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#### About the art in this issue:

Dina Potter is a contemporary abstract artist based in Seattle, Washington. Her artistic inclinations have driven her since childhood to pursue an education and career in the arts, and she has continued to seek out her artistic passions in design, creation, and painting. Inspiration for her art comes from music, sounds, nature, and the visuals of everyday life. Ms Potter seeks to create the illusion of depth, bold movement, and rhythmic-flowing pattern, exaggerating and abstracting those images and making them her own in each of her creations. Her art has been showcased in numerous exhibits, art walks, and galleries, and has been placed in commercial and residential permanent installations. She also volunteers as an art docent instructor to elementary-level students, and enjoys spending time with her family, trail running, and gardening in her beautiful Pacific Northwest backyard. Learn more about Ms Potter and her work at www.dinapotter.com.

**Cover:**  
*Ghost Tree.* Acrylic on canvas. 60" × 48".

**Articles:**  
*Branchings.* Acrylic on canvas. 48" × 60".
*Sunny Tree.* Acrylic on canvas. 48" × 60".
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*Tunnel of Trees – Gold.* Acrylic on board. 16" × 20".
*Tunnel of Trees – Green.* Acrylic on board. 16" × 20".
Art, Science, and Personalized Cancer Care Through Regional Therapeutics

“…To enable a new era of medicine through research, technology, and policies that empower patients, researchers, and providers to work together toward development of individualized treatments.”

— President Barack Obama

This issue highlights a variety of regional therapies aimed at offering durable disease control, improving quality of life, and impacting survival for patients who present with recurrent, metastatic, or, most often, unresectable disease. Covered herein is a heterogenous group of tumor sites, histologies, patterns of spread, and disease stages, but the intertwining theme is the regional targeting of disease that has failed primary or conventional first-line therapies. In this era of rapidly advancing technology in medicine, clinical oncologists have a wide range of tools for offering cutting-edge treatments to patients. However, no 2 patients with advanced or recurrent disease are exactly alike.

Personalized medicine, especially as it relates to cancer care, is the most important element for successful treatment moving forward. Proof of this came when President Barack Obama announced in 2015 a multimillion-dollar investment from the US federal government aimed at improving our understanding of and ability to develop strategies for treating diseases (most notably, cancers) in a way that takes into account personal differences in genetics and environment. That initiative is focused on promoting discoveries of the specific patient and tumor factors that could predict an individual’s response to treatment. This issue of Cancer Control aims to highlight the personalized care necessary for complex patients with cancer through novel and targeted approaches to difficult and oftentimes incurable disease.

Dr Strom and colleagues detail the difficulties in approaching the clinical scenario of recurrent, unresectable disease in patients with head and neck cancers. With specific and challenging regional anatomical considerations, such as the carotid artery and spinal cord, they show that limiting doses to surrounding structures while providing high doses through conformal stereotactic radiation to recurrent sites in the head and neck can provide outcomes at least as good as standard palliative methods while potentially limiting toxicity and possibly improving quality of life by eliminating the need for prolonged treatments. Outcomes for patients with recurrent, unresectable head and neck cancers still remain poor, with a median survival rate limited to 1 year or less.1

Thus, significant work in the arena of personalized approaches in recurrent head and neck cancers is vital.

In one of the most notable areas in the development of personalized methods, Dr Sloot and coauthors provide an insightful update on intralvesional strategies for the treatment of metastatic or unresectable melanomas. In their article reviewing treatment options for lesions not amenable to surgery, they provide compelling evidence to support the injection of a variety of agents directly into the tumor to reduce tumor volume and overall disease burden, as well as to improve rates of progression-free and, in some cases, overall survival. In addition to the direct antitumor effects observed in the injected primary lesion, many of the proposed agents provide a “bystander effect” — that is, noninjected, anatomically separate lesions show a measurable response likely through an activated immune system.2 Their article highlights the breadth of possibilities in the realm of personalized medicine by proposing treatments (eg, injection in combination with systemic therapies) that take into account tumor genetics, tumor characteristics (eg, sites of metastases, disease-free interval), and patient factors (eg, medical comorbidities, performance status), potentially providing the optimal individualized formula for future treatment.

Historically, distant metastatic disease was considered to be a state of incurability and called for palliative approaches. However, during the past 20 years, advances in radiation and surgical techniques have, in many cases, reversed the mindset regarding treatment of oligometastatic disease to one of potential curative intent. Drs Ahmed and Torres-Roca describe the techniques and outcomes related to the primary use of stereotactic body radiotherapy for the treatment of lung, liver, and spine metastases from a variety of primary tumor sites, including colorectal, breast, prostate, and sarcomas. With careful patient selection based on site, location, disease burden, and histology, personalized dosing and fractionation strategies can lead to favorable local control rates and improved toxicity profiles over conventional palliative regimens.

Keeping in mind the targeted management of oligometastatic disease with strategies aimed at limiting the traditional toxicities of open surgical techniques, Drs Wong and Cooper present the rationale for local ablative approaches for disease metastatic...
to the liver. They review thermal ablative techniques and electroporation and how these techniques, when used alone or in combination with systemic therapy, can meaningfully prolong rates of survival. By assessing each lesion and its anatomical confines, the best-suited, individualized approach can be selected to limit liver toxicity and postablation syndromes while still providing durable response.

When minimally invasive or ablative techniques fail, aggressive multimodality therapies may be necessary to help increase survival, even in the setting of incurable disease. Drs Rajeev and Turaga review use of cytoreductive surgery in combination with hyperthermic intraperitoneal chemotherapy in the setting of peritoneal carcinomatosis. They present favorable median survival data with aggressive application of the cytoreductive surgery/hyperthermic intraperitoneal chemotherapy technique compared with historical palliative approaches. Controversy still exists on the optimal timing, technique, and regimen to be used, but few would argue that reduced disease burden and perfusion of the peritoneal cavity with regional chemotherapy can lead to improved survival and quality of life in patients with this often poor diagnosis. In addition, with individualized selection, applying these strategies as a preventive measure in high-risk disease has been proposed.

Although we are moving toward an era in which abundant information and updated and new technology are available to all, it is still important to remember that our patients are individuals with unique diagnoses, tumor and staging considerations, genetics, lifestyles, ethics, and belief systems. Personalizing our treatment approaches and keeping the individual in mind will allow oncologists to keep the science of medicine at the forefront of innovation.

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References

The Department of Head and Neck and Endocrine Oncology at H. Lee Moffitt Cancer Center is a uniquely specialized group offering individualized treatment for malignancies of the head and neck. With our team of skilled surgeons, medical oncologists, radiation oncologists, pathologists, endocrinologists, radiologists, and supportive care professionals — all of whom only treat head and neck cancers — we take a truly integrative approach to treatment, enabling patients to consult with numerous experts through a single referral.

The department offers an unsurpassed level of expertise by exclusively focusing on the following diagnoses: sarcomas of the head and neck; squamous cell carcinomas and other cutaneous malignancies of the head and neck; oral, laryngeal, oropharyngeal, hypopharyngeal, and nasopharyngeal cancers; nasal and paranasal sinus cancers; major/minor salivary tumors; benign and malignant skull-base tumors; orbital tumors; thyroid and parathyroid tumors; and ear and temporal bone cancers.

We provide each patient with an individualized treatment plan, using evidence-based pathways to select the most appropriate options from our broad spectrum of therapeutic services. From novel chemotherapy agents and highly targeted radiotherapy delivery techniques to minimally invasive, image-guided, and robotic-assisted surgeries, numerous treatments can be combined to address each patient's unique needs. Diagnostic procedures, pain management support, speech and swallowing rehabilitation services, and clinical trials are also available. In addition, due to our emphasis on translation research, we make it possible for patients to benefit from breakthroughs we achieve in our on-site laboratories as soon as possible. Our tumor board meets weekly to explore additional treatment and support options for each patient, ensuring that his or her ever-evolving needs are consistently being met.

To schedule a patient appointment with a physician in the Department of Head and Neck and Endocrine Oncology, call the New Patient Appointment Center at 813-745-3980 or 1-888-860-2778 (during normal business hours).

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Stereotactic Body Radiotherapy for Recurrent Unresectable Head and Neck Cancers

Tobin Strom, MD, Christian Wishka, and Jimmy J. Caudell, MD, PhD

Background
In 2016, an estimated 61,760 new head and neck cancer cases were diagnosed and an estimated 13,190 deaths occurred due to head and neck cancers in the United States. Most patients with head and neck cancers present with locoregional advanced disease and require a multimodal approach to treatment, including a combination of surgery, radiation, or systemic therapy. Despite aggressive management, 40% to 50% of patients will experience locoregional recurrence and, of patients who recur, 50% to 60% will die from disease progression. Following recurrence, surgery is the preferred approach, although fewer than 50% of patients are candidates for salvage surgery because of the location, extent of recurrence, or both, as well as medical comorbidities that may preclude surgery. For patients who are not candidates for salvage surgery, few treatment options remain, including reirradiation, supportive care, or palliative systemic therapy.

Systemic Therapy
Palliative systemic therapy results in a 12-month survival rate ranging from 40% to 50%, but it comes at the
cost of high rates of toxicity.4 A phase 3 trial that included participants with recurrent or metastatic squamous cell carcinoma of the head and neck showed that systemic therapy with cisplatin or carboplatin in combination with fluorouracil and cetuximab, a targeted inhibitor of epidermal growth factor receptor, resulted in a median survival rate of 10 months and an 82% rate of grade 3/4 adverse events.4 Following this study cetuximab in combination with cisplatin was approved for the treatment of metastatic head and neck squamous cell carcinoma. Pemetrexed, a folate antimetabolite, was also tested in a large, randomized, phase 3 trial in combination with cisplatin for recurrent or metastatic head and neck cancers but did not improve overall or progression-free survival rates when compared with combination cisplatin/placebo.6 However, on subgroup analysis, an improvement in survival was seen with pemetrexed/cisplatin among patients with a performance status of 0 or 1 (8.4 vs 6.7 months, respectively) and patients with oropharyngeal cancers (9.9 vs 6.1 months, respectively) compared with cisplatin alone.6 A phase 2 trial assessed combination cisplatin, cetuximab, and pemetrexed among 66 participants with recurrent or metastatic head and neck cancers and reported an overall survival rate of 9.7 months.7 Survival outcomes with this triplet therapy were similar to those seen with other systemic therapy alternatives, although treatment-related deaths were higher than expected; therefore, this triplet combination was not recommended for routine use.7

Conventionally fractionated radiotherapy in combination with systemic therapy has also been attempted for locoregional control of recurrent head and neck cancers with moderate success. In the early 1990s, Vokes et al8 first investigated concurrent chemoradiotherapy with 5-fluorouracil and hydroxyurea in study patients who had previously received irradiation for their recurrent head and neck cancers. The researchers reported treatment-related deaths in 5 of 45 study patients (11%) and concluded that reirradiation was feasible but that further research was needed.8

Subsequently, the first multi-institutional, phase 2 prospective reirradiation head and neck trial was reported by Spencer et al.9 This benchmark trial included 79 participants who had previously received radiotherapy for recurrent or new primary head and neck cancers. The study patients were treated with 60 Gy in 1.5 Gy twice-daily fractions concurrently with fluorouracil and hydroxyurea.9 The rates of median and 1-year survival were 8.5 months and 40.5%, respectively, with 14 participants (17.7%) experiencing grades 4/6 toxicity and 7.6% with grade 5 acute toxicity.9 Langer et al10 performed a separate prospective phase 2 trial that enrolled 105 patients who were similarly treated to a total dose of 60 Gy with twice-daily 1.5 Gy fractions, 5 days per week every other week; study participants were also treated with concurrent cisplatin and paclitaxel as radiosensitizers. The median survival rate was 12 months, and the rate of toxicity was similar to previous reirradiation studies; 8 deaths (8%) were also attributed to the treatment, including 2 cases of late carotid hemorrhage.10 A trial update in 2012 reported a 5-year survival rate of 15% and an estimated 10-year survival rate of 5% with 6 study patients alive.11 These results were promising, yet room for improvement remains.

