and laser ablations, and radiation and systemic therapies.

COLORECTAL CANCER

18 Current and Future Roles of Molecular Profiling in Colorectal Cancer
  Rona Yaeger, MD, and Leonard Saltz, MD

This review describes the role of molecular profiling of colorectal cancer, including its strengths and limitations in clinical practice today and future directions for molecular studies of colorectal cancer.

PER CONFERENCE HIGHLIGHTS

18th Annual International Congress on Hematologic Malignancies®

25 Case Study in Del(17p) Chronic Lymphocytic Leukemia

28 Challenges in the Treatment of Elderly Patients With Diffuse Large B-Cell Lymphoma

BREAST CANCER – CME

31 Best of the 31st Annual Miami Breast Cancer Conference®

The 31st Annual Miami Breast Cancer Conference convened surgical, medical, and radiation oncologists to foster awareness of state-of-the-art treatments in each therapeutic area. Highlights from some key presentations are provided here in a CME-certified enduring material sponsored by Physicians’ Education Resource®, LLC.
From the Editor

In this issue of The American Journal of Hematology/Oncology, we turn our focus to new developments in a number of cancers, starting with a look at immunotherapy, with “Immunotherapy for Gynecologic Malignancies: The Way Forward,” by Christine H. Kim, MD, and Russell J. Schilder, MD. Looking at metastatic melanoma, Jonathan S. Zager, MD, FACS, presents “The Role of Regional Perfusion for Locoregionally Metastatic Melanoma.”

An understanding of the genetic alterations in colorectal cancer has become crucial to improving patient outcomes, and in “Current and Future Roles of Molecular Profiling in Colorectal Cancer,” Rona Yaeger, MD, and Leonard Saltz, MD, look at the strengths and limitations of molecular profiling in colorectal cancer, both in terms of its role in clinical practice today and the direction of future studies.

This issue’s CME-accredited article covers highlights of the 31st Annual Miami Breast Cancer Conference®, from Physicians’ Education Resource®, LLC. The conference helps foster awareness of state-of-the-art treatments in surgical, medical, and radiation oncology in the field of breast cancer.

Finally, we highlight two discussions from the 18th Annual International Congress on Hematologic Malignancies®: Focus on Leukemias, Lymphomas, and Myeloma. In this issue we present a “Case Study in Del(17p) Chronic Lymphocytic Leukemia,” and “Challenges in the Treatment of Elderly Patients With Diffuse Large B-Cell Lymphoma.”

We hope you enjoy this issue, and please feel free to direct your feedback and comments to the editorial staff at moconnell@gotoper.com.

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Immunotherapy for Gynecologic Malignancies: The Way Forward

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Abstract
Further understanding of tumor immune escape mechanisms has allowed targeting of specific immunosuppressive pathways that are ubiquitous among different tumor types, thus allowing the treatment of gynecologic malignancies to benefit from basic science and clinical research established in other solid tumors. Discovery of novel inhibitors targeting tryptophan metabolism, various immune checkpoint T cell receptors and their corresponding ligands, as well as other immunomodulatory agents using viral proteins have created exciting new treatment possibilities that harness a patient’s own immune system to better recognize tumor cells.

Background
Recent advances in understanding the microenvironment of T cells and their intricate stimulatory and inhibitory interactions with other cells have allowed new immunomodulatory agents to be at the forefront of cancer therapy development. Until the last decade, most immunotherapeutic strategies had focused on stimulating immune effector cells with tumor-specific antigens or exogenous cytokines to activate the host’s immune system with limited benefit. In contrast, a more recent understanding of tumor immune escape mechanisms has allowed targeting of specific immunosuppressive pathways that are often present among different tumor types. These new immunomodulatory agents have yielded durable results in preliminary clinical studies. These agents are potentially useful in malignancies not traditionally thought to be responsive to immunotherapy. This review describes new immune checkpoint pathway inhibitors and other compounds with novel mechanisms of action that have shown clinical activity and may serve as the basis for new combination strategies in the treatment of gynecologic cancers.

Historically, certain gynecologic malignancies such as epithelial ovarian cancers and human papilloma virus (HPV)-associated cervical cancers have been considered immunogenic tumors. In ovarian cancer, cytotoxic T cells have demonstrated antitumor activity, and the presence of tumor-infiltrating lymphocytes (TIL) have been associated with improved survival. Despite encouraging laboratory findings, strategies to enhance antigen presentation to T cells with tumor-specific peptide vaccination, antigen-pulsed dendritic cells (DC), or antibodies targeting tumor antigens have been of limited clinical benefit. Understanding the mechanisms of immune regulation are essential to understanding how tumors are able to escape the host immune surveillance.

Role of IDO1 in Immune Tolerance
One mechanism of immune tolerance involves indoleamine 2,3-dioxygenase-1 (IDO1), an intracellular enzyme that catalyzes the rate-limiting step in metabolizing tryptophan, an essential amino acid. Prior to identifying IDO1, tryptophan 2,3-dioxygenase (TDO) was initially isolated in the liver and found to metabolize tryptophan. TDO is a liver-specific enzyme that regulates dietary tryptophan catabolism. IDO1, on the other hand, remains absent or inactive in cells of the immune system until activated or induced in macrophages and DC subsets by cytokines, particularly interferon-gamma (IFN-γ).

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Munn and colleagues demonstrated the biologic significance of the role of IDO1 in immune tolerance by demonstrating the fundamental importance of IDO1 for maternal-fetal immune tolerance in the placenta of pregnant mice. The activity of IDO1 in depleting the local placental microenvironment of tryptophan is critical to establishing maternal T-cell tolerance of fetal alloantigens.

Additional studies showed that IDO1-induced immune suppression not only by depleting tryptophan from the local microenvironment but also by accumulating immunosuppressive metabolites such as kynurenine. T cells undergoing antigen-dependent activation are extremely sensitive to local tryptophan concentrations such that a decrease in tryptophan can lead to effector T-cell cycle arrest and anergy. Downstream tryptophan metabolites, such as kynurenine, have also been shown to modulate natural killer (NK) cell cytotoxicity by selectively interfering with NK receptors and thereby modifying NK cell responses.

Uyttenhove and associates first demonstrated that the expression of IDO1 in tumor cells allowed the malignant cells to resist host immune rejection by preventing activation of alloreactive T-cells. Several studies further demonstrated that IDO1 expression is inversely correlated with the presence of TILs, suggesting IDO1 expression may be associated with poor prognosis due to IDO1-mediated TIL and/or NK suppression.

Increased IDO1 expression is associated with poor clinical outcomes in multiple solid tumors including melanoma, renal cell, colon, pancreatic, hepatic, and squamous cell carcinomas. Increased IDO1 expression in tumors of patients with gynecologic cancers has been correlated with a worse prognosis compared to those patients whose tumors have limited or negative IDO1 expression. Overexpression of IDO1 in patients with serous ovarian tumors has been correlated with paclitaxel resistance and poor survival outcomes. High IDO1 expression in one study was found in over 70% of patients with stage II-IV disease and was significantly correlated with low intratumoral CD8+ TILs. Additionally, in vitro studies have shown that IDO1-expressing ovarian cancer cells suppress T-cell proliferation. Increased IDO1 expression was correlated with a poor prognosis in endometrial and cervical cancer patients as well. Taken together, these findings as well as similar findings in other solid tumors suggest that IDO1 plays a key role in creating an immunosuppressive microenvironment potentially tolerant to tumors.

Preclinical mouse studies demonstrated IDO1 inhibitors could slow tumor growth and potentiate cancer chemotherapy. Inaba and co-investigators showed increased peritoneal metastases in mice bearing IDO1 transfected SKOV3 ovarian cancer xenografts compared to control mice bearing IDO1 negative xenografts. Administering an oral IDO1 inhibitor, 1-methyl-tryptophan (1-MT), abrogated the effect. Additionally, prolonged survival was found when IDO1 inhibition was combined with chemotherapy compared to chemotherapy alone.

A significantly more potent IDO1 inhibitor, INCB-24360, was investigated in a phase I study in patients with advanced malignancies; results were presented at the American Society of Clinical Oncology (ASCO) 2013 Annual Meeting. Most of the enrolled patients had colorectal (55.8%) cancer or melanoma (13.5%). Although no patients experienced a complete or partial response, 15 patients experienced stable disease for at least 8 weeks, and 8 patients experienced stable disease for at least 16 weeks. In 10 patients, the duration of INCB-24360 stable response exceeded that of their last prior therapy, including ipilimumab in 2 patients with melanoma. In this phase I study, the maximum tolerated dose (MTD) was not obtained; however, doses of ≥300 mg twice daily were able to inhibit IDO1 activity by more than 90% at all time points and were found to effectively normalize kynurenine plasma concentrations. Common adverse events (AEs) were grade 1-2 fatigue and gastrointestinal disturbances. Two patients had grade 3-4 ALT or AST elevations that did not appear to be dose related. The recommended phase II dosage is 600 mg orally twice daily.

There currently is an ongoing trial in women with ovarian, fallopian tube, or primary peritoneal cancer who have had a biochemical recurrence defined as two successively increasing CA 125 values that are greater than the upper limit of normal and without evidence of disease by RECIST 1.1 (NCT01685255). These patients are being randomized in a 1:1 fashion to receive oral medications INCB-24360 (600 mg) or tamoxifen (20 mg) twice daily.

**Targeting CTLA-4**

Immunotherapy involving T cells provides long-lasting tumor responses in patients with melanoma. However, less than 20% of patients achieve an objective response, and the addition of cytokine-based treatments were found to either increase the toxicity profile or to not be effective. To generate antitumor responses, T cells must be both specific for cancer cell antigens and have the potential to exert effector activity. Thus, in addition to T cell receptor (TCR) recognition of specific tumor antigens, a second costimulatory signal, such as the one between receptor CD28 and B7 ligand, is needed for full activation of T cells. This costimulation is tightly regulated through specific stimulatory and inhibitory receptor-ligand relationships. Recently, several inhibitory receptors and ligands found on antigen presenting cells (APCs), T cells, and tumor cells have been identified as targets for cancer immunotherapy, as they play critical roles in immune suppression within the tumor microenvironment.

These novel immunotherapy strategies targeting negative regulatory pathways in T-cell activation are considered immune checkpoint inhibitors. These checkpoint inhibitors interfere with endogenous T-cell regulation in order to prevent the development of immune tolerance to tumors. Ipilimumab was the first immune checkpoint inhibitor that the US Food and Drug Administration (FDA) approved for clinical use. Ipilimumab, a human monoclonal IgG1 antibody, binds and blocks inhibitory signaling mediated by cytotoxic T-lymphocyte antigen-4 (CTLA-4) found on T-cell surfaces. As the mechanism of...
action is not specific to one tumor type and because preclinical data support immunotherapy as a potential treatment for various malignancies, ipilimumab is actively being investigated as a treatment option for patients with prostate, breast, renal, and lung cancers, in addition to other tumor types including cervical cancer.43,44

CTLA-4 is a critical negative regulator of early T-cell expansion, opposing the actions of CD28 receptor co-activation when bound to B7-1 (CD80) and B7-2 (CD86) ligands (anergy).45 CTLA-4 induces inhibitory downstream T-cell receptor signaling while also upregulating CTLA-4 expression and competitively inhibiting CD28 co-activation.46 CTLA-4 is also expressed on CD25+FOXP3+ T regulatory cells (Treg) and is important to T reg function.47 CTLA-4 interactions occur more centrally at an earlier step of interaction between T cells and APCs in lymphatic tissue. Specifically, CTLA-4 blockade with ipilimumab leads to T-cell activation and intratumoral T reg depletion.47

In 2010, Hodi and co-investigators reported a landmark phase III trial in patients with recurrent unresectable melanoma. Patients were randomized to ipilimumab 3 mg/kg with or without gp100 peptide vaccine versus gp100 peptide vaccine alone. Although all patients received prior treatment, patients who received the ipilimumab with or without gp100 had a significant overall survival (OS) advantage of approximately 3.7 months (10.1, 10.2 months, respectively) to those in the gp100 control arm (6.4 months, hazard ratio [HR] = 0.68, P <.001).48

A subsequent phase III trial in patients with untreated melanoma comparing dacarbazine +/- ipilimumab showed improved OS in the regimen containing ipilimumab. Increased liver toxicity was noted, potentially due to additive or synergistic enhancement of known single-agent hepatotoxicity for each drug. The median OS in the ipilimumab-dacarbazine group was 11.2 months compared with 9.1 months in the dacarbazine-placebo patients (HR = 0.72, P = .006).49 One of the impressive findings of CTLA-4 blockade has been the durability of objective tumor responses that are found in approximately 10% of patients with melanoma. Monoclonal antibodies targeting programmed death protein-1 (PD-1) and programmed death ligand-1 (PD-L1), which are earlier in their development, seem to follow a similar pattern (discussed in the next section).