One possible explanation for suboptimal outcomes with reirradiation is that cancer cells from previously radiated, recurrent head and neck tumors are radioresistant.12 To overcome the inherent radioresistance of recurrent head and neck cancers and to limit the rate of toxicity to healthy, surrounding tissues, dose-escalated and focused reirradiation using stereotactic body radiotherapy (SBRT) has been investigated.

What Is Stereotactic Body Radiotherapy?
SBRT is the delivery of large daily fractions of highly focal radiotherapy, typically 5 to 30 Gy per fraction in 1 to 5 fractions, using image guidance and other techniques to account for tumor motion.13 Initial reports of SBRT in early-stage, inoperable lung cancer had local control rates that exceeded 90%.14 SBRT has also shown good local control rates with 1 to 3 metastatic lesions of the liver, oligometastatic lung cancer, renal cell carcinoma, and metastatic lesions to the spine, as well as when administered after a prior course of SBRT for locally recurrent disease or limited metastatic disease progression.15-19 Given that SBRT delivers a high dose of radiation per fraction, the mechanism of tumor cell death was initially hypothesized to be a result of the effects of radiation on the tumor vasculature, thus causing endothelial cell death at doses of more than 7 Gy/fraction.20 However, new evidence suggests that SBRT maintains the classic pathways of radiation-induced cellular death along with possible enhanced antitumor immunity from high fractional doses.15

Reirradiation
Experiences with SBRT for the treatment of locally recurrent head and neck cancers following prior radiation are presented in the Table.4,9,10,21-28 SBRT provides a potential advantage over other salvage therapies because it requires a shorter treatment course and has potentially fewer systemic adverse events compared with conventional chemoradiotherapy or protracted courses of systemic therapy alone.4,10,28 Heron et al29 performed the first phase 1 dose-escalation trial with SBRT for recurrent squamous cell carcinoma of the head and neck in the reirradiation setting. A total of 25 study participants received radiation doses starting at 5 Gy that escalated to 8.8 Gy per fractions for 5 fractions over 2 weeks.29 The researchers initially reported a maxi-
mum tolerated total dose of 44 Gy with no grade 3/4 acute toxicities and an overall response rate of 76%, a median duration of response of 4 months, and a median survival rate of 6 months.29 An update of the report, which included 85 enrolled patients, revealed a radiation dose response with improved local control at reirradiation doses of at least 35 Gy (71% vs 59%, respectively) compared with less than 35 Gy.25 Following this study and reports of improved survival rates with the addition of cetuximab to systemic therapy, another group investigated concurrent cetuximab with SBRT and compared it with SBRT alone (both treatment groups, n = 35); both study arms were given a median radiation dose of 40 Gy (range, 20–44 Gy).4,25,30 The researchers reported improved rates of survival in the combined cetuximab and SBRT group compared with those assigned to SBRT alone (median 24.5 vs 14.8 months, respectively).30 In 2014, the trial data were updated to include more enrollees (132 participants) treated to a median dose of 44 Gy (range, 35–50 Gy) and had a short median follow-up of 6 months.28 Following these results, Lartigau et al23 headed a phase 2 multi-institutional trial to assess reirradiation using SBRT with concurrent cetuximab in 56 patients with recurrent or new primary head and neck cancers. Participants — all with squamous cell carcinoma — were treated with 36 Gy in 6 fractions for 11 to 12 days.23 Use of SBRT resulted in consistent median and 1-year survival rates of 11.8 months and 25 mL.28 Thus, these prospective studies of reirradiation using SBRT helped to refine the optimal radiation dose and fractionation for reirradiation using SBRT, and the survival results for treatment with concomitant SBRT and cetuximab surpassed previous results with chemoradiation or systemic therapy alone.

Two sites in Europe have also reported outcomes with reirradiation using SBRT. A group in France led by Comet et al22 performed a feasibility study with salvage reirradiation using SBRT with or without cetuximab for the treatment of locally recurrent or new primary head and neck cancers. In this phase 1 trial, 40 study patients with 43 lesions were treated to a total dose of 36 Gy in 6 fractions prescribed to the 85% isodose line, 15 (37.5%) were treated with concurrent cetuximab, and 1 was treated with concurrent cisplatin.22 One-half of the study patients had squamous cell carcinoma histology.22 Rates of median and 1-year survival were 13.6 months and 58%, respectively. Of the 34 study patients who were evaluable for response, 15 (44%) had a complete response, 12 (35%) had a partial response, and 7 (21%) had stable disease.22 Of the 14 evaluable study patients treated with concurrent cetuximab, 75% had an overall objective response.22

Following these results, Lartigau et al23 headed a phase 2 multi-institutional trial to assess reirradiation using SBRT with concurrent cetuximab in 56 patients with recurrent or new primary head and neck cancers. Participants — all with squamous cell carcinoma — were treated with 36 Gy in 6 fractions for 11 to 12 days.23 Use of SBRT resulted in consistent median and 1-year survival rates of 11.8 months and

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Table. — Select Studies for the Treatment of Locoregional, Recurrent Head and Neck Cancers After Prior RT

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>No. of Participants</th>
<th>Median Survival Rate, mo</th>
<th>1-y Overall Survival Rate, %</th>
<th>Grade 4/5 Late Toxicity, %</th>
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</thead>
<tbody>
<tr>
<td>Cengiz21</td>
<td>SBRT</td>
<td>46</td>
<td>11.9</td>
<td>47</td>
<td>15.6</td>
</tr>
<tr>
<td>Comet22</td>
<td>SBRT, SBRT + cetuximab</td>
<td>40</td>
<td>13.6</td>
<td>58</td>
<td>0</td>
</tr>
<tr>
<td>Langer10</td>
<td>RT + cisplatin, paclitaxel</td>
<td>105</td>
<td>12.1</td>
<td>50.2</td>
<td>20</td>
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<tr>
<td>Lartigau23</td>
<td>SBRT + cetuximab</td>
<td>56</td>
<td>11.8</td>
<td>47.5</td>
<td>2</td>
</tr>
<tr>
<td>Roh24</td>
<td>SBRT</td>
<td>36</td>
<td>16.2</td>
<td>52.1</td>
<td>8.6</td>
</tr>
<tr>
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<td>SBRT</td>
<td>85</td>
<td>11.5</td>
<td>48.5</td>
<td>0</td>
</tr>
<tr>
<td>Spencer2</td>
<td>RT + fluorouracil, hydroxyurea</td>
<td>79</td>
<td>8.5</td>
<td>40.5</td>
<td>3</td>
</tr>
<tr>
<td>Siddiqui28</td>
<td>SBRT</td>
<td>44</td>
<td>6.7 (recurrent)</td>
<td>38.1 (recurrent)</td>
<td>9.1</td>
</tr>
<tr>
<td>Unger27</td>
<td>SBRT</td>
<td>65</td>
<td>12</td>
<td>~ 50</td>
<td>9</td>
</tr>
<tr>
<td>Vargo28</td>
<td>SBRT + cetuximab</td>
<td>132</td>
<td>7</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>Vermorken4</td>
<td>Cisplatin, fluorouracil, cetuximab × 6</td>
<td>222</td>
<td>10.1</td>
<td>40</td>
<td>31</td>
</tr>
</tbody>
</table>

RT = radiotherapy, SBRT = stereotactic body radiotherapy.
of the 49 evaluable study participants, the objective response rate was 69%, complete response was seen in 24 (49%), partial response in 10 (20%), and stable disease in 11 (23%).

Eighteen study patients (32%) experienced toxicities of grade 3 or higher and 1 patient died from arterial rupture. These results were comparable with those seen in the study conducted by Heron et al,30 Lartigau et al23 attributed the low rate of carotid rupture (ie, 1 occurrence) to the careful selection of patients without tumor encasement of less than one-third of the carotid artery.

Cengiz et al21 also performed a retrospective analysis of 46 study participants with locally recurrent head and neck cancers treated with reirradiation using SBRT to a median dose of 30 Gy (range, 18–35 Gy) for 1 to 5 fractions. Most of those enrolled in the study had squamous cell carcinoma; 16 participants (35%) had nonsquamous histologies.21 The median overall survival rate was 46%,21 A total of 10 of 37 evaluable study patients (27%) had a complete response, 11 (30%) had a partial response, and 10 (27%) had stable disease on imaging.21 Despite these comparable survival outcomes with other studies, the late-grade (≥4) toxicity rate was higher: 8 study patients (17%) experienced late carotid blowout, 7 (15%) of whom died from carotid hemorrhage.21 It has been suggested that the relatively high rate of late toxicity in this study was a result of daily SBRT fractionation, rather than an every-other-day fractionation scheme, as seen in most other studies.28

Two other studies addressing reirradiation using SBRT have provided consistent 1-year survival rates between 40% and 50%, although each has had subtle variations in treatment regimens, patient selection criteria, and treatment regimens.24,27 Unger et al27 reported their experience with 65 study patients treated with palliative reirradiation with SBRT for recurrent head and neck cancers. The study included 27 participants (42%) with metastatic disease or untreated local disease, 11 (17%) with nonsquamous histologies, 19 (29%) treated with surgery prior to reirradiation, and 21 (32%) treated with concurrent chemoradiation; the SBRT doses ranged from 21 to 35 Gy in 2 to 5 fractions.27 The group reported an overall response rate of 80%, a complete response rate of 54%, a partial response rate of 27%, and no response of 27% by laryngopharyngoscopy, biopsy, imaging, or all 3 procedures.27 The median rate of survival was 12 months and the 2-year survival rate for participants with nonmetastatic cancer at the time of treatment was 41%.27 Seven enrollees (11%) experienced late toxicities related to reirradiation, and 1 study patient died due to treatment.27

A Korean trial conducted by Roh et al24 enrolled 36 patients (totalling 44 lesions) who were treated for locally recurrent head and neck cancers using SBRT at 18 to 40 Gy (median, 30 Gy) in 3 to 5 fractions. More than one-half of the lesions were squamous cell carcinoma, although other histologies were included, and a median survival rate of 16 months was reported with a complete response rate of 43%, a partial response rate of 37%, and stable disease in 9%.34 Grade 3 acute complications were reported in 13 participants (36%) and late complications were reported in 3 (8%).34 The median survival rate in this study was 16 months; however, a high rate of late grade (≥4) toxicities was seen, which others have suggested were likely the result of daily radiation rather than every-other-day delivery.24,28

Reirradiation experiences with or without cetuximab offer promising and relatively consistent rates of median survival (~12 months) comparable with previously reported, protracted courses of chemoradiation and chemotherapy alone (see Table).4,10 However, despite this, subtle differences in selection criteria and tumor histology exist between studies, along with slightly different radiation doses and fractionations, making it difficult to directly compare each study. As a result, a large, multi-institutional phase 3 trial examining reirradiation using SBRT would be useful, especially to evaluate whether the 24-month rate of median survival reported by Heron et al30 is reproducible in a multi-institutional setting.

Toxicity

With reirradiation of normal tissues, it is important to be aware of possible late toxicities and to limit doses to normal tissue in order to minimize late effects. Both carotid blowout syndrome and spinal cord myelopathy are rare, but they are highly morbid conditions that can present as fatal, late complications of reirradiation.31,32 An animal model for studying reirradiation to the spinal cord was performed by Medin et al,35 in which 23 pigs were treated with 30 Gy in 10 fractions to 10 cm of the spinal cord and then stratified into 6 groups, with mean maximum reirradiation doses escalating from 14.9 to 25.4 Gy. The group reported a steep dose-response relationship with no neurological changes when using a maximum spinal cord dose below 18.8 Gy and 100% with neurological changes when using a dose higher than 21.3 Gy; they also found a 50% incidence of paralysis at a dose of 19.7 Gy (ie, median effective dose).33 When compared with other pigs treated with de novo, single-dose spinal cord irradiation, Medin et al34 found a nearly identical median effective dose of 20.0 Gy.