The most frequently reported AEs associated with treatment with ipilimumab were immune-related, grade 1-2, and primarily affected the skin (pruritis, rash), and gastrointestinal tract (diarrhea, nausea, vomiting, and colitis). A dose-dependent increase in immune-related AEs of any grade was seen with increasing dosages of ipilimumab. In the study by Hodi and colleagues, grade 3 or 4 immune-related AEs occurred in 10% to 15% of patients who received ipilimumab and resolved over a median time of 4.9 weeks (95% CI, 3.1 to 6.4 weeks).48 It is important to also be aware of endocrinopathies such as thyroiditis and hypophysitis that can develop when treating with immunotherapy agents. Most high-grade AEs were able to be medically treated and resolved in approximately 4 weeks.

GOG 9929 is an ongoing phase I study investigating the use of ipilimumab for the primary treatment of high-risk cervical cancer after chemoradiation (NCT01711515). Patients must have squamous, adenosquamous, or adenocarcinoma histology and at least stage IB2 or IIA disease with positive para-aortic lymph nodes or stage IIB and higher with positive pelvic and/or para-aortic lymph nodes. HPV contains immunogenic viral E6 and E7 oncoproteins that are capable of inducing an immune response in most immunocompetent patients. However, failure to generate an effective immune response in some women facilitates HPV persistence, which ultimately increases the risk of cervical cancer development.50,51 After enrolled patients are treated with pelvic and extended field radiation with concurrent cisplatin (40 mg/m²) weekly and intracavitary brachytherapy, dif-
ferent cohorts then receive increasing doses of ipilimumab to determine the MTD, feasibility of treatment, dose-limiting toxicities, and disease outcomes. A subcohort of patients will continue to be treated with an extended regimen for an extra 48 weeks, with a dose given every 12 weeks for 4 weeks. The underlying hypothesis of the trial is that chemoradiation would induce an antigen release in patients whose immune response would be boosted by receiving ipilimumab. There is a second National Cancer Institute (NCI) trial that involves administering single agent ipilimumab in patients with metastatic or recurrent HPV-related cervical cancer (NCT01693783).

**Anti-PD-1/Anti-PD-L1 Inhibitors**

While CTLA-4 is involved in early T-cell activation in lymphatic tissues, PD-1 receptor signaling functions in regulating T cell activation in peripheral tissues (Figure 3). PD-1 is an immunoinhibitory receptor expressed on numerous cell types that have had long-term exposure to antigens including activated T cells, Treg, activated B cells and NK cells (Figure 4). The primary ligand of PD-1, PD-L1 (also known as B7-H1 or CD274), is frequently expressed within the tumor microenvironment, including tumor cells and tumor-infiltrating macrophages. PD-1/PD-L1 interactions decrease the risk of collateral tissue damage by activated T cells.52-53 On the other hand, PD-L2 (also known as B7-DC or CD273) is the second ligand of the PD-1 receptor and is restricted largely to APCs.54 The interaction of PD-1 with its two ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC), occurs mainly in peripheral tissues within the tumor microenvironment, leading to apoptosis and downregulation of T-cell effector function.55 Tumors expressing PD-L1/L2 have been found to suppress TILs by activating PD-1/PD-L1, L2 interactions.56 Targeting these interactions with therapeutic antibodies against PD-1/PD-L1 enhanced the T-cell response and stimulated antitumor activity.57

The first anti-PD-1 inhibitor to be evaluated was nivolumab, a human IgG4 monoclonal inhibiting antibody directed against the PD-1 protein.58 A phase I study in patients with selected advanced solid tumors showed that nivolumab was tolerable and effective with an objective response rate of 16% to 31% in heavily pretreated patients across diverse tumor types. Also notable was the durability of objective responses for >1 year after treatment. These results demonstrated that immunotherapy via PD-1 blockade could be expanded beyond targeting usual immunogenic tumor types, such as melanoma and renal cell cancer, to include treatment-refractory metastatic non-small cell lung cancer (NSCLC), particularly squamous cell carcinoma. These unexpected findings emphasized the possibility that any tumor type could be immunogenic with the appropriate immune activation.58 Similar to findings with ipilimumab, some patients experienced apparent progression or stable disease prior to ultimately responding to therapy, and responses have been observed with re-induction therapy.59 Measuring objective responses to immunotherapeutic agents has proved to be quite different from measuring responses to traditional cytotoxic chemotherapeutic agents. Thus, Wolchok and colleagues have summarized new immune-related response criteria that are considered an appropriate alternative to traditional methods for measuring objective responses mediated by these new immunomodulatory agents.60

Although AEs and efficacy with ipilimumab seem to be dose dependent, this correlation was not observed in patients treated with nivolumab, which may be explained by high receptor-antibody occupancy even at smaller doses.61 In 2 clinical trials, common AEs associated with anti-PD-1 blockade included fatigue (30%), rash (21%), pruritus (21%), diarrhea (20%), and myalgia (12%), with some rare serious AEs including pneumonitis (4%) and interstitial nephritis (4%).62,63

Two anti-PD-L1 inhibitory antibodies, BMS-936559 and MPDL3280A, have been clinically investigated. These agents are thought to function by specifically blocking PD1/PD-L1 signaling. Unlike PD-1 antibodies, PD-L1 antibodies spare potential interactions between PD-L2 and PD-1, but additionally block interactions between PD-L1 and CD80.64 The therapeutic significance of these particular interactions remains to be determined.

**FIGURE 3. Simultaneous Inhibition of CTLA-4 and PD-1 Pathways**

CTLA-4 acts to dampen the immune response at the level of the APC and the T-cell, thus decreasing the early activation of T-cells. PD-1, on the other hand, helps modulate T-cell activity in peripheral tissues via its interactions with PD-L1 and PD-L2. Since the two pathways act at different points in the immune response, therapeutically blocking both pathways with ipilimumab and nivolumab, monoclonal antibodies targeting CTLA-4 and PD-1 respectively, may have a synergistic effect.
A multi-institutional phase I study showed that BMS-936559 was tolerable and clinically active across multiple advanced tumor types. Blocking the immune inhibitory ligand PD-L1 with a monoclonal antibody produced both objective tumor regression with an objective response rate (ORR) of 6% to 17% and a durability of response across tumor types in these heavily pretreated patients. Anti-PD-L1 blockade generated 1 response in 17 (6%) enrolled patients with recurrent ovarian cancer. Also the 10% ORR was observed in patients with advanced NSCLC who received anti-PD-L1 therapy.

Although the 2 studies targeting anti-PD-1 and anti-PD-L1 are similar in patterns of clinical activity, the molecular interactions involved are not identical. For example, PD-L1 exerts inhibitory signals to T cells through PD-1 and B7-1. Thus, an antibody that specifically blocks PD-L1 would inhibit the interaction between PD-1 and its two ligands PD-L1 and PD-L2. However, PD-L1 would be able to send inhibitory signals through B7-1. In contrast, an antibody only directed at PD-L1 would block the inhibitory signals through PD-1 and B7-1, but PD-L2 would still be available to bind to PD-1 (Figure 5). The latter interaction has been found to downregulate T cell responses in vitro and in vivo. As immune checkpoint inhibitor research continues, the potential benefit of combining immunotherapeutic agents is being considered. Although monotherapeutic approaches to PD-1 blockade have shown some success, preclinical models indicate that combination therapies may generate greater clinical impact. For example, when combining ipilimumab with nivolumab, more rapid and greater magnitude responses were seen in patients treated with the combination regimen compared to that seen with either agent alone with up to 15% grade 3 or 4 toxicity depending on the type of AE.

Application of Lm-LLO for HPV-Associated Disease

HPV-associated cervical cancer is one of the most well-established associations in medicine where an infection with a virus is the cause of malignancy. Normal cell cycle regulation becomes disrupted when HPV oncoprotein E6 complexes with the tumor inhibitor gene p53, and HPV oncoprotein E7 complexes with the tumor suppressor protein retinoblastoma (pRb). These events lead to genomic instability and subsequent neoplasia. Immunologic activation of the HPV proteins expressed by transformed...
cells have been associated with increased numbers of CD8+ T cells and a high ratio of CD8+ T cells to FOXP3+ Tregs. A similar therapeutic change in the ratio of CD8+ TILs to Tregs has been seen after the administration of *Listeria monocytogenes* protein listeriolysin O (*Lm*-LLO) in various models.76,77 Studies have also demonstrated that bioengineered *Listeria monocytogenes* (*Lm*) is a potent vector in both infectious diseases and when applied to cancer immunotherapy.78-80 *Lm*-LLO-E7 (ADXS11-001) is a live attenuated *Lm* vector that secretes an antigen-adjuvant (*Lm*-LLO) fused to HPV16-E7.81 *Lm*-LLO-E7 induces E7-specific cytotoxic T cells and mature dendritic cells while decreasing intratumoral regulatory T cells and inhibiting angiogenesis.81

A phase I study investigating the safety and feasibility of ADXS11-001 was performed in 15 patients with previously treated metastatic, recurrent, or refractory cervical carcinoma who had failed chemotherapy, radiotherapy and/or surgery.84 Patients in the first 2 dose levels of 1 x 10<sup>9</sup> CFU and 3.3 x 10<sup>9</sup> CFU experienced a tolerable safety profile. Dose-limiting toxicities of grade 2 hypotension occurred in the 1 x 10<sup>10</sup> CFU group within hours after receiving the *Lm*-LLO-E7 infusion, requiring therapeutic intervention and resulting in study discontinuation as per protocol. Patients were given ampicillin to clear the *Lm* vector. No patients manifested any serious symptoms of *Lm* infection. All 15 patients experienced AEs during the study with the most common being pyrexia (100%), vomiting (60%), chills (53%), headaches (53%), anemia (53%), nausea (47%), tachycardia (47%),...
and musculoskeletal pain (28%). Six (40%) patients experienced grade 3 toxicities considered related to receiving Lm-LLO-E7: 3 (20%) were related to pyrexia, 2 (13%) had significant transaminitis, and 1 (7%) fatigue. These toxicities resolved in the first 12 hours after treatment. No drug-related grade 4 AE occurred. In this heavily pre-treated population, there was 1 (7%) patient who had a partial response and 7 (47%) patients who experienced stable disease. Additional phase II studies are currently active or will be initiated in the near future.

Conclusions
The future clinical application of immunotherapy in gynecologic malignancies is upon us. Several trials with immune checkpoint inhibitors should start late this year in ovarian and cervical cancers in response to the recent mass solicitation by the Cancer Therapy Evaluation Program (CTEP) of the NCI. With increased understanding of the tumor microenvironment and the complex immunoregulatory interactions between tumor cells and the host immune system, clinical trials involving immune checkpoint inhibitors and other immune regulating agents in gynecologic cancers are already under way (Table).

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Disclosure: Drs Kim and Schilder have no relevant financial conflicts of interest to disclose.

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Abstract
Locoregional recurrence of a melanoma is a spectrum of recurrent disease encompassing a true local recurrence which is in or near a scar from a previous melanoma wide excision, as well as macro satellite and in-transit metastases. In the most recent edition of the American Joint Committee on Cancer (AJCC) staging system (AJCC 7th edition), macro satellitosis and/or in-transit metastases are grouped together in the nodal (N) staging classification as stage IIIB (without past or present regional nodal disease) or stage IIIC (with past or present regional nodal disease). Ideally, these patients should be discussed in a multidisciplinary tumor board type setting in order to review all potential treatment options that can be tailored to the individual patient. This article focuses on the role of regional perfusion therapy for locoregionally metastatic melanoma.

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Initial Work-Up
One of the key aspects in the decision-making process when faced with a patient with a locoregional recurrence (LR) from melanoma is, can all the disease be removed? The initial workup of patients with documented LR should include full body imaging with either a full body PET/CT fusion or CT of the chest/abdomen/pelvis and extremities, as well as dedicated imaging of the brain (CT or MRI). We prefer a full body PET scan, as this imaging modality includes the extremities and can help identify smaller volume in-transit disease in an extremity or truncal that routine CT imaging may miss. A study by Beasley et al explored the clinical utility of PET/CT fusion in patients with AJCC stage IIIB or IIIC extremity melanoma.3 Although this study looked specifically at patients after ILI for extremity in-transit metastases and the utility of restaging PET/CT scans, the authors concluded that PET/CT appears to identify a subgroup of patients after ILI that have residual or recurrent disease not seen on exam that would potentially be surgically resectable.1 The same principle goes for initial staging, where PET/CT often gives a clear picture of the burden of disease in the limb that might not otherwise be appreciated on physical exam or CT imaging of chest/abdomen and pelvis. It should be noted that micro satellitosis is a diagnosis seen on pathology with separate nodules near the primary tumor under the microscope, not clinically identified, and is part of the key pathologic factors reported. For the purposes of this paper, we will be addressing treatment options for macro satellitosis—or clinically obvious satellitosis.

Surgical Resection
When contemplating surgical resection of isolated LR metastases, one has to ask whether the entire recurrence/tumor can be removed safely, with minimal morbidity, and the patient rendered no evidence of disease (NED)? Complete surgical resection in the absence of extensive disease is currently considered the standard of care by oncologists treating melanoma.2 In a large series by Dong et al looking at 648 patients with primary melanomas and true local recurrence in or around the primary excision scar, 124 (19%) had no further recurrences after surgical resection of the recurrent disease. A total of 196 (30%) developed another local recurrence, 178 (27%) developed further recurrences in the form of in-transit disease, and 150 (23%) eventually developed distant disease.4 The authors concluded that close to 20% of patients would benefit from surgical resection alone of a local recurrence. Over 50% of the patients in the series were alive at 5 years, many of whom who had additional recurrences beyond the initial LR and went on to get aggressive local, intra-arterial perfusion-based or systemic therapies.4

Jonathan S. Zager, MD, FACS
Regional Perfusion-Based Therapies

Isolated Limb Infusion (ILI)

ILI as originally described by John Thompson and colleagues from the Melanoma Institute of Australia (formerly Sydney Melanoma Unit) is the minimally invasive counterpart to HILP.3 ILI is essentially a low flow, acidic, hypoxic HILP.5,8 The chemotherapeutic agents most widely used in ILI and HILP are melphalan (ILI and HILP, United States and Europe) combined with tumor necrosis factor-alpha (TNF), (HILP, Europe) and dactinomycin (ILI, United States and Australia).9-13 A key difference between ILI and HILP is the initial access to the vasculature of the extremity. HILP is a complex, open surgical exposure and cannulation of the femoral/iliac or axillary/subclavian vessels, whereas ILI is performed with small caliber catheters and tourniquet isolation, vascular access is obtained via fluoroscopy with a percutaneous route via the groin (the contralateral groin if dealing with lower extremity in-transit metastases) and into the vessels feeding the affected limb.6,8,34 The leg is warmed with a warming blanket or liquid gel pad warmers prior to bringing the patient to the operating room and starting the ILI procedure. A pneumatic eschar tourniquet is placed on the proximal aspect of the limb to isolate the limb from the systemic circulation. The temperature is monitored through probes placed in the extremity, and once temperatures of greater than 37°C are achieved, the tourniquet is inflated (300 mm Hg for lower and 250 mm Hg for upper extremity ILIs), the arterial and venous catheters are hooked up to the circuit, and infusion of chemotherapy is begun.5,8,15-17 Like HILP, the chemotherapy is circulated through an extracorporeal circuit (preferably with an in-line heating source), then into the arterial catheter, and subsequently removed through the venous catheter–warmed and recirculated for 30 minutes in a closed circuit, low-flow perfusion. Reported results for ILI demonstrate complete response (CR) rates ranging from 23% to 44% and partial response (PR) rates from 27% to 56%. Median duration of response has been reported to range between 12 to 18 months.18-21 In a large, single-institution study conducted by Kroon et al, a CR was associated with a median duration of response of 24 months compared with 9 months if PR was achieved.18 Median disease-specific survival times were significantly longer if CR was achieved versus PR (42 vs 32 months, P = .04).34 It is important to note that in the Kroon 14-year experience, the results are mixed with early-stage (MD Anderson stage I and II) patients as well as some patients who received more than 1 ILI. Another large, single-institution trial by Wong et al looked at 54 initial and 23 repeat ILIs for LR extremity melanoma. The authors showed similar response rates to the Australian study by Kroon et al, with an overall response rate (ORR) after ILI for melanoma of 72% (CR of 32% and PR 40%) at the initial 3-month restaging assessment.6 An additional 10% of patients demonstrated stable disease. Beasley et al also reported on a large multi-institutional United States ILI trial and demonstrated an ORR of 64% (CR 31% and PR 33%) after initial ILI for LR extremity melanoma.21 The Beasley study also mentioned that the use of papaverine in the ILI circuit for vasodilation was associated with a better ORR (P < .001) but a higher risk of grade 3 toxicity (P = .001). Correction of the dose for ideal body weight (IBW) did not alter response rates but led to a marked reduction in toxicity (P < .001). Table 1.

Hyperthermic Isolated Limb Perfusion (HILP)

HILP as described by Creech et al in 1958 is a surgical method of isolating an affected extremity and treating the in-transit metastases using high-dose chemotherapy and bypassing systemic exposure.22 In HILP, the vessels at the root of the extremity are directly, openly cannulated, and the limb is isolated via a tourniquet and tying off of all collateral vessels as to limit systemic exposure to chemotherapy. The chemotherapy is then infused and circulated throughout the limb via a cardiopulmonary bypass machine, which is used to reheat and oxygenate the effluent. HILP is a high-flow procedure with flow rates greater than 400 cc to 500 cc per minute (ILI is about 100 cc per minute).23,24 After the 60-minute perfusion, the perfusate is washed out from the limb with 2 liters of a balanced electrolyte solution.13 As with ILI, regional hyperthermia (39°C to 41°C) is achieved, which has been shown to augment the effects of the delivered chemotherapy.15,25 Results from multiple studies show ORR after HILP to be 80% to 90% and CR rates to be as high as 60% to 70%.11,13,23,26 When tumor necrosis factor (TNF) is added to melphalan, improved CR rates of up to 60% to 80% have been reported, but the data are not consistent throughout the literature, with a large multicenter clinical trial in the United States (ACOSOG Trial Z0020) of HILP with melphalan versus melphalan plus TNF and a study in Europe using the same groups failing to show significant differences in CR rates between treatment groups.9,21 The ACOSOG Z0020 trial showed CR rates of only 25% and 26% in the melphalan alone and melphalan plus TNF groups, respectively.12 The ACOSOG Z0020 trial also reported a significantly higher number of complications in the melphalan plus TNF group versus melphalan alone (16% vs 4% grade IV adverse events, P = .04).21 At this time, however, TNF is not approved for regional therapy of in-transit metastases in the United States. Considering that ILI is a less invasive alternative to HILP, some investigators have turned to using ILI before attempting HILP for regional therapy treatment-naïve patients. The Table lists some contemporary ILI and HILP publications and describes results achieved.

Morbidity From ILI and HILP

The morbidity from ILI appears to differ significantly from HILP. Surgical access is not required, and patients with medical comorbidities or anatomical constraints that preclude large dissections and longer operative times of HILP may still be candidates to undergo ILI. The systemic toxicities between HILP and ILI also do not appear to be equivalent.19,27 The routine use of a pneumatic tourniquet prevents the systemic exposure to chemotherapy, and the washout of the chemotherapy prior to deflating the tourniquet removes the vast majority of the remain-
The Role of Regional Perfusion for Locoregionally Metastatic Melanoma

Santillan and colleagues specifically looked at toxicity and its relation to perioperative factors from 171 patients who underwent ILI. The Wieberdink regional limb toxicity (WBD) scale and creatine phosphokinase (CK) levels were used to measure regional and systemic toxicity. 

Mild (grades I–II) and severe (>grade III) WBD limb toxicity developed in 68% and 32% of patients, respectively. Median peak CK for all patients was 563 U/L, and median peak occurred on postoperative day 4. On univariate analysis, intraoperative use of papaverine and high CK levels (>563 U/L) were significantly associated with higher WBD toxicity. On the contrary, melphalan dose correction based on ideal body weight (IBW) was significantly associated with a lower risk of severe (>grade III) toxicity. Perfusate blood gas analysis at 30 min (pH, PaO₂, and base excess [BE]), limb temperature, and ischemia time were not predictive of limb toxicity. On multivariate analysis, severe toxicity was associated with female sex (P = .01), papaverine use (P = .01), and high peak CK levels (P <.01). Morbidities from HILP with or without TNF can be significant and can be attributed to the local effects of the chemotherapy itself, the addition of hyperthermia, systemic leak of the chemotherapy, or the surgical intervention itself.  

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<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Modality Used (ILI or HILP; number of patients studied)</th>
<th>Complete Response</th>
<th>Partial Response</th>
<th>Duration of Response</th>
<th>Regional Toxicity Noted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beasley21</td>
<td>2009</td>
<td>ILI (n = 128)</td>
<td>31%</td>
<td>33%</td>
<td>N/A</td>
<td>46% with ≥Grade III</td>
</tr>
<tr>
<td>Wong26</td>
<td>2013</td>
<td>ILI (n = 49 initial ILI)</td>
<td>Initial ILI only: 35% LE 42% UE</td>
<td>Initial ILI only: 38% LE 33% UE</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Kroon18</td>
<td>2008</td>
<td>ILI (n = 185)</td>
<td>38%</td>
<td>46%</td>
<td>13 months; overall 22 months after CR</td>
<td>N/A</td>
</tr>
<tr>
<td>Santillan19</td>
<td>2009</td>
<td>ILI (n = 171)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>32% with ≥Grade III</td>
</tr>
<tr>
<td>Brady25</td>
<td>2006</td>
<td>ILI (n = 32 in 25 patients)</td>
<td>23%</td>
<td>27%</td>
<td>12 months</td>
<td>None with ≥Grade III</td>
</tr>
<tr>
<td>Kroon24</td>
<td>2009</td>
<td>All repeat ILIs</td>
<td>23%</td>
<td>60%</td>
<td>11 months overall; 10 months after CR</td>
<td>52% with ≥Grade III</td>
</tr>
<tr>
<td>Chai21</td>
<td>2011</td>
<td>All after repeat perfusions: ILI → ILI (n = 25)</td>
<td>16%</td>
<td>24%</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ILI → HILP (n = 10)</td>
<td>30%</td>
<td>10%</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HILP → ILI (n = 12)</td>
<td>N/A</td>
<td>N/A</td>
<td>33%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HILP → HILP (n = 3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noorda1</td>
<td>2004</td>
<td>HILP (n = 40 with melphalan, n = 90 with melphalan and TNF)</td>
<td>45% (melphalan alone); 59% (melphalan and TNF)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Grünhagen46</td>
<td>2004</td>
<td>HILP (n = 100, melphalan and TNF)</td>
<td>69%</td>
<td>26%</td>
<td>16 months overall; 22 months after CR</td>
<td>81 % with &lt;Grade III 3% Grade IV 3% Grade V</td>
</tr>
<tr>
<td>Cornett12</td>
<td>2006</td>
<td>HILP (n = 64, melphalan, n = 65 melphalan and TNF)</td>
<td>25% melphalan; 26% melphalan and TNF</td>
<td>39% melphalan; 43% melphalan and TNF</td>
<td>N/A</td>
<td>16% vs 4% Grade IV adverse events in melphalan vs melphalan plus TNF</td>
</tr>
<tr>
<td>Aloia22</td>
<td>2005</td>
<td>HILP (n = 59, melphalan)</td>
<td>57%</td>
<td>31%</td>
<td>13.4 months</td>
<td>44% were ≥Grade III</td>
</tr>
</tbody>
</table>

Toxicity based on Wieberdink regional toxicity scale. CR, complete response; HILP, hyperthermic isolated limb perfusion; ILI, isolated limb infusion; LE, lower extremity; N/A, not applicable; TNF, tumor necrosis factor; UE, upper extremity;
repeat procedures in the same limb with ILI with relative ease and minimal morbidity, whereas repeat HILP is very challenging. Repeat regional chemotherapy in the form of ILI is technically much easier to perform and appears to be better tolerated than HILP. Response rates from repeat regional chemotherapy can be as high as 60% to 70% for ORR, with 20% to 40% for CR. Limb salvage rates have been reported after repeat regional perfusions (ILI or HILP) as high as 95%.30,31 Chai et al proposed an algorithm for initial and repeat regional perfusions.31 The authors suggest that ILI be used in most cases for initial regional perfusions and HILP be used initially for high volume in-transit disease. HILP was suggested as a salvage regional perfusion procedure in patients who progressed rapidly after ILI. If the patient had a good response with ILI and then recurred with in-transit disease, a repeat ILI was suggested as a treatment option.31

**Surgery After ILI**


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**Disclosure:** Dr Zager has served as an advisory board member and consultant for Amgen, and as an advisory board member for Provectus.