In a feasibility study for reirradiation of the head and neck with concurrent chemotherapy, Balermpas et al35 treated 5 study patients with reirradiation and limited the cumulative spinal cord maximum dose to 67 Gy without observing any late cord toxicities. Other groups have reported successfully limiting the
The maximum dose of spinal cord reirradiation to 8 Gy in 1 fraction, 12 Gy in 2 fractions, 8 Gy in 5 fractions, and 6 Gy in 6 fractions without a single reported late case of radiation-induced myelopathy.\textsuperscript{23,25,29}

By contrast, carotid blowout syndrome occurs more frequently with reirradiation of the head and neck, but few risk factors for carotid blowout syndrome have been identified. A Japanese study led by Yamazaki et al\textsuperscript{36} published the results of a trial of 381 participants who were treated with 484 reirradiation sessions to a median dose of 30 Gy in 5 fractions for recurrent head and neck tumors. Of those participants, 32 (8.4\%) experienced carotid blowout syndrome, and the researchers reported a 1-year survival rate of 37.5\% among these study patients.\textsuperscript{36} Tumor invasion of the overlying skin was the single significant risk factor associated with carotid blowout syndrome on multivariate analysis (odds ratio, 1.96; $P$ = .04).\textsuperscript{36} A literature review of carotid blowout syndrome following reirradiation of the head and neck reported an increased risk of carotid blowout syndrome with an accelerated hyperfractionated (1.5 Gy twice daily, 5 days per week, alternating weeks) or delayed hyperfractionated radiation regimen, compared with conventional fractionation, although the overall incidence of carotid blowout syndrome remained low (4.5\% vs 1.3\%, respectively); however, studies of reirradiation using SBRT were not included in the review.\textsuperscript{29} In an attempt to reduce the risk of carotid blowout syndrome, 1 study of reirradiation using SBRT limited the carotid radiation dose to less than 20 Gy and reported no late cases of carotid blowout syndrome.\textsuperscript{23} Another study by Yaciz et al\textsuperscript{27} retrospectively compared daily (n = 43) with every-other-day (n = 32) reirradiation using SBRT for the treatment of head and neck cancers and reported improved survival rates and no incidence of carotid blowout syndrome when irradiation was provided as an every-other-day regimen (23 vs 11 months and 9 vs 23 months, respectively). No events related to carotid blowout syndrome were reported in study patients who received a maximum carotid artery dose (< 34 Gy).\textsuperscript{57}

**Conclusions**

Stereotactic body radiotherapy is a promising but still evolving strategy for the treatment of locally recurrent, unresectable head and neck cancers following definitive radiotherapy. In general, the 1-year survival rates following treatment are similar to both chemotherapy alone and concurrently, conventionally fractionated radiotherapy and chemotherapy. Treatment-related adverse events of reirradiation using stereotactic body radiotherapy are difficult to compare head-to-head with systemic palliative therapies given the relatively small volume of tissue treated, although it is now known that late carotid rupture is a rare — but concerning — late adverse event. Reirradiation using stereotactic body radiotherapy offers patients a potential logistical advantage compared with other palliative treatments, because it requires 1 to 2 weeks of treatment, rather than up to 18 weeks of maintenance cetuximab or 6 to 7 weeks of daily treatment with conventional chemoradiation. Initial experiences with combined cetuximab and reirradiation also appear to have benefit in the reirradiation setting, although larger, confirmatory studies are needed.

**References**


Intralesional therapy is a possible treatment option for patients with metastatic melanoma due to its good local response and tolerable adverse-event profile.

Developments in Intralesional Therapy for Metastatic Melanoma
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Background: Locoregional advanced melanoma poses a complex clinical challenge that requires a multidisciplinary, patient-centered approach. Numerous agents have been studied for their suitability as intralesional therapy in the past decades, but few have successfully completed phase 3 clinical trial testing.

Methods: The relevant medical literature was searched for articles regarding use of intralesional therapies in metastatic melanoma. Therapies with data from phase 2 or higher studies were selected for review. This review also summarizes the mechanisms of action, adverse-event profiles, and clinical data for these agents.

Results: Intralesional therapies demonstrate promising effects in select patients with advanced melanoma. The optimal approach should be individually tailored and consist of a combination of intralesional therapies, regional perfusions, systemic immunotherapies, targeted therapies, and surgery, if necessary.

Conclusions: Due to its relatively good local response rates and tolerable adverse-event profile, intralesional therapy may be a treatment option for select patients with unresectable, locally advanced or metastatic melanoma.

Introduction
Melanoma is accountable for most deaths related to skin cancer.1 In 2016, an estimated 76,380 new cases of melanoma will be diagnosed and approximately 10,130 people will die from the disease in the United States alone.1 Although cure rates are high if the disease is discovered when confined to its primary location, metastasis frequently occurs.1 A unique clinical challenge posed by locoregional metastasis, also known as intralymphatic metastasis, occurs when metastasis develops between the primary melanoma and the draining lymph-node basin. This type of metastasis, which occurs in 5% to 10% of patients with melanoma, has traditionally been classified into 2 categories: satellite metastases (located < 2 cm from the primary tumor) and in-transit metastasis (located ≥ 2 cm from the primary tumor).2,3

Surgical resection is the standard of care for patients whose disease is limited enough to be rendered with no evidence of disease. If disease is confined to the limb, then unresectable disease can be amenable to locoregional treatment. For example, regional perfusion therapies, such as isolated limb infusion or hyperthermic-isolated limb perfusion, have demonstrated objective response rates (ORRs) of 50% to 90%.4,5 These
treatments can be repeated multiple times, depending on response and rate of toxicity. The disadvantages of limb infusion and perfusion include associated regional toxicity, morbidity from a surgical procedure, and applicability to disease confined to the extremities alone (eg, not applicable to in-transit metastasis on the trunk). Although radiotherapy is frequently used to treat microscopic disease in an adjuvant setting, macroscopic melanoma is difficult to treat with radiotherapy and has been used to treat individual lesions or localized clusters with anecdotal success; however, wide-field irradiation is associated with morbidity and is not a preferred first-line modality.6,7

Patients with limited locoregional disease often have few symptoms. Consequently, physicians are less likely to recommend systemic or regional perfusion-based therapy that could expose asymptomatic patients to considerable toxicity. These patients may benefit from intralesional therapy, where the active agent is immediately injected into the tumor, exerting mainly local effects, with fewer adverse events than systemic or regional therapy.3 Intralesional therapies have been extensively studied, but effective agents have not been available until recently.6 However, similar to the rapid development of multiple new systemic treatments for stage 3/4 metastatic melanoma (nivolumab, ipilimumab, trametinib, dabrafenib, vemurafenib, pembrolizumab, cobimetinib, pegylated interferon), intralesional injections and topical therapies have seen major advances.8,9 Due to their rate of efficacy and relatively low toxicity profile, these treatment modalities may be promising in select patients with locoregional disease.3

Intralesional therapy was first reported in 1893 by Coley,10 which was prior to the report published by Handley11 on wide local excision as the mainstay of melanoma treatment. Local therapy increases rates of efficacy and lowers rates of toxicity when compared with systemic administration by delivering an increased concentration of the drug locally.3,12 A so-called “bystander effect” has been reported in select agents, including velimogene aliplasmid, 10% rose bengal, and talimogene laherparepvec, where noninjected (both visceral and nonvisceral) distant lesions respond to the locally injected drug.2,3 Although the exact mechanism of action is under investigation, tumor antigens in the injected lesions may serve as an autologous vaccine, stimulating systemic immunity.12,14 The occurrence of the bystander effect makes intralesional therapies appealing because local injections have been associated with a systemic reduction in tumor burden.15

Generally, lesions are treated using a 25- to 30-gauge needle using a “fanning” technique, where the needle is moved in multiple directions within the same lesion. Preferably, the same needle entry and needle stick are used to keep the number of needle tracks and cavities in the tumor limited to prevent intralesional injectate from leaking out and to maximize the delivered dose. Visible or palpable lesions can be injected in the ambulatory clinic, whereas deeper lesions can be injected using ultrasonographic guidance. Tumor response may be measured using caliper measurements, ultrasonography, or cross-sectional imaging (magnetic resonance imaging/computed tomography), depending on tumor size and location.9 Evidence suggests that subcutaneous lesions are less responsive than cutaneous lesions, and tumors with smaller bulk are more likely to regress under treatment.16-18 Investigators have attempted to limit intralesional volumes to 1 mL or less to minimize the local adverse events that result from injecting higher volumes.16

This review will summarize the mechanisms of action, adverse-event profiles, and clinical data for all agents currently in use and of historic importance (Tables 1 and 2).9,12,13,16,18-41

**Velimogene Aliplasmid**

Velimogene aliplasmid is an intralesional agent that advanced to phase 3 clinical trial testing based on results seen in phase 1/2 trials; however, both phase 3 trials conducted with velimogene aliplasmid failed to reach their primary end point (NCT00395070).24,25 Velimogene aliplasmid is classified as a gene therapy because it contains plasmid DNA encoding for HLA-B7.25 It recruits macrophages and T cells, which attack injected and noninjected lesions alike, bringing about immune responses against the alloantigen. Most of the initial studies were limited to study participants negative for HLA-B7; however, after no correlation between HLA status and response rate was found, other studies did not incorporate HLA status as an inclusion criterion.16 Reported adverse events include paresthesias, asthenia, myalgias, fatigue, injection-site pain, rigors, and flulike symptoms.16

Velimogene aliplasmid was first investigated in 4 small phase 1 trials with up to 17 study participants and reported response rates reaching 50%.20-23 The study of this drug advanced to 4 phase 2 trials that reported ORRs ranging from 10% to 28%.16,25,27 The most frequently reported schemes used 2 mg velimogene aliplasmid per lesion with 1- to 2-week intervals.16,27 The largest study was a dose-escalation/efficacy trial conducted by Bedikian et al,16 who enrolled 133 patients and assigned them to groups that received 0.5 to 2 mg velimogene aliplasmid for 6 weeks with 1-week intervals. A total of 127 participants were treated with the highest dose; efficacy data were also available for all enrollees.16 Complete response (CR) was reached in 3% and partial response (PR) in 9%.16