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Current and Future Roles of Molecular Profiling in Colorectal Cancer

Rona Yaeger, MD, and Leonard Saltz, MD

Introduction

Molecular alterations in colorectal cancer (CRC) have been extensively studied, with seminal work from Bert Vogelstein defining the molecular events that underlie the adenoma-carcinoma sequence and the common genetic alterations that make up the “mountains” of the CRC genomic landscape. An understanding of these molecular alterations is directly applicable to patient care, and molecular profiling is now part of standard management for patients with CRC. This review describes the role of molecular profiling, including its strengths and limitations, in CRC management today.

Types of Molecular Markers

Molecular profiling consists of identification of specific changes, or “markers,” in the tumor DNA that impact tumor growth and behavior. Such markers can be separated into two types: predictive and prognostic. Prognostic markers tell us who has a better or worse chance of a favorable outcome, but they do not tell us whether we are able to influence that outcome. Thus, prognostic markers do not offer clear guidance as to what management choices to make for an individual patient. Predictive markers, on the other hand, tell us who is more or less likely to respond to a certain intervention, and are highly useful in guiding individual treatment decisions. As such, predictive markers are a routine part of management in CRC care today.

Predictive markers can be broken down into two major categories: inclusionary markers and exclusionary markers. Exclusionary markers are markers, such as RAS mutations (discussed later), that identify patients who should not receive a treatment that would otherwise be considered for patients with CRC. Unfortunately, this is the sort of marker that is in routine use in CRC management today. Inclusionary markers are markers that identify a mutation which, when present, would offer an otherwise unavailable treatment strategy for a patient. The identification of such inclusionary markers represents an active area of investigation, but inclusionary markers do not currently play a role in standard care.

Mismatch Repair Enzymes and Their Role in Stage II and III Decisions

Impaired DNA mismatch repair (MMR), resulting from mutations in MMR genes that cause a malfunctioning gene product or from promoter hypermethylation causing epigenetic silencing of MMR protein expression, is seen in about 15% of patients with CRC. MMR deficiency (the phenotypic manifestation of which is microsatellite instability) is both prognostic and predictive. MMR-deficient CRC has been associated with a more favorable prognosis, being less likely to metastasize to regional nodes and distant sites. A recent study by Sinicrope et al, however, suggests a more nuanced situation, in which the prognosis in patients with stage III MMR-deficient CRC varies by primary tumor site (left versus right-sided CRC). The researchers analyzed the impact of MMR deficiency on disease-free survival (DFS) of patients participating in the North Central Cancer Treatment
Group (NCCTG) N0147 trial. The trial randomized patients with stage III colon cancer to adjuvant treatment with FOLFOX (leucovorin, fluorouracil, and oxaliplatin) with or without cetuximab, and found that MMR-deficient tumors located in the right side of the colon had favorable outcome, while those MMR-deficient tumors arising in the left side of the colon not only did not have a favorable prognosis, but actually had a worse prognosis than those with intact MMR proteins. The study also found that having 4 or more positive lymph nodes (N2 disease) was prognostic of a poor outcome, regardless of MMR status.

From a predictive perspective, however, MMR-deficient CRC appears to be resistant to fluoropyrimidines, and the benefit of adjuvant 5-fluorouracil (5FU)-based chemotherapy for MMR-deficient tumors has come into question. Ribic et al analyzed the impact of microsatellite instability in a series of 570 patients with colon cancer enrolled in 5 prior phase III trials of 5FU-based adjuvant chemotherapy following curative resection for stage II and III colon cancer. A total of 18% of patients in this series had colon tumors with microsatellite instability. In the untreated arms, microsatellite instability was associated with better survival (hence prognostic), but in the 5FU arms, microsatellite instability was not prognostic and not associated with improved survival.

From a predictive perspective, however, adjuvant chemotherapy with 5FU improved survival only among patients whose tumors did not exhibit microsatellite instability. Thus, microsatellite instability, or MMR-deficiency, was predictive of non-benefit from 5FU, and absence of microsatellite instability (microsatellite-stable, or MMR-proficiency) was predictive of benefit from adjuvant 5FU. Sargent et al extended these results by assembling a larger dataset that combined 4 of the 5 studies analyzed by Ribic with an additional randomized trial of 5FU adjuvant chemotherapy in stage II and III colon cancer. Patients with MMR-deficient CRC experienced no benefit from adjuvant 5-FU chemotherapy in either stage II or III disease, while patients with proficient MMR with stage III colon cancer had improved survival with adjuvant chemotherapy. However, a larger-still dataset from the same group demonstrated that while MMR deficiency remained a favorable prognostic indicator in stage II and III patients, patients with MMR-deficient stage III disease did benefit further from receiving adjuvant 5FU. Currently, the best available evidence indicates that there is no benefit to the use of adjuvant 5FU or capecitabine in patients with MMR-deficient stage II colon cancer. Patients with stage III colon cancer do appear to benefit from adjuvant therapy and should be treated similarly regardless of MMR status, with oxaliplatin-fluoropyrimidine combinations such as FOLFOX or CapeOx, and with fluoropyrimidine alone in those patients with contraindications to oxaliplatin.

MMR deficiency is also an appropriate screen for Lynch syndrome, the identification of which has important screening and surveillance implications for both the patient and his/her immediate family members. Currently National Comprehensive Cancer Network (NCCN) guidelines recommend routine MMR screening for all CRC tumors in patients who are either less than 70 years old and/or who have a suggestive family history.

**Multigene Assays**

There has been intense effort to develop a biomarker that can identify patients with stage II colon cancer who will benefit from adjuvant chemotherapy. Gene expression profiling with either the Oncotype DX Colon Cancer test or the ColoPrint assay has been validated to risk-stratify stage II colon cancer patients. These biomarkers are prognostic, and can identify patients with stage II disease with a higher or lower risk of recurrence, but do not predict who will benefit from adjuvant chemotherapy and therefore do not currently guide treatment decisions. Use of such assays is not currently recommended in NCCN guidelines, as they add expense without providing a basis for treatment decisions in the management of patients with CRC.

**RAS**

RAS proteins are small GTPases that are active when bound to GTP and regulate cell proliferation, survival, and differentiation. RAS proteins exist in 3 isoforms: KRAS, NRAS, and HRAS. Activating mutations in the genes encoding the KRAS isoform are seen in about 45% of colorectal tumors. Up to an additional 5% of CRC tumors harbor NRAS mutations. HRAS mutations for all practical purposes do not occur in CRC. RAS mutations occur in hotspots and lead to constitutative activation of RAS by preventing hydrolysis of GTP. Mutations in the different RAS isoforms are nearly always mutually exclusive.

Mutations in KRAS most commonly occur at exons 2 and 3, with KRAS G12D, G12V, and G13D mutations occurring in about 17%, 10%, and 8% of CRC, respectively. An additional 5% of KRAS mutations occur at exon 3, primarily at codon 61, and at exon 4, at codons 117 and 146. KRAS mutation is an early event in the development of colorectal adenocarcinoma; these mutations can be identified in early adenomas. Primary colorectal tumors and metastases exhibit an extremely high rate of concordance for KRAS mutation status, so RAS mutation determination from archived primary material is satisfactory; there is no indication to rebiopsy a metastasis for the purpose of RAS genotyping.

There are conflicting reports regarding the prognostic impact of KRAS mutation, with many studies describing worse outcomes for patients with KRAS mutant tumors, but others unable to confirm these findings. Several series suggest that KRAS G12V mutations, in particular, are associated with more aggressive disease with increased risk of recurrence and worse CRC-specific survival. KRAS G13D mutation has been proposed as a poor prognostic factor in CRC, however the prognostic role of this mutation could not be confirmed in other datasets. The presence of a KRAS mutation has been associated with early recurrence and worse survival after curative resection of colorectal liver metastases.
While the prognostic role of KRAS mutation is controversial, the predictive value of RAS mutations as a firm exclusionary marker against the use of anti-EGFR agents (panitumumab and cetuximab) is now a well-established part of standard practice. The presence of an activating RAS mutation leads to EGFR-independent activation of mitogen-activated protein kinase (MAPK) signaling and results in lack of response to the anti-EGFR monoclonal antibodies cetuximab and panitumumab. Historically, Ford et al initially found that virtually all patients who responded to cetuximab did not have an exon 2 KRAS mutation within their tumors, and subsequently Lievre et al validated this finding in an expanded dataset with 89 patients treated with cetuximab where none of the responders had mutated KRAS. A similar analysis of 427 patients treated with panitumumab monotherapy by Amado et al indicated that wild-type KRAS was required for response. More recently, De Roock et al performed a retrospective analysis of a pooled dataset of patients with chemotherapy-refractory CRC treated with cetuximab that suggested that patients with G13D KRAS mutations treated with cetuximab had longer overall survival (OS) and progression-free survival (PFS). However, a subsequent, substantially larger retrospective pooled analysis of 3 phase III trials of panitumumab demonstrated no benefit for panitumumab treatment in patients with KRAS G13D CRC, confirming that anti-EGFR antibodies should be limited to patients with RAS wild-type colorectal tumors.

Non-exon 2 KRAS mutations are less common and were not included in the earlier studies of predictive factors for anti-EGFR antibodies. Analysis of a large, genetically annotated dataset first suggested that non-exon 2 KRAS mutations may lead to insensitivity to anti-EGFR antibodies. More recently, Douillard et al performed a prospective-retrospective analysis of the efficacy and safety of panitumumab in combination with FOLFOX compared with FOLFOX alone in the PRImE study according to RAS mutation status. They found that non-exon 2 KRAS mutations were associated with inferior progression-free survival (PFS) and overall survival (OS) for panitumumab-FOLFOX compared with FOLFOX. Based on these data, extended RAS sequencing for hotspot mutations in exons 3 and 4 is now recommended in the National Comprehensive Cancer Network (NCCN) guidelines and should be regarded as standard practice prior to consideration of initiation of an anti-EGFR antibody-containing regimen.

The NRAS isoform of RAS is hyperactivated in about 2% to 5% of metastatic CRC cases. Activating mutations occur in the same hotspots as mutations in KRAS, and also lead to constitutive activation. The analysis of any RAS mutation in the PRImE study recently demonstrated that the presence of a NRAS mutation also precludes response to anti-EGFR antibody therapy.

NRAS mutation has been associated with worse OS in metastatic CRC and has been implicated in tumorigenesis in the context of inflammation. Preclinical data suggest that NRAS-mutant tumors may be more sensitive to selective MEK inhibition than KRAS-mutant tumors, and current clinical trials are testing the clinical efficacy of combined MEK and EGFR inhibition in NRAS-mutant CRC.

The analysis of the PRImE study also confirmed earlier series that suggested that anti-EGFR antibody treatment is actually associated with worse outcomes in patients with RAS-mutant tumors. The etiology of these inferior outcomes is not well understood and highlights the need for further studies of the impact of RAS mutation on EGFR signaling in CRC and the effect of wild-type RAS isoforms in tumors with RAS mutations. CRC cell lines with KRAS mutations depend on RAS activity for proliferation, and knockdown of RAS prevents tumor growth. Thus far RAS has eluded pharmacologic inhibition, but the development of new therapies that target RAS-activated CRC is an area of intense research efforts and was recently prioritized by the National Institutes of Health (NIH) as an area of clinical need.

**BRAF**

BRAF encodes a protein directly downstream from RAS in the canonical mitogen-activated protein kinase cascade. BRAF mutations in CRC occur most commonly at the V600 hotspot and lead to constitutive activation of BRAF independent of RAS activity. BRAF V600 mutations are nearly always mutually exclusive with RAS mutations. BRAF mutations are more common in adenomas and early-stage colorectal tumors, with a frequency of about 30%. In metastatic disease, BRAF mutations are seen in 5% to 11% of cases. BRAF mutation is commonly associated with sporadic microsatellite instability and BRAF-mutant, microsatellite unstable tumors are thought to develop through the serrated neoplastic pathway. These tumors are characterized by MLH1 promoter hypermethylation and silencing, more commonly occur in the right side of the colon, and have more subtle endoscopic features, such as a flat shape and indistinct borders, making them harder to see. They occur more commonly in female and older patients. BRAF mutation does not clearly affect prognosis in early-stage disease, although it does have a negative prognostic impact in some series.

BRAF mutation is an independent, poor prognostic factor in metastatic CRC. Survival for BRAF-mutant metastatic CRC is estimated at 11 to 14 months. Microsatellite instability is seen in about one-third of BRAF-mutant metastatic CRC cases. BRAF-mutant metastatic CRC commonly spreads to the peritoneum, a pattern of spread that may contribute to the poor outcome for these patients. BRAF-mutant tumors less commonly present with liver-limited metastatic disease, and when patients with BRAF-mutant metastatic CRC undergo complete metastasectomy, they are more likely to recur and have a shorter OS than wild-type cases.

Many studies have evaluated BRAF mutation as a predictive marker for anti-EGFR antibodies, and the sum of the data strongly suggest that BRAF mutation predicts for lack of benefit.
from these agents. Responses to cetuximab or panitumumab in BRAF-mutant CRC are so extremely rare as to be essentially non-existent. In the chemotherapy-refractory setting, Di Nicolantonio et al reported no responses to cetuximab or panitumumab in 11 patients with BRAF-mutant CRC.40 Laurent-Puig et al reported no responses to cetuximab in 5 patients with BRAF-mutant CRC,41 and De Roock et al reported responses in 2 of 24 patients with BRAF-mutant CRC.20 In the first-line setting, retrospective analyses have examined the impact of BRAF mutation on outcomes after first-line treatment with FOLFOX or FOLFIRI plus cetuximab in the OPUS and CRYSTAL trials, respectively, and FOLFIRI plus panitumumab in the PRIME trial, and have confirmed that BRAF mutation is a consistently poor prognostic marker.22,23 An unplanned retrospective subset analysis that combined the OPUS and CRYSTAL trials suggested that patients who received cetuximab appeared to fare better in terms of OS and PFS than those who received chemotherapy alone, but the differences between the 2 groups were not significant. Analysis of BRAF-mutant CRC in the PRIME trial similarly suggested an improvement in PFS and OS with the addition of panitumumab to FOLFOX, but again the differences between the 2 groups were not statistically significant.

BRAF activation is a driver of tumor growth that has been validated as a target in melanoma. However, in a CRC extension cohort, the selective BRAF inhibitor vemurafenib showed minimal activity.33 Subsequent preclinical work suggested that MAPK signaling is not durably suppressed with BRAF inhibition in CRC. High ERK activity leads to profound feedback suppression of upstream signaling through negative feedback loops, and BRAF inhibition inhibits ERK, releasing upstream signaling from this negative feedback suppression and leading to a rebound in activated ERK levels. The rebound in ERK activity is mediated by reactivation of RAS and therefore is insensitive to BRAF inhibitors that selectively inhibit V600-mutant BRAF, which signals as monomer, further attenuating the effect of the BRAF inhibitor. In CRC, high levels of EGFR lead to more rapid rebound in ERK activity than in melanoma, and Prahallad et al and Corcoran et al recently showed that combined RAF and EGFR inhibition more durably inhibits ERK and inhibits proliferation of BRAF-mutant CRC cell lines.14,15 Trials testing combinations aimed at targeting both RAF and the upstream signaling released with BRAF inhibition are currently ongoing.

**PIK3CA**

PIK3CA encodes the catalytic subunit of phosphatidylinositol 3-kinase (PI3K), a lipid kinase that plays a central role in cell growth and survival. Hotspot mutations in the helical and kinase domains of PIK3CA that lead to PI3K constitutive activation are seen in about 10% to 20% of colorectal tumors. PIK3CA mutations are usually concurrent with KRAS or BRAF mutations. Only about one-third of PIK3CA-mutant CRC is wild-type for KRAS and BRAF. PIK3CA mutation is thought to occur in the late adenoma stage.16,37 Studies evaluating the impact of PIK3CA mutation on prognosis have had conflicting results, with some noting worse survival for patients with PIK3CA-mutant CRC and others failing to confirm this association.38,39 The effect of PIK3CA mutation on clinical outcomes may be difficult to isolate because PIK3CA mutation often co-occurs with other driver mutations, and thus PIK3CA-mutant CRC is likely not a homogenous group. Additionally, exon 9 and 20 mutations may have different biologic effects, further complicating our understanding of PIK3CA mutation in CRC.

Two observational studies have linked PIK3CA mutation with benefit from aspirin in the prevention of CRC recurrence in early-stage disease. An analysis by Liao et al of the relationship between regular aspirin use and CRC PIK3CA mutation status among participants of the Nurses’ Health Study and Health Professionals Follow-up Study found that aspirin use conferred superior CRC-specific survival and OS only in patients with PIK3CA-mutated tumors.40 Domingo et al analyzed data from the VICTOR (Vioxx in Colorectal Cancer Therapy: Definition of Optimal Regime) trial, a randomized trial of rofecoxib in patients with stage II/III disease, and did not find greater benefit from rofecoxib treatment in patients with PIK3CA-mutant tumors.41 In this trial, aspirin use was documented at the time of randomization, and analysis by aspirin use found an association between regular aspirin use after CRC diagnosis, with a reduced rate of CRC recurrence in patients with PIK3CA-mutant tumors and not in those with PIK3CA-wild-type tumors. These data suggest that PIK3CA mutation status may be a biomarker for benefit from adjuvant aspirin therapy, and we await prospective trials to corroborate these findings. Two ongoing randomized trials, ASCOLT (Aspirin in Dukes C and High-Risk Dukes B CRCs) and CALGB (Cancer and Leukemia Group B) 80702, should provide insight on the use of adjuvant aspirin and celecoxib therapy, respectively. At present, however, these results should be regarded as hypothesis-generating only, and PIK3CA analysis is not currently part of standard management of CRC.

**Future Directions**

Recent data suggest a possible future role for serial molecular profiling in patients who develop secondary resistance to anti-EGFR antibody therapy to identify mechanisms of resistance and guide subsequent treatment. Preclinical models and small patient series have identified the emergence of KRAS mutations,42,43 HER2 activation,44 MET amplification,45 and EGFR mutation46 in tumors from patients who develop secondary resistance to anti-EGFR antibodies. Current trials are evaluating combined MEK and EGFR inhibition or anti-MET antibodies in patients with CRC who develop resistance to anti-EGFR antibodies. Such approaches remain investigational at this time, however.

Our growing understanding of secondary resistance to anti-EGFR antibodies suggests that patients might, at some time in the future, benefit from serial molecular profiling of their tu-
mors to guide the use of anti-EGFR antibodies and then to identify mechanisms of resistance in patients who develop resistance to these agents. However, the costs, discomfort, and risks associated with tumor biopsy limit the ability to obtain serial tumor specimen for analysis in the clinical care of patients with CRC. New, noninvasive methods to detect and monitor CRC that use digital polymerase-based technologies to analyze circulating tumor DNA may provide a safe and easy means to detect tumor mutations, quantify the proportion of mutant alleles, and monitor tumor burden. This so-called “liquid biopsy” has been shown to be quite sensitive for CRC and capable of identifying new mutations that emerge with anti-EGFR antibody-acquired resistance.42,43,47 As we expand our understanding of the molecular events in CRC and apply targeted therapy to genetically defined subsets of CRC, these liquid biopsies may allow us to witness the dynamic molecular changes in CRC.

Conclusion
Several predictive markers, including MMR, RAS, and BRAF, are now part of standard management in CRC. All patients with CRC who are 70 years old or less and/or have a positive family history should have tumors evaluated for MMR deficiency. Stage II patients with MMR deficiency should not receive adjuvant therapy. All patients with metastatic CRC (but not stages I, II, or III) should have tumors profiled for RAS and BRAF status. Tumors harboring any KRAS or NRAS mutation not only do not benefit from anti-EGFR therapies, but may actually experience accelerated growth as a result of treatment with such agents, and so neither cetuximab nor panitumumab should be used in these patients. At the present time, further molecular profiling is not routinely recommended, and should be regarded as a research tool only. It is hoped that in the near future, identification of validated inclusionary markers will open up additional treatment options for specific molecularly characterized subsets of patients with colorectal cancer.

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Disclosure: Dr Yaeger has served as a consultant to or on a paid advisory board for Amgen. Dr Saltz has served as a consultant to or on a paid advisory board for Roche/Genentech, Pfizer, Inc. and Taiho Pharmaceutical Co., Ltd. Dr Saltz has served as a consultant to or on a paid advisory board for Roche/Genentech, Bristol-Myers Squibb, Pfizer, Inc. and Taiho Pharmaceutical Co., Ltd.

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18th Annual International Congress on Hematologic Malignancies®: Focus on Leukemias, Lymphomas, and Myeloma

Case Study in Del(17p) Chronic Lymphocytic Leukemia

At the 18th Annual International Congress on Hematologic Malignancies®: Focus on Leukemias, Lymphomas, and Myeloma, held February 14-15, 2014, in New York City, William Wierda, MD, PhD, Professor of Medicine, The University of Texas MD Anderson Cancer Center in Houston, presented case studies on 17p deletions in chronic lymphocytic leukemia (CLL). Joining Dr. Wierda in the discussion panel were Thomas J. Kipps, MD, PhD, Deputy Director for Research, UC San Diego Moores Cancer Center in California; Jennifer Brown, MD, PhD, Director of the Chronic Lymphocytic Leukemia Center at Dana-Farber Cancer Institute in Boston, Massachusetts; and Alessandra Ferrajoli, MD, Associate Professor, Leukemia, The University of Texas MD Anderson Cancer Center. In this article, we highlight some of the case study discussion.

Case Study: Frontline Therapy

65-year-old male with incidentally noted lymphocytosis on routine complete blood count (CBC)

- White blood cells: 45 k/µL; 79% lymphocytes; hemoglobin: 13 gm/dL; platelets: 140 k/µL.
- Flow cytometry: CD19+; CD20+(dim); CD5+; CD23+; slg+(dim); ZAP70+.
- FISH: trisomy 12 and del(17p); IGHV-unmutated.
- Beta-2 microglobulin: 3.0 mg/L (mildly elevated).
- Bone marrow aspiration/biopsy: 80% lymphocytes, diffuse pattern.
- Physical examination: 1 cm cervical node, no palpable spleen.
- Rai stage I, Binet A, asymptomatic.