In the first phase 3 study, Richards et al24 randomized 202 patients to either systemic dacarbazine/velimogene aliplasmid on days 3 and 10 out of 28 to the chemotherapeutic cycle (n = 98) or dacarbazine alone
Response rates were 13.2% and 11.6%, respectively. Adding velimogene aliplasmid did not cause any significant difference in median time to progression (1.9 vs 1.6 months) or survival (10.8 vs 9.2 months). The second phase 3 trial was stopped early when no difference was shown in ORR at more than 24 weeks and in overall survival rate for the 390 study participants, who were randomized 2:1 to either velimogene aliplasmid or physician’s choice of chemotherapy (dacarbazine or temozolomide; NCT00395070). No new trials are planned for velimogene aliplasmid.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Treatment</th>
<th>Documented Bystander Effect</th>
<th>No. of Participants</th>
<th>Dosing</th>
<th>Dosing Interval</th>
<th>Treatment Duration</th>
<th>CR, %</th>
<th>PR, %</th>
<th>SD, %</th>
<th>PD, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bedikian16</td>
<td>Velimogene aliplasmid</td>
<td>Yes</td>
<td>127</td>
<td>0.5–2 mg</td>
<td>Once weekly</td>
<td>6 wk</td>
<td>3</td>
<td>9</td>
<td>25</td>
<td>63</td>
</tr>
<tr>
<td>Stopeck19</td>
<td>Velimogene aliplasmid</td>
<td>No</td>
<td>51</td>
<td>10 µg</td>
<td>Wk 1–4, 8, 9</td>
<td>≤ 6 cycles</td>
<td>2</td>
<td>16</td>
<td>24</td>
<td>59</td>
</tr>
<tr>
<td>Gonzalez27</td>
<td>Velimogene aliplasmid</td>
<td>No</td>
<td>77</td>
<td>10 µg</td>
<td>Once weekly/6 wk</td>
<td>≤ 3 cycles</td>
<td>3</td>
<td>7</td>
<td>23</td>
<td>68</td>
</tr>
<tr>
<td>Karakousis28</td>
<td>BCG</td>
<td>No</td>
<td>8</td>
<td>0.1 mL of 4 × 10^9 to 9 × 10^9 viable organisms/mL</td>
<td>NA</td>
<td>Once</td>
<td>75</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Kidner41</td>
<td>BCG/imiquimod</td>
<td>No</td>
<td>19</td>
<td>3 × 106 cfu/5%</td>
<td>5–7 d/wk for every 2 wk</td>
<td>2 injections titrated to local inflammation</td>
<td>56</td>
<td>11</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>Marty23</td>
<td>ECT/Bleo</td>
<td>No</td>
<td>41b</td>
<td>≤ 1000 IU/cm², depending on tumor size</td>
<td>NA</td>
<td>Once</td>
<td>73</td>
<td>11</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>ECT/Cis</td>
<td></td>
<td></td>
<td>≤ 2 mg/cm², depending on tumor size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Byrne30</td>
<td>ECT/Bleo vs Bleo vs ECT</td>
<td>No</td>
<td>19</td>
<td>1 U/mL tumor volume</td>
<td>4, 8, or 12 wk</td>
<td>4, 8, or 12 wk</td>
<td>72</td>
<td>5</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td>Heller22</td>
<td>ECT/Bleo vs Bleo vs electroeptoration</td>
<td>No</td>
<td>34</td>
<td>0.025 U, 1250 V/cm</td>
<td>Once</td>
<td>Once</td>
<td>89</td>
<td>10</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Mir31</td>
<td>ECT/Bleo</td>
<td>No</td>
<td>20</td>
<td>18 or 27 U/m², 1300 V/cm</td>
<td>Once</td>
<td>Once</td>
<td>53</td>
<td>39</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Ridolfi24</td>
<td>GMCSF, IL-2</td>
<td>No</td>
<td>16</td>
<td>150 ng, 3 million IU</td>
<td>Every 21 d</td>
<td>6 cycles</td>
<td>0</td>
<td>13</td>
<td>69</td>
<td>19</td>
</tr>
<tr>
<td>Boyd46</td>
<td>IL-2</td>
<td>No</td>
<td>39</td>
<td>10.4 MIU</td>
<td>Biweekly</td>
<td>4 cycles</td>
<td>51</td>
<td>31</td>
<td>18</td>
<td>(SD/PD)c</td>
</tr>
<tr>
<td>Weide18</td>
<td>IL-2</td>
<td>No</td>
<td>48</td>
<td>0.3–6.0 MIU</td>
<td>3 × wk</td>
<td>1–32 wk</td>
<td>69</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Thompson23</td>
<td>10% rose bengal</td>
<td>Yes</td>
<td>80</td>
<td>NA</td>
<td>Wk 1, 8, 12, 16</td>
<td>≤ 4 cycles</td>
<td>26</td>
<td>25</td>
<td>18</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>20</td>
<td>NA</td>
<td>Once</td>
<td>1 cycle</td>
<td>20</td>
<td>20</td>
<td>35</td>
<td>25</td>
</tr>
<tr>
<td>Senzer38</td>
<td>Talimogene laherparepvec</td>
<td>Yes</td>
<td>50</td>
<td>10^4 PFU first dose, then 10^4 PFU thereafter</td>
<td>First interval 3 wk, then every 2 wk</td>
<td>≤ 24</td>
<td>16</td>
<td>10</td>
<td>24</td>
<td>50</td>
</tr>
<tr>
<td>Andtbacka39</td>
<td>Talimogene laherparepvec</td>
<td>No</td>
<td>295</td>
<td>10^4 PFU first dose, then 10^4 PFU thereafter</td>
<td>First interval 3 wk, then every 2 wk</td>
<td>NR</td>
<td>11</td>
<td>16</td>
<td>73</td>
<td>(SD/PD)c</td>
</tr>
<tr>
<td></td>
<td>GMCSF</td>
<td>No</td>
<td>141</td>
<td>125 µg/m²</td>
<td>Daily × 14 d every 4 wk</td>
<td>NR</td>
<td>1</td>
<td>5</td>
<td>94</td>
<td>(SD/PD)c</td>
</tr>
</tbody>
</table>

*Only studies with sufficient data regarding responses are included.
*Multiple tumor types are included, but responses are not split for study patients with melanoma and without melanoma. Bleo/Cis is equally effective.
*Responses were not split out.
Bleo = bleomycin, Cis = cisplatin, CR = complete response, ECT = electrochemotherapy, GMCSF = granulocyte macrophage colony-stimulating factor, IL-2 = interleukin 2, NA = not applicable, NR = not reported, PD = progression of disease, PFU = plaque-forming unit, PR = partial response, SD = stable disease.

(n = 104). (n = 104). Response rates were 13.2% and 11.6%, respectively. Adding velimogene aliplasmid did not cause any significant difference in median time to progression (1.9 vs 1.6 months) or survival (10.8 vs 9.2 months). The second phase 3 trial was stopped early when no difference was shown in ORR at more than 24 weeks and in overall survival rate for the 390 study participants, who were randomized 2:1 to either velimogene aliplasmid or physician’s choice of chemotherapy (dacarbazine or temozolomide; NCT00395070). No new trials are planned for velimogene aliplasmid.
Bacille-Calmette-Guerin

Bacille-Calmette-Guerin (BCG) has been historically used in intralesional therapy, but it has a severe adverse-event profile. The aim of using BCG for intralesional therapy against metastatic melanoma is to stimulate an immune reaction to eliminate the tumor using the patient’s own immune system. BCG is a live, attenuated strain of *Mycobacterium bovis*, which is an antigen that can trigger an immune reaction. In animal models, BCG produces a nonspecific immune response. In humans, it has been used for intralesional therapy in patients who have already demonstrated an immune reaction to BCG to stimulate an immune response against the injected lesion. Adverse events include fevers, chills, diaphoresis, arthralgias, malaise, and angioedema in patients positive for tuberculin and those with lymphadenopathy, pneumonitis, BCG granulomas, and granulomatous hepatitis. Toxicity is caused by the patient having an immune response to BCG; thus, patients who have no immunity against BCG cannot demonstrate adverse events.

Seigler et al recruited 160 patients with locally recurrent melanoma who were treated with intralesional BCG using a 4-stage approach. In the first stage, participants who were immune sensitive to BCG were selected; in the second stage, a delayed hypersensitivity reaction to BCG was stimulated in participants with booster therapy; in the third stage, adoptive immunity was achieved by harvesting participant lymphocytes, which were exposed to tumor cell samples and re injected into the participants; and, in the fourth stage, to further increase antitumor responsiveness, the participants were injected with a vaccine of tumor cells and BCG. Of the 70 study patients evaluated in stage 1, 44% (31) were sensitive to BCG, and, as those study patients progressed through the 4 stages, they demonstrated increased rates of antitumor immune responsiveness. Of the 62 participants examined for cell-mediated, tumor-specific immunity, 69% (n = 43) had a prolonged response, with 60% mean tumor lysis. Of the 19 study patients who never developed immunity against melanoma, all of them progressed and died of complications from diffuse, distant metastatic disease. Although results from early clinical trials correlated well with the rationale for BCG intralesional therapy, the adverse-event profile of BCG is a limitation to its broad implementation. And, although BCG uses *M. bovis* to stimulate an immune, antitumor response, it also produces complications associated with that same immune response, leading to adverse events and disseminated intravascular coagulation at a rate of 12%. Because of these inflammatory reactions and the concomitant high risk of morbidity, BCG treatment requires that patients be closely observed. Prophylactic treatment should be provided, such as antihistamines and isoniazid, because of the morbidity of these adverse events. In addition, to minimize the morbidity of these reactions when they do occur, signs or symptoms of these complications should be treated with hydration, antituberculosis therapy, steroids, antihistamines, and supportive care.

**Electrochemotherapy**

Electrochemotherapy (ECT) is used as an intralesional therapy that delivers agents into the treated lesion. ECT applies high-intensity, pulsed electrical current to the treated lesion that renders the tumor cells permissive for the uptake of drugs, viruses, or genetic material. By contrast, electroporation delivers the current to the lesion without the need of additional agents. Therefore, ECT can be used to deliver therapeutic agents.

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### Table 2. — Select Treatment-Related Adverse Events of Intralesional Therapy

<table>
<thead>
<tr>
<th>Type of Therapy</th>
<th>Adverse Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacille-Calmette-Guerin&lt;sup&gt;21,28-30&lt;/sup&gt;</td>
<td>Angioedema (with positive tuberculin test)</td>
</tr>
<tr>
<td></td>
<td>Bacille-Calmette-Guerin granulomas</td>
</tr>
<tr>
<td></td>
<td>Disseminated intravascular coagulation</td>
</tr>
<tr>
<td></td>
<td>Lymphadenopathy</td>
</tr>
<tr>
<td></td>
<td>Pneumonitis</td>
</tr>
<tr>
<td>Electrochemotherapy + bleomycin/cisplatin&lt;sup&gt;22,35&lt;/sup&gt;</td>
<td>Pain at injection site</td>
</tr>
<tr>
<td>Granulocyte macrophage colony-stimulating factor&lt;sup&gt;34&lt;/sup&gt;</td>
<td>Flulike symptoms</td>
</tr>
<tr>
<td>Interleukin 2&lt;sup&gt;24,36&lt;/sup&gt;</td>
<td>Flulike symptoms</td>
</tr>
<tr>
<td>Rose bengal 10%&lt;sup&gt;12,13,23,37&lt;/sup&gt;</td>
<td>Blistering</td>
</tr>
<tr>
<td></td>
<td>Headache</td>
</tr>
<tr>
<td></td>
<td>Inflammation</td>
</tr>
<tr>
<td></td>
<td>Skin discoloration</td>
</tr>
<tr>
<td>Talimogene laverparepvec&lt;sup&gt;9,39,40&lt;/sup&gt;</td>
<td>Cellulitis</td>
</tr>
<tr>
<td></td>
<td>Fatigue</td>
</tr>
<tr>
<td>Velimogene aliplasmid&lt;sup&gt;16,19,24-27&lt;/sup&gt;</td>
<td>Asthenia</td>
</tr>
<tr>
<td></td>
<td>Flulike symptoms</td>
</tr>
<tr>
<td></td>
<td>Myalgia</td>
</tr>
<tr>
<td></td>
<td>Pyrexia</td>
</tr>
</tbody>
</table>
Of all the agents used in combination with ECT, bleomycin is the most commonly reported (0.025 units delivered with ECT at 1250 V/cm).\textsuperscript{32} ORRs up to 98% have been reported and CR in more than 50%; however, case series have been small and limited by short follow-up periods.\textsuperscript{33} No significant adverse events have been noted.\textsuperscript{22, 35} Marty et al\textsuperscript{33} conducted the European Standard Operating Procedures of Electrochemotherapy study, based on the experience of leading European cancer centers, that has been a landmark trial in the field.\textsuperscript{33} Prior to the report by Marty et al,\textsuperscript{33} which was published in 2006, different study groups used a variety of protocols with different pulse parameters, pulse generators, electrode types, and dosages of chemotherapy. Marty et al\textsuperscript{33} generated standard operating procedures in a prospective study with 2 years of follow-up using bleomycin or cisplatin. For bleomycin, they used either intravenous 15,000 IU/m² in a bolus lasting 30 to 45 seconds or various intratumoral doses, depending on the tumor size. Cisplatin was administered based on tumor size.\textsuperscript{33} Depending on the number of nodules treated, study participants either received local anesthesia or general anesthesia.\textsuperscript{35} Procedures were performed on an outpatient basis or during a 1-day admission.\textsuperscript{33} Using 5000 Hz electric pulses was more effective than using 1 Hz.\textsuperscript{33} Melanoma nodules showed a lesional response of 80% and a CR rate of 66.3%.\textsuperscript{33}

Subsequently, a meta-analysis of 44 studies analyzed intralesional treatment with ECT on 1,894 lesions.\textsuperscript{46} Results were reported for both bleomycin and cisplatin.\textsuperscript{46} When the clinical responses in all histological diagnoses were evaluated, the CR rate was 59.4% and the ORR was 84.1%.\textsuperscript{46} When the melanoma results were evaluated, the rate of CR and ORR of treated melanoma tumors were 56.8% and 80.6%, respectively.\textsuperscript{46} No adverse events were reported.\textsuperscript{29} Although these results are encouraging, the data are limited due to their small size and lack of long-term follow-up. Therefore, further studies are required to determine which patients may benefit from ECT.

**Granulocyte Macrophage Colony-Stimulating Factor**

Use of granulocyte macrophage colony-stimulating factor (GMCSF) for intralesional therapy against metastatic melanoma is based on 2 mechanisms.\textsuperscript{47} GMCSF stimulates dendritic cells that then induce antitumor immune responsiveness.\textsuperscript{47} The result is twofold: direct destruction of the injected lesion and enhanced antigen presentation, leading to an immune response against metastatic melanoma. T cells treated with GMCSF have demonstrated increased antitumor responsiveness.\textsuperscript{47} Reported adverse events have generally been tolerable and typically constitute flu-like symptoms.\textsuperscript{3, 34, 47}

In addition to increasing the antitumor responsiveness of T cells, GMCSF also appears to reduce the immune-inhibitory effects of metastatic melanoma by having an effect on the cells implicated as mediators of decreasing the immune response against cancer.\textsuperscript{5, 47} GMCSF has been shown to decrease T-regulator, suppressor, and myeloid-derived suppressor cells, which are all mediators of decreased T-cell antitumor activity.\textsuperscript{47} Patients with a higher T-cell composition of the tumor infiltrate with higher interleukin 2 (IL-2) receptor expression are more likely to demonstrate a clinical response to therapy.\textsuperscript{5, 47} Phase 1 data showed increased CD4, CD8, lymphocyte, histiocyte, and eosinophil tumor infiltrate in the injected lesions and a higher likelihood of clinical response in patients with a higher T-cell composition of the tumor infiltrate with a higher IL-2 receptor expression.\textsuperscript{48} Phase 1/2 studies showed ORRs up to 26%,\textsuperscript{3, 34, 45, 48} Efforts are underway to further evaluate mechanisms to enhance the immune response against melanoma.

**Interleukin 2**

IL-2 is a naturally occurring glycoprotein secreted by T cells to augment the immune response and was first used in clinical cancer studies in the early 1980s.\textsuperscript{49} This glycoprotein promotes T-lymphocyte proliferation and stimulates cytotoxic T cells and natural killer cells.\textsuperscript{50} IL-2 has been used as immunotherapy for nearly 40 years, although it has mostly been employed as an intravenous agent.\textsuperscript{50} Its use for intralesional therapy is limited due to logistical problems because patients require multiple injections per lesion and IL-2 is costly.\textsuperscript{50}

The immune-stimulating mechanism of IL-2 has already been applied to melanoma and other solid tumors as a systemic therapy.\textsuperscript{50, 52} It produces a relatively high rate of morbidity when considering its relatively low response rates, which range from 10% to 15%.\textsuperscript{52} Because IL-2 has the potential to induce durable responses, high-dose systemic IL-2 was the mainstay for the treatment of tumors like melanoma and renal cell carcinoma up until the 2000s.\textsuperscript{51, 52} Although its usage has recently tapered off as more effective drugs are now available, IL-2 is still considered a treatment option for unresectable melanoma.\textsuperscript{51, 52} Treatment of tumors has been reported using intralesional and perilesional injections of IL-2, whereby an IL-2 injection into the tumor has been shown to be effective.\textsuperscript{55} Intralesional IL-2 has been studied in many forms, including use as viral vectors, xenogeneic monkey fibroblasts, and IL-2 cultured lymphocytes harvested from patients with melanoma, as well as adjunctive therapy with other systemic therapy and topical agents.\textsuperscript{17, 49, 54-57} Response rates were low and erratic until human recombinant IL-2 was developed, which has provided consistent and promising results.

Unlike systemic IL-2, which has a morbid adverse-event profile, intralesional IL-2 typically produc-
es flulike symptoms alone. The number of study patients in published reports has been small: 7 participants treated in 1 documented case series and 23, 39, and 48 study patients in 3 phase 2 studies. Response rates consistently exceed 80%. Boyd et al reported improved overall 5-year-survival rates in study patients with CR (51% of 39 patients) and study patients with PR (21% of 39 patients). The reported 5-year survival rates were 80% and 33%, respectively. Complete responders had a significant overall survival benefit when compared with partial responders (P = .012). Despite demonstrating a high response rate with minimal rates of morbidity, IL-2 has not demonstrated a significant bystander effect, despite its immune-mediated mechanism. Studies so far conducted have used an onerous administration scheme requiring multiple injections each week; furthermore, because IL-2 is a costly drug to purchase, it is not broadly pursued in research.

**Rose Bengal**

Rose bengal (10%) is an investigational agent for use as an intralesional therapy. The 10% rose bengal solution is a water-soluble stain used to diagnose liver and eye cancers and ocular damage, as well as in food coloring in Japan and as an insecticide, with medical reports being published as early as the 1920s. Because of the wide variety of its application, experience with the drug is extensive, and its safety profile has been well established. As an xanthine dye, the hypothesized mechanism of action of 10% rose bengal is that it creates reactive oxygen by reacting with visible and ultraviolet light, thereby mediating phototoxic reactions. It is selectively absorbed by lysosomes of cancer cells, inducing autolysis and 10% rose bengal is currently under investigation for melanoma and liver tumors (NCT00986661, NCT02557321, NCT02288897). Responses have been reported in study patients refractory to previous systemic ipilimumab, anti–programmed death ligand 1, and vemurafenib, and therapeutic responses have been seen in study patients progressing after a median of 6 treatments.

A bystander effect has been observed in 10% rose bengal. Use of 10% rose bengal leads to increased tumor-specific, interferon-γ secretion in a mouse model, induces an increase in circulating, cytotoxic CD56/CD8 T cells, and recruits dendritic cells to drain lymph nodes. Injection into the non–tumor-bearing flanks of mice had no effect on distant lesions. Rather, the agent must be injected into a tumor lesion to induce a bystander effect. The rate of morbidity is generally considered to be low, although most patients report some local adverse events, most commonly pain (≤ 80%). Local blistering (40%) has been correlated with a better outcome. Other reported adverse events include vesicles, edema, skin discoloration, inflammation, headache, and pruritus around the treatment site.

The first phase 1 trial of 10% rose bengal included 11 study patients. Treatment with 0.5 mL/cc per lesion induced an ORR in more than one-half of participants (both CR and PR, 27%). The effect was dose-dependent, as target lesions receiving less than 0.2 mL 10% rose bengal had a significantly lower response rate than lesions receiving a higher dose (25% vs 69%). A bystander effect was seen in 27% of the study patients and correlated with the response of the injected lesion. In another phase 1 trial, Thompson et al enrolled 20 patients, injecting a single dose of 10% rose bengal in up to 20 lesions per participant. Response rates were comparable with those seen the first phase 1 study. ORR was achieved in 40% of study patients, including a 20% complete response rate, and a bystander effect was reported in 15% of study patients.

Thompson et al injected up to 20 lesions per study patient at day 0 and repeated the injection if needed after 8, 12, and 16 weeks. A total of 80 study patients were included, the majority of whom responded after fewer than 2 injections, resulting in an ORR of 51%, of which the CR rate was 26%. A bystander effect was seen in 40% of 35 evaluable study patients and was correlated with the response of injected lesions (CR rate, 31%; PR rate, 9%). Both visceral and cutaneous lesions were susceptible to this effect. Overall responses were correlated with initial treatment of all discernible disease, with a CR rate seen in 50% of study patients for whom all baseline disease was treated; CR was not seen in study patients with stage 4 melanoma.

Based on these results, expanded access of this trial became available (NCT02288897). As of publication, more than 100 patients with melanoma have been enrolled in this trial. In the phase 3 trial, patients with stage 2C/3B disease will be randomized 2:1 to either 10% rose bengal or systemic chemotherapy, allowing crossover, with progression-free survival as the study's primary end point.

**Talimogene Laherparepvec**

Talimogene lahAFP was approved by the US Food and Drug Administration in 2015. It shows a trend toward improved survival rates and a robust bystander effect. Talimogene lahAFP is an oncolytic, immune-enhanced herpes simplex virus type 1, and its various genetic modifications include deletions of ICP34.5, ICP47, and insertion of GMCSF. Oncolytic viruses like talimogene lahAFP are designed to selectively multiply in tumor cells.

...
least 9 virus groups are being investigated in clinical trials. Oncolytic viruses have direct effects on the metabolic processes of cancer cells. They selectively replicate in tumors, thereby destroying and infecting cancer cells due to their direct effects on the metabolic processes in the cell as well as their ability to induce immune responses that target the cancer cell. This action is thought to be aided by the activation of nuclear factor KB and the release of chemokines and cytokines from the cancer cell. Oncolytic viruses demonstrate limited systemic applicability due to the immune responses of the host, but they are suitable for intralesional injection. Specifically with talimogene laherparepvec, ICP47 deletion helps to prevent blocking antigen presentation and enhances virus growth and replication in tumor cells. Replacing the coding sequence for neurovirulence factor ICP34.5 with the human cytokine GMCSF enables talimogene laherparepvec to initiate a systemic antitumor response by enhancing immune response to tumor antigens. The most common adverse events seen with this agent are fatigue, chills, and pyrexia.

Senzer et al investigated the effectiveness of talimogene laherparepvec in study patients with stages 3 (n = 10) and 4 (n = 40) melanoma in a single-arm, phase 2 trial. Study patients received intralesional injections of either talimogene laherparepvec or GMCSF. The initial injection had a volume of up to 4 mL of 10^6 pfu/mL followed 3 weeks later by 4 mL of 10^8 pfu/mL, every 2 weeks, for up to 24 treatments. The protocol allowed injection with or without ultrasonographic guidance and included cutaneous, subcutaneous, and nodal lesions. An ORR based on Response Evaluation Criteria In Solid Tumors was 26% (CR rate, 8%; PR rate, 5%). After 1 and 2 years, the overall survival rates were 58% and 52%, respectively. Based on these data, a phase 3 study was conducted. This study randomized 436 patients 2:1 to intralesional talimogene laherparepvec (n = 295) or subcutaneous GMCSF (n = 141) and used the same talimogene laherparepvec regimen as the phase 2 trial. The ORRs were 26.4% for those assigned to talimogene laherparepvec and 5.7% for those assigned to GMCSF. The results showed a significant difference in durable response rates (ie, PR or CR rate for > 6 months), with 16.3% in the talimogene laherparepvec group and 2.1% in the GMCSF group (P < .001); durable response rates were higher in study patients with stage 3B/C melanoma (53% for the talimogene laherparepvec group vs 0% for those in the GMCSF group). Six previously unresectable study patients were converted to resectable. Fewer than 3% of study patients experienced grade 3/4 adverse events. For the entire patient population, the overall survival rates trended toward statistical significance (23.3 months for the talimogene laherparepvec group vs 18.9 months for the GMCSF group; P = .051).

A subgroup analysis showed survival benefit in patients with stage 3B/C and 4 M1a disease, and the effect was stronger when talimogene laherparepvec was given as first-line therapy as opposed to second-line therapy or higher.

A lesion-level analysis of the phase 3 trial of 3,219 lesions in 286 patients showed a 50% reduction in 64% of the injected lesions, 32% of the uninjected nonvisceral lesions, and 16% of the uninjected visceral lesions. These findings indicate a bystander effect and, thus, a systemic immune response from the local injection of talimogene laherparepvec.

A phase 1b study of talimogene laherparepvec added to ipilimumab in 19 participants suggested a higher CR rate for the combination than for either agent alone. Grade 3/4 adverse events occurred in 32%. Two study patients had possible immune-related grade 3/4 adverse events, and, of the 17 study patients with investigator-assessed response, the ORR was 41% (CR rate, 24%; PR rate, 18%) and stable disease was seen in 35%. Median time to response was 2.9 months (NCT01740297).