Dr. Wierda: This is a case study of a 65-year-old male incidentally noted to have lymphocytosis on a routine CBC. White count was 45,000/µL, 79% lymphocytes; hemoglobin was 13 gm/dL; platelet count was 140,000/µL. Flow cytometry was typical for CLL: CD5, CD19, and CD23 positive population of monoclonal B cells. ZAP70 expression was positive. Fluorescence in situ hybridization (FISH) was done at diagnosis, which is not required but in this case it was done. And this patient had trisomy 12 and del(17p) and had an unmutated IGHV gene. Beta-2 microglobulin was 3.0mg/L. Bone marrow was done, which is also not required at the time of diagnosis, and which showed 80% lymphocytes, diffuse pattern. On physical exam, the patient had small adenopathy, 1 cm cervical node that was palpable, no hepatosplenomegaly. Therefore, this asymptomatic patient was diagnosed with Rai stage I disease, with del(17p) by the hierarchical categorization, and unmutated IGHV gene.

So which factor indicates to you that this patient will likely eventually need treatment: age, Rai stage, beta-2 microglobulin, cytogenetics—del(17p), IGHV mutation status, or nothing? And it will be sooner than later. Incidentally, the frequency of patients who are newly diagnosed and noted to have 17p is very rare; it’s less than 5%.

[The majority of the audience responded 17p.]

Dr. Kipps: I disagree [with the audience response of 17p]. The cytogenetics does not indicate that the patient will require therapy sooner than later. It’s actually the mutation status. I think a second [choice] would be the beta-2 microglobulin level, but the mutation status is the most likely to be a good predictor for the patient requiring therapy. We have patients who have del(17p) at diagnosis who have unmutated antibody genes that we have left untouched and not treated, and they’ve gone on for several years without having any complications related to their disease. Of course, they express mutated antibody genes and lack ZAP70 expression. I think you can change that if you take a patient with 17p who has very indolent disease and you impose therapy based upon the discussion that we had earlier about evolution. You can change the disease and not for the better with treatment. So I disagree with the audience’s conclusion here. It’s not the 17p minus that will indicate the patient requiring therapy sooner or later but the mutation status of the antibody genes. And just because you have deletion at 17p does not mean that you have...
Dr. Wierda: Let me show you some data and we’ll move on. So what’s the treatment or lack of treatment for this patient:

- Fludarabine, cyclophosphamide, and rituximab (FCR)
- Fludarabine and rituximab (FR)
- Bendamustine and rituximab (BR)
- Alemtuzumab
- High-dose steroids with CD20 monoclonal antibody
- Referral for clinical trial
- Observation

Dr. Wierda: So I would agree with the audience in this case; the majority of individuals indicate that this patient should be observed. Twenty-two percent say BR, which I would disagree with. Refer for clinical trial—I would also agree with this choice.

So let’s go through a little bit of data, [which was] a combined effort for MD Anderson and the Mayo Clinic. [Among] individuals who presented to one of our institutions with a del(17p) previously untreated and without necessarily having indications to start treatment—there were about 100 patients in this group. Three fourths of them ended up initiating therapy, whereas a third of the patients from the Mayo group ended up initiating therapy.

So one of the questions in this analysis was, what were those features that correlated with time to first therapy. If you have a patient who is asymptomatic, doesn’t necessarily have any of the indications to initiate therapy by the formal criteria we use—those are symptoms, anemia, or thrombocytopenia—then you can have a long treatment-free period. And, in fact, with the standard treatments that we’ve had so far, you may do patients an injustice by initiating them on ineffective treatments, particularly chemoimmunotherapy if they don’t have an indication to start on treatment. Fifty percent of these patients at year 3 progressed to needing first therapy. So the point here is that not all the 17p patients are high risk and have active, progressive disease and need to start treatment right away. In a multivariate model for time to first therapy, features that correlated with earlier time to first treatment [were]: advanced Rai stage, stage 1 or beyond, and/or having an unmutated IGHV gene.

So this patient had an unmutated IGHV gene; he also had Rai stage 1 disease. It’s likely he’ll need treatment within the next 3 years, and the correct answer that I would agree with would be that he should either be referred for a clinical trial or he should be observed.

The patient is monitored for 2 years and at age 67, he returns to the oncologist with fatigue, shortness of breath, dyspnea on exertion. White count at that time was 160,000/µL with 95% lymphocytes, hemoglobin 11gm/dL, platelets were 95,000/µL, he had diffuse adenopathy roughly 3 cm, spleen was palpable, performance status was 2, and FISH was repeated and showed trisomy 12 and del(17p). What’s the treatment strategy for this patient? At this time, [options are] FCR; FR; BR; obinutuzumab

Dr. Kipps: I think there’s some confusion out there because a lot of physicians will get the idea that you have a cytogenetics report. You have may be 15%, 17p minus. The patient has mutated antibody genes, is ZAP70 negative, and boy, that patient needs therapy—we’ll orchestrate a bone marrow transplant. This is going on all the time. There’s a lot of misconceptions here, so I think it’s important.

Dr. Wierda: I would clarify your statement for a couple reasons. I would agree with the audience. I have some data that I’m going to show you. There is also other data that I didn’t include in this session. We did a model for time to first therapy for patients who presented to MD Anderson with the Mayo clinic, particularly in patients with del(17p). This patient has several factors that indicate he will need treatment soon, including Rai stage >0, unmutated IGHV and del(17p). For this patient, if you have to pick one of these factors, for me it would be 17p, specifically del(17p) with the other mentioned factors indicate that it is very likely that this patient will need treatment within 3 years of presentation.

Table. Response With First-Line FCR-Based Treatment by FISH

<table>
<thead>
<tr>
<th>FISH</th>
<th>N</th>
<th>% Patients</th>
<th>% Complete Response</th>
<th>% Overall Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Del(17p)</td>
<td>41</td>
<td>10</td>
<td>39 P&lt;.001</td>
<td>76 P&lt;.001</td>
</tr>
<tr>
<td>Del(11q)</td>
<td>87</td>
<td>20</td>
<td>83 P=.03</td>
<td>99</td>
</tr>
<tr>
<td>+12</td>
<td>79</td>
<td>19</td>
<td>86 P&lt;.001</td>
<td>100</td>
</tr>
<tr>
<td>None</td>
<td>90</td>
<td>21</td>
<td>67</td>
<td>98</td>
</tr>
<tr>
<td>Del(13q)</td>
<td>125</td>
<td>30</td>
<td>65</td>
<td>98</td>
</tr>
<tr>
<td>Overall</td>
<td>422</td>
<td>70</td>
<td></td>
<td>96</td>
</tr>
</tbody>
</table>

*Includes FCR, FCR3, FCMR, FCR+GM-CSF, and CTAR.

Courtesy Dr. Wierda.

p53 mutation. About two thirds to three fourths of those will have p53 mutation in the remaining allele. But it doesn’t govern progression. It governs the overall response and some of the tools we use. It dictates the ability to induce these death responses upon genotoxic stress. So it dictates a response to therapy and not the kinetics or the need for therapy. That’s very important.

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plus bendamustine; alemtuzumab; high-dose steroids with CD20 antibody; allogeneic stem cell transplant; or refer for clinical trial.

I would agree with the audience in this situation that there really isn’t a standard treatment frontline for del(17p). We don’t have ibrutinib approved for that indication yet, and there are very little data available regarding ibrutinib in this setting, although I do think that that’s probably going to be the preferred treatment or the optimal treatment even in the first-line setting for this population because of some of the data that I’ll show you in a second. Other options such as FCR, BR—all of those I think are ineffective treatment frontline for del(17p).

Dr. Kipps: I think it’s worth pointing out, though, because you have a patient that likely does not have functional p53. So the cells are going to be more or less resistant to the chemotherapy, but their hematopoietic cells are going to be sensitive. They’re already under siege right now because their niches are being occupied by leukemic cells. So what happens typically when, in this setting, you give chemotherapy, is that you may get more profound myelosuppression that does not want to quit. And the patient’s leukemic cells are not responding in a fashion that allows for those niches to open up again. So it compounds the problem and I think ultimately can make it worse. I think chemotherapy in this setting actually could hasten the patient’s demise.

Dr. Wierda: Another option that I would propose, although there are not a lot of data for it in the frontline setting, is lenalidomide with CD20 monoclonal antibody, which would be a reasonable option. But I would argue to anybody that there’s not a standard first-line therapy for del(17p) disease. And so far, we’ve had very ineffective treatment for that population.

We’ve done a lot of work with chemoimmunotherapy; the regimens that we have developed and used at Anderson are FCR-based treatments. If you look at these studies in aggregate (Table), there were 422 patients in this analysis. With FCR-based therapies, the complete remission rate is 39% and overall response rate is 76% in patients with del(17p) CLL. The problem, in my opinion, for this group is not only getting them in complete remission or getting some response—it’s that nothing lasts, and they have a very rapid progression.

Among del(17p) patients who received first-line FCR therapy, 14 months is the median progression-free survival. Median overall survival is about 54 months in this population. These patients did not have ibrutinib available as salvage treatment and none were subsequently put on an ibrutinib trial at the time that this analysis was done, but I think we’ll see with this population, particularly the survival curves shifting out as patients are treated with del(17p) with ibrutinib. There have been other regimens that have been evaluated in frontline with del(17p), and all of them are similarly ineffective, I would say, with very short progression-free survival.

So these were some of the comments that I wanted to come away with. Newly diagnosed patients with del(17p) are rare patients. They should be monitored closely and not given early treatment, and should be started on treatment when they have an indication to start treatment. Frontline and salvage patients with del(17p) are high-risk. There is no standard treatment in the first-line setting and no role for chemoimmunotherapy.
Case Study
An 83-year-old male presented with an obstructing tonsillar mass but no stridor; with weight loss and fatigue.

- Physical examination: Karnofsky performance scale (KPS) 70%, obstructing supraglottic base-of-tongue mass, cervical nodes
- Pathology: DLBCL, non-germinal center phenotype by Hans: CD10—, BCL6—, MUM1+; Ki67 = 60%
- Staging: High-risk IVb
  - Lymphadenopathy, Waldeyer’s ring, gastric mass, weight loss
  - Age-adjusted International Prognostic Index (IPI)
- Previous medical history: hypertension, hyperlipidemia, basal cell carcinoma (BCC), prostate cancer (treated with brachytherapy), vasovagal syncope, and hearing loss
- Cardiac evaluation: normal left ventricular function (LVF); ejection fraction (EF) 69%

Dr. Zelenetz: This is a case of an 83-year-old man who presented with an obstructing tonsillar mass but no stridor, with weight loss and fatigue. His performance status was 70%. Exam revealed an obstructing supraglottic base-of-tongue mass and cervical adenopathy. Biopsy demonstrated DLBCL, non-germinal center by Hans (CD10—, BCL6—, MUM1+). The proliferation index was pretty much close to the median for large cell lymphoma at 60%. The staging studies demonstrated lymphadenopathy and Waldeyer’s ring. There was a gastric mass; therefore the patient had stage IV disease and, because of the weight loss, it was IVb. By the age-adjusted IPI, the patient had high-risk stage IVb disease. There was some hypertension, hyperlipidemia, some BCC, some prostate cancer that was treated with brachytherapy, a vasovagal syncope, and hearing loss. Cardiac evaluation revealed a normal LVF. Paul, what more should we be asking about an 83-year-old man before we start thinking about what we do about treatment?

Dr. Hamlin: We have to keep the concept of life expectancy in this person front and center. We do this in daily practice, but we’re trying to codify it. Large cell lymphoma is clearly the biggest threat to him, but surprisingly, at 83 years, with the other medical problems that he has, his life expectancy without the lymphoma is still probably over 5 years based on our expected life tables.

We clearly have to confront the lymphoma and query whether or not we’re going to try to address curative intent. Interestingly, across the U.S., about 40% of patients in this age category get no therapy at all. I’m always thinking about what’s the likelihood of treatment-related mortality and morbidity up front in this patient. Can we give them an anthracycline-based therapy? Can we parse some of that out by using some of the tools that the geriatric community has been using to give us a better handle above and beyond performance status? I think that’s yet to be seen.

Dr. Zelenetz: Upon comprehensive geriatric assessment, he has moderate comorbidities, he’s independent in his activities of daily living (ADL) and instrumental activities of daily living (IADL). What are the differences between ADL and IADL?

Dr. Hamlin: Simply put, ADL are those things that allow you to function in the house, for example, taking care of your daily dressing and cleaning, and caring for yourself. IADL are probably best thought of as what it takes to go to college. Can you pay your bills? Can you function on a higher order with telephones, with banking, and those sorts of activities? They have been dem-
onstrated. So, comorbidities, ADL, and IADL all have predictive value in a geriatric general population for survival at 2 and 5 years. There is a great website called e-prognosis that integrates a lot of this information that is helpful, aside from the lymphoma.

**Dr. Zelenetz:** Moving on, he has a timed get-up-and-go (GUG) of 10 seconds. Would you comment on that?

**Dr. Hamlin:** That’s actually pretty good. So, he is relatively spry at 83 years of age and that value has not been associated with worse outcomes. This is a relatively easy assessment; it takes a few seconds. If somebody is sitting in a chair, they get up from the chair, walk 10 feet, and return. The timing is not clear but above 20 seconds is associated with worse outcomes and maybe even associated with some early morbidity and death.

**Dr. Zelenetz:** He has a CARG score of 52%. By CRASH score, he has medium-high predicted risk for hematologic toxicity and high predicted total risk and risk for non-hematologic toxicity. Can you comment on the CARG and CRASH scores?

**Dr. Hamlin:** CARG stands for the Cancer and Aging Research Group, part of the CALGB that was a national endeavor to look at geriatric assessments that patients can do in the waiting area. It gives a readout in terms of risk of grade 3 through 5 toxicity. In a similar vein, the CRASH score stands for the Chemotherapy Risk Assessment Scale for High-Age Patients and was led by investigators at the Moffitt Cancer Center.

The CARG and CRASH are attempts to take geriatric assessment tools and make them approachable for oncologists. The reason that this is gaining a lot more traction is that our gestalt for who’s frail and who’s going to have toxicity and reliance on performance status really is imperfect. We guess wrong about 25% to 33% of the time. The hope is that these tools can better codify this and give us something that’s actionable. There is an online CRASH score calculator. With both the CARG and the CRASH, this patient is predicted to have grade ≥3 toxicity at a relatively high amount.

**Dr. Zelenetz:** Now that he has been interpreted, Andre, how would you approach a patient like this?

**Dr. Goy:** The incidence of DLBCL in the elderly (>80 years of age) has increased 500% over the last 10 years. This is a real problem and we see a lot of these patients. Regarding the scoring, I don’t know details of the scoring. It might be interesting, in this situation, to use what’s called pre-phase chemotherapy, sort of per the German data. We give vincristine initially and 5 days of prednisone. We actually cheat a bit, we give vincristine and some-thing rituximab before 5 days of prednisone. Patients are tuned up this way and that potentially removes a lot of the early toxicity and then they go on R-CHOP. When we give R-CHOP, we do every 21 days and reduce the vincristine, cap it at 1 mg most of the time. In patients who are in relatively good condition, but high-risk, in some situations when they are really on the high-risk side, I have used dose-adjusted R-EPOCH because you have one shot with those patients and you want to get the most mileage that you can. That’s the way I would treat.

**Dr. Zelenetz:** Does everyone get growth factors?

**Dr. Goy:** Yes, everyone gets growth factors and antibiotic prophylaxis because of the risks.

**Dr. Zelenetz:** Craig, any thoughts?

**Dr. Moskowitz:** I would certainly start the patients with steroids, for sure, probably for 5 to 7 days. I don’t have a lot of experience with the pre-phase, but I think that our group tends to do that. I would treat with curative intent, though. I would try my best to give R-CHOP every 3 weeks with growth factor support and then see how the patient is doing after cycle number 1. On the flip side, shockingly, a patient like this with an ABC phenotype is actually eligible for the phase III study of R-CHOP plus or minus ibrutinib, which is currently enrolling (NCT01855750). I don’t know enough about all of these scores, maybe Paul can tell us whether a patient like this would meet the eligibility criteria for a study like that. I assume you can probably get pre-phase for that study?

**Dr. Hamlin:** On that study you can’t get pre-phase, but he would have been eligible, it wasn’t up and running at that moment. The performance status is how we typically arbitrate whether patients are eligible for clinical trial. So using a geriatric assessment as eligibility has not been something that we’ve built into most studies. We’re actually prospectively trying to look at that right now.

**Dr. Zelenetz:** So, it sounds like you want to give him full-dose CHOP, no mini-CHOP, after some pre-phase. What happens with the same patient, except the EF is lower, at 45%, instead of 55%, with known mitral valve regurgitation and poor LVF on echo?

**Dr. Moskowitz:** If this patient were 73 years of age, this exact patient, you might be able to get away with giving dose-adjusted R-EPOCH. The National Cancer Institute (NCI) has an experience of giving EPOCH with EF of 35%. If you speak to Wyndham Wilson [head of the Lymphoma Therapeutics Section of the Center for Cancer Research, NCI] about this, he historically would do an echo after every single cycle and I have done that. You might be able to get in a number of cycles. Now, at 83 years of age, I don’t have enough experience in this, and I’ve typically given etoposide-based treatment without doxorubicin.

**Dr. Goy:** There have been some data replacing etoposide, small series of patients, replacing etoposide for doxorubicin. I will agree with Craig, and actually I have given R-EPOCH in all the patients, try not to up-escalate and then actually giving only 2 cycles and repeat a PET scan early on, and sometimes either stop or just go rituximab or R-CHOP.

**Dr. Zelenetz:** A phase II study was published in the *Journal of Clinical Oncology* last year that gave rituximab, cyclophosphamide, vincristine, gemcitabine, and prednisolone (R-GCVP) in patients with either impaired cardiac function or who were on the borderline with high risk factors. About half of these patients had a low EF and the median age was 76 years. Interest-ingly, if you look at the impact of left ventricular ejection frac-tion (LVEF) with this regimen, there was no difference. So, the
substitution of gemcitabine for anthracycline maintained a good outcome and there was no significant adverse effect for the patients with low EF. I think that was the most important thing there.

Dr. Hamlin: I’d agree with all those thoughts. That study is to be commended. I think that study has provocative results with the gemcitabine substitution and 50% overall survival going out for patients just like this.

Dr. Zelenetz: So, back to our case, the original good EF patient enrolled on protocol 13-028, which is geriatric assessment and pre-phase therapy with prednisone and rituximab. This is Dr. Hamlin’s twist on pre-phase (NCT01829958). We actually all scratched our heads originally with the German pre-phase. Why would you give vincristine, one of the more toxic drugs, to elderly patients as part of their cytoreduction? So we changed it. So there’s an improvement—he’s KPS improved from 70% to 80%. But, we didn’t see much change in his CARG or CRASH. Would we expect that with pre-phase?

Dr. Hamlin: I don’t know that those tools are going to be sensitive enough to see changes within a week. As part of the study we are looking at correlative signs to see whether or not there are changes in the inflammatory milieu and cytokine profile that recently appear to be prognostic in large cell lymphoma. And, if you believe that pre-phase is decreasing mortality within a week, it likely is through some modulation of those inflammatory markers that gives patients back some of their reserve. He had a marked reduction in the mass in his throat and then was able to get full dose R-CHOP chemotherapy, which he completed.

Dr. Hamlin: The question of whether dose intensity being maintained at 80% or higher needs to be our goal in the current era of chemoimmunotherapy, I think, is one that we have some data now that really suggest that it may not be as necessary. The French have looked at R-mini-CHOP where they give a 50% dose of cyclophosphamide and doxorubicin. The Japanese groups have looked at a 70% dose reduction and substitution of a non-doxorubicin epirubicin. And...the median age has crept up to 76 and 83 years in studies. Overall response rates are 75% to 80% and event-free survivals seem to be, I think, very acceptable, about a 10% reduction in overall survival. So I think that is a concept that we frequently should be willing to employ and still feel that there’s curative intent.

Dr. Zelenetz: There are some clinical trials. We heard about the R-CHOP plus or minus ibrutinib trial. There’s another important trial we would really like to see accrual to. If you have a patient, they have a DLBCL, you finished treatment, you’re saying, gee, I don’t want this patient to relapse, then send them to Dr. Hamlin. He will be happy to put them on the ZEAL study, which is a randomized study of adjuvant treatment with ibritumomab tiuxetan versus observation (NCT01510184).

REFERENCES

Best of the 31st Annual Miami Breast Cancer Conference®

A review of some of the presentations with the greatest impact on clinical practice.

Dates of Certification:
May 16, 2014–May 16, 2015

Medium: Print with online posttest, evaluation, and request for credit

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Overview

The optimal care of patients with breast cancer is rapidly changing as a result of advances in all aspects of patient care, including prevention, screening, and detection, as well as in the primary treatment modalities of surgery, radiation therapy, endocrine therapy, cytotoxic chemotherapy, and novel biologic therapies. There is an increasing focus on providing multidisciplinary care for patients with complex diseases such as breast cancer through the use of integrated teams of professionals representing the relevant treatment modalities, including surgery, radiation therapy, and systemic medical interventions. The multidisciplinary Miami Breast Cancer Conference® has been bringing together surgical, medical, and radiation oncologists for 31 years, with the aim of fostering awareness of the state-of-the-art treatments in each therapeutic area and encouraging crossteam cooperation in the clinic. This article covers some of the more impactful presentations from the 31st Annual Miami Breast Cancer Conference®.

Target Audience

This educational activity is directed toward medical, surgical, and radiation oncologists interested in the treatment of patients with breast cancer. Fellows, nurse practitioners, nurses, physician assistants, pharmacists, researchers, and other health care professionals interested in the treatment of breast cancer may also participate.

Learning Objectives

As a result of their participation in this activity, the target audience members should be better prepared to:

1. Integrate current guidelines and recent data on local therapy, including new strategies for surgical and radiation therapy, into the treatment of patients with breast cancer.
2. Incorporate biomarkers, molecular assays, and other risk assessment tools in treatment decision making for breast cancer when appropriate.
3. Utilize optimal treatment based on patient characteristics, molecular data, and extent/aggressiveness of disease.

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From March 6 to 9, 2014, the 31st Annual Miami Breast Cancer Conference® convened surgical, medical, and radiation oncologists to foster awareness of state-of-the-art treatments in each therapeutic area. The pioneers of innovative approaches in subspecialties within breast cancer provided insight into the optimal multidisciplinary management of breast cancer, and highlights from some key presentations are provided here.

Individualizing Therapy in Metastatic Breast Cancer Through Genomics and Proteomics

Lance A. Liotta, MD, PhD

In the future, it should be possible to biopsy a metastasis and then molecularly profile the metastasized cells at both the genomic and proteomic levels in order to synthesize that information. In this way, we can discover not only what is overexpressed but also what signaling pathways are activated within the setting of the unique microenvironment of the metastatic site and use that combination of information to tailor therapy to that individual patient. Therapy strategies for recurrent and metastatic breast cancer have been based on profiling the primary tumor, not the metastases, but it makes more sense to profile metastases, which are the lethal aspects of the disease. When tumor cells leave the primary site, they travel to a new microenvironment and encounter different growth factors, immune cells, and extracellular matrix in the secondary organ tissue.