Topical Therapies

Topical therapies have shown some success in superficial lesions and are generally associated with low rates of morbidity. Typically, they are more suited for thinner lesions. Topical diphencyprone cream is a synthetic contact sensitizer that has been used to treat alopecia and warts. The largest trial to date was conducted by Damian et al, who studied 58 patients, 50 of whom were treated for more than 1 month. A total of 46% achieved CR and 46% achieved PR; however, the results of this study should be interpreted with caution, as the majority of results came from the same research group.

Imiquimod is a toll-like receptor agonist approved by the US Food and Drug Administration for the treatment of genital warts, keratoses, and superficial basal cell carcinomas. A treatment regimen for melanoma has not been established, as the application of imiquimod ranges from once weekly to twice daily and from 2 to 88 weeks. Since 2000, it has been used for advanced melanoma in various case reports and small case series. The largest case series is of 5 patients treated with combination topical imiquimod/fluorouracil; a response was elicited in 44 of 45 lesions. Combined treatments with IL-2 and BCG have also been reported. More evidence is available for patients with lentigo maligna, including a large case reporting that more than 90% of study patients with lentigo maligna experience regression with daily or twice-daily application of an imiquimod cream.

Conclusions

The standard of care for patients with locoregional
advanced or metastasized melanoma is to render a patient free of disease as long as the disease is sufficiently limited. When this is no longer feasible, intralesional therapy is a possible option due to its good local response and tolerable adverse-event profile, as well as the option to provide outpatient treatment. A bystander effect observed in various agents adds to its appeal. During the last few decades, other agents have been tested for intralesional therapy with varying success. Many intralesional compounds now available produce a broad range of local response rates. The ideal agent should have a low toxicity profile, be easy to administer, lead to fast responses, and trigger a systemic immune response, thereby creating a bystander effect. These criteria were predominantly met in the results of trials using 10% rose bengal and talimogene laherparepvec in up to 40% of study patients.

Most agents (Bacille-Calmette-Guerin, interferon, granulocyte macrophage colony-stimulating factor) demonstrated inconsistent rates of efficacy, but the treatment field changed when velimogene aliplasmid, 10% rose bengal, and talimogene laherparepvec were introduced. Velimogene aliplasmid did not meet its primary end point in a phase 3 trial, but talimogene laherparepvec did meet its phase 3 trial objectives, demonstrating a survival benefit in select study patients. The results of phase 2 results of 10% rose bengal trials are also promising and a phase 3 is still recruiting (NCT02288897). Other options include combinations of intralesional therapies and systemic therapies, including ipilimumab/talimogene laherparepvec and pembrolizumab/rose bengal (NCT02557321).

Our treatment approach should be individualized per patient, based on the extent of disease, tumor characteristics, and disease-free interval, as well as patient characteristics such as age, performance status, and comorbidities, and work to maintain quality of life for as long as possible. An appropriate approach is often not a single therapy but rather a combination of injectable treatments, regional perfusion therapies, and systemic therapies.

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11. Handleby WS. The pathologic of melanotic growths in relation to their Reactions of erysipelas: with a report of ten original cases. 1893.

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SBRT can be considered as a treatment option for patients with oligometastases.

Stereotactic Body Radiotherapy in the Management of Oligometastatic Disease
Kamran A. Ahmed, MD, and Javier F. Torres-Roca, MD

Background: The treatment of oligometastatic disease has become common as imaging techniques have advanced and the management of systemic disease has improved. Use of highly targeted, hypofractionated regimens of stereotactic body radiotherapy (SBRT) is now a primary management option for patients with oligometastatic disease.

Methods: The properties of SBRT are summarized and the results of retrospective and prospective studies of SBRT use in the management of oligometastases are reviewed. Future directions of SBRT, including optimizing dose and fractionation schedules, are also discussed.

Results: SBRT can deliver highly conformal, dosed radiation treatments for ablative tumors in a few treatment sessions. Phase 1/2 trials and retrospective institutional results support use of SBRT as a treatment option for oligometastatic disease metastasized to the lung, liver, and spine, and SBRT offers adequate toxicity profiles with good rates of local control. Future directions will involve optimizing dose and fractionation schedules for select histologies to improve rates of local control while limiting toxicity to normal structures.

Conclusions: SBRT offers an excellent management option for patients with oligometastases. However, additional research is still needed to optimize dose and fractionation schedules.

Introduction
The concept of the oligometastatic state in cancer management was first proposed in 1995 by Hellman and Weichselbaum,1 who hypothesized a subset of patients with metastatic disease presenting with limited metastatic disease that might be amenable to curative therapy. The validity of this concept has been demonstrated by multiple studies demonstrating long-term survival rates in a subset of patients when treated with aggressive local therapy, including surgery and radiotherapy.2-5

Various radiation techniques have been used for the treatment of oligometastases. Use of high doses of single-session stereotactic radiosurgery has been employed in the management of brain metastases.6,7 Treatment with single-session stereotactic radiosurgery has also been extended to treat patients with at least 4 brain metastases (range, 4–18).8 A similar approach has been used with either a hypofractionated treatment regimen, decreasing the number of fractions delivered by increasing daily treatment doses, or single-session regimens with ablative doses of radiation delivered to extracranial disease sites.

The American Society of Radiation Oncology de-
fines stereotactic body radiotherapy (SBRT) as external-beam radiation used to precisely deliver high doses of radiation to an extracranial target within the body, either as a single dose or a small limited number of radiation fractions.9 The most studied and reported institutional experiences involve treatment to metastases in the lung, liver, and spine. In this review, we summarize the major studies reported in the management of oligometastatic disease and the management of lung, liver, and spine metastases with SBRT.

Oligometastatic Disease
Assessment of the oligometastatic state has historically been limited by imaging capabilities, and many patients previously treated for oligometastatic disease may have had underlying and undetected widespread metastases. Use of positron emission tomography/computed tomography has significantly improved the identification of patients with limited metastatic disease.10-12

Clinical experience has been significant in documenting the effectiveness of surgical resection as a treatment option for these patients. Reports have discussed surgery as the management option for lung metastases, metastases to the liver from colorectal primaries, and soft-tissue sarcoma metastases.2,3,13 In the management of lung metastases, surgery has produced good, long-term outcomes in the resection of renal, colorectal, sarcoma, and breast cancer histologies.14-17 Watanabe et al14 reported a 5-year survival rate of 68% following an R0 resection of colorectal metastases to the lung in 113 study patients. The 5-year survival rates in the resection of lung metastases of primary breast cancer have ranged from 36% to 54%, with a similar 5-year survival rate of 31% reported in the resection of 52 bone sarcoma pulmonary metastases.15,16

Other studies have reported results in the surgical management of liver metastases of colorectal primaries. In a review from Simmonds et al3 of 30 studies, they reported that the 5-year survival rate was approximately 30% and the postoperative mortality rates were relatively low (median rate, 2.8%). Thus, from the data of this surgical series, we can conclude that patients with both lung and liver metastases might achieve long-term survival with definitive surgical management.5

Stereotactic Body Radiotherapy
Historically, use of radiation for the treatment of metastatic disease was restricted to palliation. Radiation to areas of metastatic deposits has been limited by a balance of delivering truly ablative doses of radiation with the risks to surrounding normal tissues. In addition, the need for extended, prolonged fractionation schedules in patients with metastatic disease has limited the feasibility of conventional radiation treatment.9

The initial trials of SBRT were conducted on 42 tumors of the liver, lung, and retroperitoneal space in 31 study patients using mean doses of 8 to 66 Gy in 1 to 4 fractions, and the researchers reported a local control rate of 80% with a follow-up period of 1.5 to 38 months.18 With such high doses of radiation treatment being delivered per fraction, patient immobilization to ensure accurate tumor targeting while limiting dose to normal structures is of paramount importance. This was achieved by a body frame holding the patient accompanied by a vacuum pillow to form a tight, reproducible seal around the patient.

Similar immobilization devices are used in the majority of SBRT treatments. Additional devices have been utilized to limit and track tumor motion in the treatment of lung and liver metastases. This has been achieved using 4-dimensional treatment planning software, allowing tumors to be tracked during the respiration cycle and restricting treatments to particular phases of the respiratory cycle.19 Tumor motion due to respiratory excursion can also be restricted using abdominal compression, thus limiting motion of the diaphragm during treatment.20 Furthermore, daily set-up verification with cone-beam computed tomography helps to ensure adequate treatment positioning for each high dose of delivered treatment.21 Together, these treatment techniques ensure accurate tumor targeting while also limiting the dose to normal structures.

SBRT is typically delivered using gantry-based linear accelerators with multileaf collimators to shape the radiation treatment field. The treatment plan is created using a number of beams to increase the dose to the target while minimizing the dose delivered to normal tissues. SBRT is often the preferred radiation treatment option for sites of oligometastatic disease due to the limited radiation dose delivered to normal tissues with highly conformal treatment planning software and the limited number of fractions needed for treatment.

Mixed Oligometastatic Sites
The definition of oligometastatic disease has varied between studies. Milano et al5 defined persons with oligometastatic disease as those with no more than 5 detectable metastases. Salama et al4 similarly defined oligometastatic disease as 1 to 5 metastatic sites. However, other institutions have limited their eligibility for SBRT to patients with no more than 3 metastatic sites.22 Other patient factors taken into account to determine SBRT eligibility traditionally include performance status, primary rate of disease control, and life expectancy.

Several studies have assessed outcomes in patients treated to mixed sites of oligometastatic disease in an effort to assess rates of local control, toxicity, and survival.5,23-26 The largest series to date of study patients treated to mixed sites of oligometastatic disease was published by Milano et al,5 who reported outcomes in 121 study patients treated with curative-intent SBRT. The majority of study patients were treated with
10 fractions of 5 Gy. The rates of 2-year overall survival (OS), progression-free survival (PFS), local control, and distant control were 50%, 26%, 67%, and 34%, respectively. The most common sites of metastases were in the lung, liver, and lymph nodes.

Other groups have reported similar outcomes in the management of oligometastatic sites. For example, Salama et al reported on SBRT in 61 study patients with 113 metastases who had life expectancies longer than 3 months. This dose-escalation study initially treated participants with a rate of 24 Gy in 3 fractions and then sequentially increased the rate to 48 Gy in 3 fractions. No dose-limiting toxicities were seen in study patients treated to 48 Gy. The 1- and 2-year PFS rates were 33.3% (95% confidence interval [CI], 22.8–46.1) and 22.0% (95% CI, 12.8–34.4), respectively, and no significant response rate between dose levels was reported. Greco et al reported on outcomes in 126 metastases in 103 study patients treated with single-dose SBRT at doses ranging from 18 to 24 Gy. The most common primary histologies were prostate, renal, and colorectal, with the majority of treated sites in the bone, lymph nodes, and soft tissue. A dose-response relationship was noted, as was a 2-year local control rate of 64% (82% if > 22 Gy, 25% for 18–20 Gy).

Taken together, these studies show treating patients with a limited number of metastases with SBRT is feasible and offers adequate PFS rates at 24 months. In addition, toxicity rates in these studies were acceptable, indicating SBRT is a safe alternative to surgical resection.

A summary of studies of SBRT for mixed oligometastatic sites is included in Table 1.

### Table 1. — Studies Evaluating Stereotactic Body Radiotherapy for Mixed Oligometastatic Sites

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of Lesions</th>
<th>Dose</th>
<th>Rate of Local Control</th>
<th>Rate of Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greco23</td>
<td>126</td>
<td>18–24 Gy in 1 fraction</td>
<td>64% at 2 y</td>
<td>Grade 3 (&lt; 4%)</td>
</tr>
<tr>
<td>Kang25</td>
<td>78</td>
<td>42 Gy in 3 fractions</td>
<td>66% at 3 y</td>
<td>Grade 4 (3%)</td>
</tr>
<tr>
<td>Milano5</td>
<td>293</td>
<td>Median 50 Gy in 10 fractions</td>
<td>77% at 2 y</td>
<td>Grade 3 (1%)</td>
</tr>
<tr>
<td>Salama4</td>
<td>113</td>
<td>24–48 Gy in 3 fractions</td>
<td>67% at 2 y</td>
<td>Grade 3 Acute: 3% Late: 10%</td>
</tr>
<tr>
<td>Stinauer26</td>
<td>53</td>
<td>40–50 Gy in 5 fractions or 42–60 Gy in 3 fractions</td>
<td>88% at 18 mo</td>
<td>Grade 3 (3%)</td>
</tr>
<tr>
<td>Wersall24</td>
<td>162</td>
<td>30–40 Gy in 3 fractions</td>
<td>Crude (90%)</td>
<td>Grade ≥ 1 toxicity (40%)</td>
</tr>
</tbody>
</table>

#### Liver

Many studies have assessed SBRT in the control of metastases to the liver, and some prospective phase 1/2 trials have established the safety and efficacy of SBRT as a treatment modality for liver metastases. A summary of the prospective trials assessing SBRT for the management of liver metastases is reported in Table 2.

Lee et al reported phase 1/2 outcomes in 68 inoperable or medically unsuitable study patients. Individualized radiation doses were chosen to maintain the same nominal risk of radiation-induced liver disease. The median SBRT dose was 41.8 Gy (range, 27.7–60 Gy) in 6 fractions over 2 weeks, and the most common histologies were colorectal (n = 40), breast (n = 12), or other (n = 16). SBRT was well tolerated: 6 (9%) acute grade 3 toxicities (gastritis, nausea, lethargy, and thrombocytopenia) and 1 (1%) grade 4 toxicity (thrombocytopenia) were seen, and the 1-year local control rate was reported to be 71% (95% CI, 58–85). Rusthoven et al also reported phase 1/2 results in 47 study patients with 63 liver metastases with a maximal diameter less than 6 cm in a dose-escalation trial of 36 to 60 Gy in 3 fractions. No cases of radiation-induced liver disease were noted, but 1 study patient did experience grade 3 or higher toxicity (2%). Local control rates at 1 and 2 years following SBRT were 95% and 92%, respectively, with a median survival rate of 20.5 months.

Although local control rates were higher in this study, the lesions were smaller, with a median volume...
of 14.9 mL, compared with 75.2 mL in Lee et al.\textsuperscript{27,30} Nevertheless, these results raise the question of whether higher radiation doses for the management of liver metastases may improve local control rates.

A study by Hoyer et al\textsuperscript{29} reported on the results of inoperable colorectal metastases treated with SBRT in the liver \((n = 44)\), lung \((n = 12)\), lymph nodes \((n = 3)\), suprarenal gland \((n = 1)\), or 2 organs \((n = 4)\) with a central dose of 15 Gy on 3 occasions within 5 to 8 days. The 2-year rates of local control by tumor and patient were 86\% and 63\%, respectively.\textsuperscript{29} Toxicities included 1 liver failure leading to death and 2 severe, late gastrointestinal toxicities.\textsuperscript{29} Despite these toxicities, the authors noted adequate toxicity profiles for the majority of study patients.\textsuperscript{29}

Fig 1 is an example of a treatment plan for SBRT in a patient with a renal cell metastasis to the liver.

**Lung**

Historically, the standard of care for early stage non–small-cell lung cancer is surgical resection. Reports of treatment with SBRT represented a novel approach for the management of inoperable early stage lung cancer; the initial phase 1 data in patients with medically inoperable stage 1 disease were published by Timmerman et al.\textsuperscript{32} Doses ranged from 8 to 20 Gy in 3 fractions.\textsuperscript{32} Researchers of a phase 2 trial treated 59 study patients with medically inoperable stage T1/T2 N0 M0 non–small-cell lung cancer with 18 Gy for 3 fractions, reporting a 3-year local control rate of 98\%.\textsuperscript{35}

The pioneering work from Timmerman et al\textsuperscript{32} was eventually translated to the setting of oligometastatic disease and lung metastases.\textsuperscript{34-38} Ricardi et al\textsuperscript{34} published a series of 61 study patients with oligometastatic lung tumors. Dose selection was 26 Gy in 1 fraction in 51 study patients, 45 Gy in 3 fractions in 22 study patients, and 36 Gy in 4 fractions in 3 study patients.\textsuperscript{34} After a median follow-up interval of 20.4 months, local control rates at 2 and 3 years were 89\% and 83.5\%, respectively, OS rates at 2 and 3 years were 66.5\% and 52.5\%, respectively, and PFS rates at 2 and 3 years were 32.4\% and 22.3\%, respectively.\textsuperscript{34}

Siva et al\textsuperscript{39} published a systematic review of the literature on oligometastatic disease to the lung treated with SBRT, assessing 334 study patients with 564 targets. The 2-year weighted local control rate was 77.9\%, the corresponding 2-year weighted OS rate was 53.7\%, and a 4\% rate was seen of radiation toxicity of grade 3 or higher.\textsuperscript{39} In addition, the series reported on single-fraction stereotactic radiosurgery to 154 study patients treated to 174 targets, and the 2-year weighted rate of local control was 78.6\%.\textsuperscript{39}

No randomized data have yet to determine whether surgical resection and SBRT are equivalent treatment options for oligometastatic disease. Several trials have been attempted in the primary lung cancer setting, but they were closed due to poor accrual (NCT00687986, NCT00840749). However, SBRT is still an accepted approach for the management of lung metastases due to its efficacy, safety, and feasibility.

Fig 2 shows a lesion in the left upper lobe of the lung treated with 50 Gy in 5 fractions, and Table 3 sum-

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**Fig 1.** — (A) Axial, (B) sagittal, and (C) coronal imaging used to plan treatment using stereotactic body radiotherapy for hypervascular renal cell metastasis in the liver.
marizes the studies evaluating the management of oligometastatic lung disease treated with SBRT.34-38

**Spine**

In addition to its use in the setting of reirradiation and radioresistant histologies, SBRT to the spine is indicated for the treatment of oligometastatic disease.40,41 Many prospective and retrospective series have reported outcomes in the management of spinal metastases with SBRT.42-46 A summary of reported series on SBRT to the spine are reported in Table 4.22,41-46

A phase 1/2 trial of 149 study patients with 166 lesions were treated with SBRT to the spine, receiving total doses of 27 to 30 Gy, typically in 3 fractions.42 The number of study patients reporting no pain from bone metastases, as measured by the Brief Pain Inventory, increased from 39 (26%) prior to SBRT to 55 (54%) 6 months after SBRT (P < .0001).42 They were followed for a median of 15.9 months and had a local control rate of 72%.42

The largest report to date of SBRT to the spine was a retrospective review from Gerszten et al,45 who reported the results of 393 study patients with mixed histology (500 total lesions). No significant neurological effects were reported.45 The researchers followed the study patients for a median of 21 months and reported a tumor control rate of 88%.45

Zelefsky et al41 reported a significant dose-response relationship in the management of renal cell carcinoma metastases to the spine. Renal cell carcinoma has traditionally been thought to be radioresistant, so Zelefsky et al41 indicated a higher biological effective dose may be necessary to achieve adequate tumor control.41 With a dose of 24 Gy in 1 fraction, a 3-year PFS rate of 88% was achieved with a rate of 21% for a dose lower than 24 Gy in 1 fraction.41 These results are intriguing and should be assessed further in randomized prospective trials.

Although a significant dose-response relationship

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**Table 3. — Retrospective and Prospective Experiences for Lung Metastases Treated With Stereotactic Body Radiotherapy**

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of Lesions</th>
<th>Dose</th>
<th>Rate of Local Control</th>
<th>Rate of Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Okuniewicz</td>
<td>125</td>
<td>50 Gy in 10 fractions</td>
<td>91% at 3 y</td>
<td>Grade 2 (6.1%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(most common)</td>
<td></td>
<td>Grade 3 (2.2%)</td>
</tr>
<tr>
<td>Onimaru</td>
<td>57</td>
<td>48–60 Gy in 8 fractions</td>
<td>70% at 3 y for 48 Gy</td>
<td>Grade 5 (2.2%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100% for 60 Gy</td>
<td></td>
</tr>
<tr>
<td>Ricardi</td>
<td>77</td>
<td>26 Gy in 1 fraction to 45 Gy</td>
<td>89% at 2 y</td>
<td>Grade 3 (1.6%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>in 3 fractions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rusthoven</td>
<td>63</td>
<td>60 Gy in 3 fractions</td>
<td>96% at 2 y</td>
<td>Grade 3 (8%)</td>
</tr>
<tr>
<td>Yoon</td>
<td>101</td>
<td>30 Gy in 3 fractions to 48 Gy</td>
<td>70% for 30 Gy</td>
<td>No cases of grade ≥ 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>in 4 fractions</td>
<td>77% for 40 Gy</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100% for 48 Gy</td>
<td></td>
</tr>
</tbody>
</table>
was reported by Zelefsky et al, caution must be taken to avoid any excess dose to the spinal cord, which could lead to acute and late toxicities. Fig 3 shows a radiation therapy plan for T11 metastases due to leiomyosarcoma that were treated with 25 Gy in 5 fractions and dose painting to 30 Gy to areas of gross disease. Dose constraints to the spinal cord have included a maximum dose of less than 22 Gy, less than 0.35 cc receiving 18 Gy, and less than 1.2 cc receiving 12.3 Gy. Radiation oncologists must cautiously select candidates for SBRT of the spine to avoid additional risks to the spine that can occur with SBRT compared with conventional external-beam radiotherapy. Following conventional external-beam radiotherapy, the risk of vertebral compression fractures has been reported to be 5%; however, several retrospective series have reported higher rates of vertebral compression fractures with SBRT (11%–39%). Decreased dose to the spinal cord can be achieved using conformal radiation treatment planning. However, myelopathy continues to be a rare but significant toxicity; the largest reported series of 1,075 study patients reported this rate to be 0.6%. Particular attention must be taken to decrease dose delivery to the spinal cord, particularly in the setting of reirradiation, to minimize the risk of myelopathy.

### Factors Affecting Outcomes

Increasing clinical evidence suggests that the management of oligometastases should differ based on primary histology. A study from Takeda et al suggested that clinical outcomes may be different between the local control of oligometastatic lung lesions and primary lung cancers. Their study assessed 21 colorectal metastatic lung lesions, 23 lesions from other origins, and 188 primary lung cancers treated with 50 Gy in 5 fractions. On multivariate analysis, they found that the origin of the tumor was significant (P < .05). The researchers reported 1-year local control rates of 80%, 94%, and 97% (P < .05) for colorectal primaries, oligometastases from other origins, and clinically diagnosed lung cancer, respectively. For the management of oligometastatic disease, Milano et al found differences in the outcomes of local control rates depending on breast primaries vs non-breast primaries with 2-, 4-, and 6-year local control rates of 87% for breast primaries and 74%, 68%, and 65% for non-breast primaries, respectively.
Taken together, these data indicate histology is a variable that might affect outcomes and should be taken into account when developing SBRT dosing and fractionation schedules.

**Formula for the Biological Effective Dose**

The biological effective dose delivered to a tumor is a mathematical formula based on the linear quadratic model. The formula helps estimate the biological effect of a delivered dose to a tumor, taking into account the number of radiation treatments, the total dose delivered, and the $\alpha:\beta$ ratio. The $\alpha:\beta$ ratio is used to describe the curvature of the cell survival curve after radiation. Low $\alpha:\beta$ ratios characterize late-responding tissues and high $\alpha:\beta$ ratios delineate early-responding tissues. The formula is given as:

\[
\text{Biological effective dose} = nd (1 + d/\alpha:\beta)
\]

In this formula, $n$ is the number of fractions and $d$ is the dose per fraction.