In the Side-Out trial, Liotta’s team performed proteomic analysis to map the signaling network of the metastatic tumor cells and understand which growth or survival pathways are functionally in use in the tissue environment. By studying the phosphorylation events in the microdissected tumor cells, they were able to reveal which signaling pathways were active and which upstream and downstream pathways were connected. The trial combined the proteomic data with genomic profiling information to select candidate therapies. Progression-free survival (PFS) was compared using therapy selected by molecular profiling versus the last therapy the patient was being treated with upon progression. The pre-specified cutoff ratio (1.3) of PFS with molecular profile therapy to PFS with prior therapy

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was met or exceeded by 10 of 25 evaluable patients (40%). The disease control rate (partial response + complete response + stable disease) was 16/25, or 72%. Importantly, molecular profiling yielded a therapy recommendation that was different from the original therapy recommended by the physician for all patients.

Side-Out II is currently enrolling patients, and physicians who believe their patients may benefit from the trial can contact Dr. Liotta at liotta@gmu.edu.2

The New Definition of Surgical Margins: What Are “Adequate” Margins of Resection for Breast-Conserving Therapy? Jay R. Harris, MD

The standard answer to the question of adequate margins of resection has always been 2 mm. However, with highly effective systemic therapy and detailed pathological and mammography evaluation, the current evidence indicates that “no ink on tumor” is sufficient for the large majority of patients.3 This finding prompted the organization of the joint SSO-ASTRO Consensus on Margins in Invasive Breast Cancer, which met in Chicago in July 2013. Upon a review of an updated meta-analysis comprising 33 studies, 28,162 patients, and 1506 local recurrences, the group decided upon several consensus statements.4 First, a positive margin, defined as ink on invasive cancer or ductal carcinoma in situ (DCIS), is associated with at least a 2-fold increase in local recurrence. Also, negative margins (no ink on tumor) optimize local control; wider margin widths do not significantly improve local control; and the routine practice of obtaining wider margins than simply “no ink on tumor” is not indicated. The word “routine” is important because it is not meant to abolish the use of re-excision in certain cases (Table). The key concept is that margins are used (as well as detailed mammographies) not to ensure there is no cancer remaining but to ensure that there is only limited residual cancer, capable of being eradicated with conventional doses of radiation combined with systemic therapy.3

### The Great Margin Counterpoint

J. Michael Dixon, MD, OBE

Dr. Dixon provided detailed counterarguments to the Consensus findings presented by Dr. Harris. According to Dixon, there is often disease at the margin that cannot be seen on radiology. Pathologists find disease that was undiscovered prior to surgery, via imaging, and could not be felt. The meta-analysis that was reviewed by the Consensus panel was the second one; the first meta-analysis showed that close margins increase ipsilateral breast tumor recurrence by an odds ratio of 1.8.3 Also, over time, the prognostic value of margin status was not diminished by a decline in local recurrence rates that was the result of increased systemic therapy use over the years. This meta-analysis concluded that it is reasonable to define a minimum distance of 1 mm as a definition of a negative margin in breast-conserving therapy for invasive breast cancer.

Hidden in the discussion of the second meta-analysis publication is a statement revealing that no tumor ink is actually worse than a wider margin: “Pairwise comparison between distance categories for negative margins (in the adjusted models) showed that the odds of local recurrence were significantly higher for studies using >0 mm relative to 5 mm (P = .021).” Furthermore, this meta-analysis relied on study B 06, in which positive margin was defined as no tumor on ink, and ipsilateral breast tumor recurrence was low. In this study, the presence of residual tumor at the margins often could not be confirmed because the pathology reports did not describe the margins precisely. There was only a 31% concordance in positive margins between central and local pathology; therefore, no reasonable means of verifying margin status existed.6,7

Dixon concluded that many members of the societies that endorse the new consensus statement feel their views were not canvassed, that 1 mm has a huge evidence base, with no good quality evidence to show that more than 1 mm is needed, and that no tumor on ink may be correct, but the evidence from the meta-analyses indicates that it is not sufficient.

### The Great Mammography Debate

Patrick I. Borgen, MD

The Canadian National Breast Screening Study caused a frenzy of media attention leading to doubts about the value of mammography screening.8 Dr. Borgen presented the lesser-known facts about this study and its design that refute the veracity of its findings. The goal of the trial was to compare breast cancer incidence and mortality in women aged 40 to 59 years who did or did not undergo screening mammography. Borgen pointed out, however, that there will never be a randomized clinical trial that shows a survival benefit in women aged 40 to 49 years, because it would require half a million people with long follow-up, as the cure rate in that age group for screen-detected cancer is about 97%.

There were numerous weaknesses in the study design. For

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### TABLE. Factors to be Considered in Re-Excision

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VOL. 10, NO. 2 THE AMERICAN JOURNAL OF HEMATOLOGY/ONCOLOGY 33
example, allocation to either the screening or control arm was left up to the discretion of the nurse or doctor who performed the clinical breast examination. Borgen surmised that no clinician who felt a lump would send a patient to the control arm, thereby skewing the randomization. The data suggest that this guess is true, as there were 666 invasive cancers in the mammography arm and only 524 in the control arm. Also, even though symptomatic patients should be excluded altogether from any screening trial, this trial included patients with advanced lymph node positive breast cancer, and they were randomized significantly more often to the screening arm. In addition, the trial was statistically underpowered, with only one-tenth of the requisite number of patients that would have been required to show a significant reduction. Furthermore, 26% of the women in the control arm had mammograms in their community.

There were also problems with the technology being used. Patients were accrued between 1980 and 1985, so the machines are at least 34 years old by now, and technology has improved tremendously in that time period. The sensitivity of mammograms was only 32% at that time, which is extremely low by today’s standards. There was no funding for modern equipment and no special training for technologists. External reviews of the mammogram quality revealed that half of the mammograms were of such poor quality that they were uninterpretable. And sadly, the authors neglected to mention that 6 other trials, most of which were larger and better performed, showed a substantial reduction in mortality ranging from 20% to more than 40% in women aged 40 to 59 years.

Prolonging the Benefit From Estrogen Blockade in Metastatic Breast Cancer
Joyce O’Shaughnessy, MD

Because clinical benefit rates decline with each subsequent line of endocrine therapy for ER+ metastatic breast cancer, new options are needed to improve patient outcomes. Fulvestrant and anastrozole combination therapy was found to be a reasonable strategy that improved overall survival in patients who were endocrine therapy naïve.9,10 It is still unknown, however, how this combination would work with the 500-mg dose of fulvestrant instead of the 250-mg dose studied. In addition, trials studying fulvestrant with or without PI3K or CDK4/6 inhibition have now entered phase III. An aromatase inhibitor or tamoxifen plus everolimus have also been shown to provide benefit, as long as a steroid mouth rinse is used to prevent the everolimus-associated oral mucositis.11,12 Dr. O’Shaughnessy indicated that this strategy can be particularly useful for patients who have already been treated with nonsteroidal aromatase inhibitors or who cannot tolerate them. She also said that the PI3K or mTORC1/2 inhibitors may be of even greater benefit. Letrozole plus CDK4/6 inhibition is a promising option being tested now in phase III trials, and O’Shaughnessy stated that this combination will probably become our new first-line standard before long.13,14 There is also an ongoing study of the CDK4/6 inhibitor LEE011 with an α-specific PI3K inhibitor. C-src inhibition using dasatinib may delay aromatase inhibitor resistance, particularly in patients who are endocrine therapy naïve. Triplet therapy may be needed to keep these patients free from progression for years.15 The HDAC inhibitor entinostat is also promising with aromatase inhibitors, potentially reexpressing tumor suppressor genes and expressing antigens that predict for immunological priming, leading to greater benefit with subsequent therapy.16 Triplet therapy may also be in store for the future, as O’Shaughnessy mentioned that investigators are now working toward triplet therapy combinations with inhibitors of PI3K, aromatase, and CDK4/6.

Targeting HER2 in 2014: Early-Stage Breast Cancer
Sunil Verma, MD, MSED, FRCPc

Although positive data for trastuzumab in the adjuvant setting have been available for years, differences exist in the neoadjuvant setting, where HR+ patients do not achieve the same level of pathological complete response as HR-/HER+ patients. In addition, patients who are HR+ retain a risk of recurrence beyond 8 years, which is not true of patients who are HR-/HER+.17-21 Further research on optimizing trastuzumab therapy has shown that there is no additional benefit to treating beyond 1 year.19,22 There may be differences in the optimal therapy duration, however, by patient subgroup. The PHARE trial did not meet its noninferiority criteria for 6-month versus 1-year trastuzumab treatment, but certain subgroups of patients, including those with HR+ tumors, did show signs that longer-term trastuzumab treatment may not add benefit.23 Dr. Verma suggested that it would be reasonable to administer non–anthracycline-based therapy for patients with cardiac risk factors, for those for whom the absolute benefit of adjuvant therapy might be low (such as those with T1a or T1b tumors), or for older patients, but stated that he still considers anthracycline to be standard of care. Therefore, he would offer weekly paclitaxel and trastuzumab to patients with T1 N0 HER2+ tumors or to patients who are not suitable candidates for anthracycline- and/or docetaxel-based chemotherapy regimens.

There are a number of exciting new drugs in the pipeline that Verma said will reshape the treatment of early HER2+ breast cancer, such as lapatinib, pertuzumab, and trastuzumab emtansine (T-DM1). Results from the ALTTO trial will be revealed at this year’s American Society of Clinical Oncology (ASCO) meeting, and it remains to be seen if HR-/HER2+ patients in the adjuvant setting can benefit from dual targeted therapy using the tyrosine kinase inhibitor lapatinib with the monoclonal antibody trastuzumab. Other trials to look out for include APHINITY, which in 3 or 4 years will have results of chemotherapy and trastuzumab with or without pertuzumab in the adjuvant setting,24,25 and the KAITLIN trial, which is studying whether...
T-DM1 and pertuzumab will allow the taxane component of chemotherapy to be eliminated. In addition, the ATEMT study trial aims to study whether chemotherapies can be eliminated completely with T-DM1 therapy in patients with tumors up to 3 cm.

Evolving Paradigms in HER2+ MBC: Strategies for Individualizing Therapy With Available Agents
Kimberly L. Blackwell, MD
The preferred regimens for first-line treatment of HER2+ metastatic breast cancer were just recently updated in the NCCN guidelines to include docetaxel + trastuzumab + pertuzumab or paclitaxel + trastuzumab + pertuzumab.27 Lapatinib was found to be detrimental to these patients in the first-line setting, with a lower progression-free survival (PFS) rate than trastuzumab, and therefore should not be considered to be interchangeable with trastuzumab.27

T-DM1 has been shown to have activity in the first-, second-, and third-line settings in patients with HER2+ tumors. While a 5-month improvement in PFS has already been shown in the first-line setting with T-DM1 compared with trastuzumab and docetaxel, results of the MARIANNE trial may lead to a more definitive standard of first-line care if, in fact, pertuzumab + T-DM1 or T-DM1 alone is found to be superior to trastuzumab + taxane.28,29 T-DM1 is the preferred treatment for patients who have seen trastuzumab in the metastatic setting. Dr. Blackwell advised that the most important thing for a patient with a HER2-driven tumor is to continue the HER2 blockade.30,31 T-DM1 has also been shown, through the TH3RESA trial, to be efficacious in a heavily pretreated population, leading to a 2.9-month improvement in median PFS over alternative treatments of the physicians’ choice.32 Besides T-DM1, it has been shown that value exists in adding everolimus to vinorelbine and trastuzumab therapy, but the combination is associated with increased toxicities such as anemia, thrombocytopenia, and febrile neutropenia.33

It is important to remember that there are patients who are both ER+ and HER2+ in the metastatic setting. In these patients, median PFS has improved by 2.4 months using anastrozole and trastuzumab and by 5.2 months using letrozole and lapatinib.34,35 The combination of letrozole and lapatinib has actually been approved by the FDA for this population.36

Another special population of HER2+ patients is those who have brain metastases. The standard of care for these patients is lapatinib and capecitabine, as there are some data showing that lapatinib can penetrate the blood-brain barrier.37 The EMILIA trial did allow for patients with established brain metastases, and did not appear to show an increased risk of progression within the brain with the use of T-DM1, so that may be an option to consider in the future.38 Some evidence exists as well to show that everolimus may penetrate the blood-brain barrier, and it is being studied further now in combination with trastuzumab and vinorelbine.39,40

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