Assuming an $\alpha:\beta$ ratio of 10, we can see 3000 cGy in 1 fraction vs 3000 cGy in 10 fractions would predict the biological effective doses to be:

\[
30 \text{ Gy} \times 1 = 30(1 + 3.0) = 120
\]
\[
3.0 \text{ Gy} \times 10 = 30(1 + 0.30) = 39
\]

Using this biological effective dose model, we can see the higher biological dose delivered using SBRT doses.

However, the linear quadratic model has limitations when assessing high-dose fractionation regimens. The $\alpha:\beta$ ratio in the linear quadratic model depends on the dose range, and parameters used for conventional fractionation cannot be applied to high dose-per-fraction situations. In addition, it fails to predict tumor or normal-tissue responses at high doses based on conventional radiotherapy experiences. Additional models, such as the generalized linear quadratic model suggested by Wang et al., may be better suited to assess the conversion of sublethal to lethal DNA damage with SBRT and high-dose-rate brachytherapy. However, because no consistent model has been developed to measure the biological effect of larger hypofractionated SBRT doses, radiation oncologists have had difficulty developing dose-fractionation regimens for SBRT and high-dose radiation brachytherapy to model biological effect.

**Dose Selection**

Various dosing schedules have been suggested for both lung and liver metastases. However, an optimal dosing schedule that takes into account factors such as lesion volume, primary histology, and biological response has yet to be determined. Data from McCammon et al. suggest a dose-response relationship in the treatment of liver and lung metastases. They reported on 246 lesions treated with SBRT for 3 fractions and lesions treated to a dose of at least 54 Gy and found 3-year local control rates of 89.3% compared with 59.0% and 8.1% for those treated to between 36 and 53.9 Gy and less than 36 Gy, respectively. Fumagalli et al. suggested a difference in outcome based on anatomical location. They assessed outcomes in hepatic or pulmonary oligometastases, of which 70% were digestive primary, and an improved disease-free survival rate on univariate analysis was noted in study patients with pulmonary metastases ($P = .02$).

Results from these groups suggest a difference may exist in SBRT outcomes based on treatment location and that dose selection plays an important role in achieving optimum control.

**Future Directions of the Radiosensitivity Index**

Differences exist in the radiosensitivity of tumors based on the $\alpha:\beta$ ratio of the cell survival curve. We previously developed the radiosensitivity index (RSI) modeled as a function of gene expression, tissue of origin, Ras, and p53 status correlated to the surviving fraction of cells at 2 Gy in a panel of 48 human cancer cell lines. In another study, we assessed RSI differences between primary and metastatic colon cancers. As suggested by the clinical experience of other groups using SBRT, we found significant differences in RSI based on the anatomical location of metastases. We found that colon cancer metastasized to sites such as the ovary, abdomen, and liver and were more radioresistant than when tumors metastasized to sites such as the lung and lymph nodes ($P < .0001$). These results were confirmed when we restricted our analysis to lesions from the same study patient.

Our clinical practice has been to treat both lung and liver metastases regardless of primary histology to 60 Gy in 5 fractions of SBRT. Assessing our own institutional experience with the management of lung and liver metastases with primary colon cancer, we have found significant differences in local control outcomes, with lung metastases fairing better than liver metastases. This correlates well to our RSI analysis, which revealed liver metastases of primary colon cancer to be more radioresistant than those of lung metastases. Thus, we propose that RSI could be used as a model to select dose and fractionation schedules for various anatomical sites and various histologies, possibly setting the stage for use in future clinical trials.

**Conclusions**

As novel, targeted agents are developed and systemic disease control is improved, local control of oligometastatic disease will become more important. Many
prospective and retrospective studies have revealed stereotactic body radiotherapy (SBRT) to be a safe management option for the control of oligometastatic disease to various disease sites with good local control.22,41-46 Due to the shortened time course and ablative radiation doses, SBRT is ideal for the management of metastatic cancer with limited sites of disease.

The optimal dose and fractionation schedule of SBRT for various sites of disease have yet to be determined. Increasing evidence suggests a uniform treatment schedule cannot be applied to all metastases, and dosing and fractionation schedules must be individualized based on tumor histology and anatomical location. As we move toward an era of personalized medicine, it will become increasingly important that dose and fractionation schedules be tailored to specific tumors.

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Local Ablation for Solid Tumor Liver Metastases: Techniques and Treatment Efficacy
Joyce Wong, MD, and Amanda Cooper, MD

Background: Treatment options for liver metastases from solid tumors, such as colon cancer, breast cancer, neuroendocrine tumors, and sarcomas, have expanded in recent years and now include nonresection methods.
Methods: The literature focused on the treatment of liver metastases was reviewed for technique, perioperative, and long-term outcomes specifically related to local ablation techniques for liver metastases.
Results: Ablation modalities have become popular as therapies for patients who are not appropriate candidates for surgical resection. Use of these techniques, alone or in combination with other liver-directed therapies (and often systemic therapy), has extended the rate of survival for patients with liver metastases and, at times, offers nearly equivalent disease-free survival rates to surgical resection.
Conclusions: Although surgical resection remains the optimal treatment for liver metastasis, local options, including microwave ablation and radiofrequency ablation, can offer similar long-term local control in appropriately selected patients.

Introduction
Liver metastases occur as the sole site of metastatic disease in many solid neoplasms, commonly from colorectal adenocarcinoma or breast carcinoma. However, other malignancies, such as pancreatic adenocarcinoma, neuroendocrine tumors, and gastrointestinal stromal tumors, can also present with liver metastases as the site of metastatic disease. The options for liver-directed therapies have expanded in the last several decades and now include intra-arterial chemotherapy, radiation, and local ablative techniques. Although this review will not address intra-arterial embolization or perfusion options, multiple techniques are often utilized in a complementary manner to treat disease.

Surgical resection, sometimes in a staged-fashion or as repeat resections, for liver-only metastatic disease has remained the gold standard of treatment, providing the best rates of disease-free survival (DFS) and overall survival (OS). However, patient or tumor factors may preclude surgical resection. Liver-directed ablation has also emerged as an important and often effective alternative to surgical resection for these patients, frequently providing a survival benefit over chemotherapy or palliative care alone.

Liver ablation was first described in the 1980s with the use of ethanol injections for primary and secondary hepatic tumors; around this time, use of cryotherapy was also described, and high-intensity ultrasonography was being investigated as a way of creating necrosis within hepatic tumors in animal studies. The past several decades have provided us
with a deeper understanding of the technology, the clinical impact of treatment for hepatic tumors from metastatic disease, and the development of newer and safer techniques.

Cryotherapy
Cryotherapy was initially reported in the late 1970s and involved using ultrasonography to guide the placement of a cryoprobe into the center of a lesion.9 The tip of the cryoprobe contains liquid nitrogen circulating at −196 °C, and freezing is continued until a 1-cm diameter of hypoechogeticity is circumferentially achieved, thus creating an “iceball.”10 A thaw cycle then commences by circulating nitrogen at room temperature, at which point the probe can be removed or additional freeze cycles can be performed.11 Adverse events from cryoablation include hepatic bleeding from parenchymal cracks, which can be potentiated by thrombocytopenia and coagulopathy seen in hepatic cryosurgery; in addition, operative times are typically long because of the technique utilized to create an “iceball.”12 However, the modality has fallen out of favor and has been largely replaced with thermal ablation techniques.

Thermal Ablation
Similar to cryotherapy, radiofrequency ablation (RFA) uses ultrasonographic guidance to place a probe into the center of the lesion; the procedure can then be performed in a monopolar or bipolar manner. High-frequency alternating currents from the electrodes cause heat to form due to ionic agitation, thus resulting in thermal ablation. Heating around the tip of the electrode can be intensified by increasing the power of the generator, thus inducing a significant volume of necrosis and char formation, thereby limiting further current flow.13 Sustained temperatures higher than 50 °C result in coagulation necrosis, and overlapping ablations are often used to ensure that the tumor area has been adequately covered. In general, contraindications to the procedure include proximity of the tumor to the bile duct or major intrahepatic vessels, coagulopathy, or tumor size above 5 cm.14 A limitation to use of RFA is the “heat sink” effect, which is related to the decrease in temperature from ablation in proximity to blood vessels, which is particularly true for large vessels with faster blood flow. Data have shown that the maximal temperature can be decreased by up to 21% when ablating in proximity to large vessels, and this phenomenon is thought to limit the efficacy of RFA around vessels.15

Microwave ablation is a modality that also achieves tumor necrosis through heat, which is produced by the conduction of microwave energy. Tissues with a higher concentration of water, such as liver, are most conducive to this type of heat because the microwave energy causes the water molecules to realign. Microwave energy can also conduct through charred or desiccated tissue, unlike RFA, and can achieve higher temperatures than RFA (up to 180 °C). Microwave ablation also seems less affected by thermal convection from adjacent vessels.16,17

Postablation Syndrome
A self-limited, flulike constellation of symptoms, including fever, pain, nausea, vomiting, malaise, and myalgia, has been described following both RFA and microwave ablation and is commonly referred to as postablation syndrome.18-21 Prospective survey studies have reported incidences of these symptoms ranging from 32% to 81% in some patients.18-21 Onset of these symptoms typically occurs within 24 to 48 hours following the procedure, and symptom resolution is typically within 10 days, although some symptoms may persist for up to 3 weeks.18-20 In most patients, these symptoms can be managed with nonsteroidal anti-inflammatory drugs.20 Factors predictive of the syndrome may include tumor volume (> 4.5 cm), ablation volume, length of ablation (> 20 minutes), and significantly elevated postablation levels of aspartate transaminase.18,21

Complications
Most complications associated with use of RFA and microwave ablation are consequences relating to thermal injury.22-25 The risk of periprocedural mortality following ablation in the absence of combined hepatic resection is low and ranges from 0.1% to 0.5%.22-24 Major complication rates range from 2.8% to 9.5%, and these types of complications are more commonly seen with open ablation and in patients with underlying cirrhosis.22-25 The most commonly reported early complications include symptomatic pleural effusion, hemorrhage, perihepatic/hepatic abscess, biloma, liver dysfunction (occurring almost exclusively in patients with cirrhosis), portal venous thrombosis, and hemothorax/pneumothorax.22-25 Thermal injury to adjacent structures, most commonly the stomach, has also been reported, but this complication is rare and can be generally avoided with careful patient selection and meticulous technique.22,23,25 Late procedure–associated complications are rare (< 2.4%) but can be significant.22,23 Such complications include liver abscess, biloma, biliary fistula, bile duct stricture, arteriovenous fistula, hepatic abscess, diaphragmatic hernia, gastric perforation, and intractable pain.22,23

Irreversible Electroporation
Irreversible electroporation (IRE) represents a recent modality that has been used for liver ablation. Electroporation involves applying high-voltage electricity that is delivered in short pulses across a cell membrane, resulting in changes in the electrochemical properties of the cell membrane.
potential of the cell membrane and subsequent instability. This instability causes pores to form in the membrane, an effect that allows the introduction of macromolecules, drugs, and, in some cases, genes into the cell. Prolonged application of the electric stimulation will result in irreversible porosity (ie, IRE) of the cell membrane, ultimately leading to cell death. The preservation of the structural integrity of nearby vital structures has also been reported, thus making IRE an ideal option for lesions located adjacent to vascular or biliary structures.

Because IRE is a relatively new technique, its associated complications and their incidence are not well described; however, several complications have been observed and are important to mention. Because IRE relies on high-voltage electrical pulses,cardiac arrhythmias and severe muscle contractions may occur. Complete neuromuscular blockade during the procedure prevents muscle contraction and patient movement. As experience with the technique has accumulated, modifications to the technique (eg, synchronization with electrocardiography so that the electrical pulses are administered during the absolute myocardial refractory period) have been developed to prevent life-threatening arrhythmias, such as ventricular fibrillation, from occurring. However, technique modification has not eliminated the incidence of transient atrial arrhythmias, which still occur in approximately 2% of patients. During the procedure, increases in both systolic and diastolic blood pressure may be seen; in addition, increases in transaminase and lactate levels following the procedure are frequently observed, although these increases typically resolve within 3 days.

Although existing data on IRE are still relatively new, 1 systematic review found no periprocedural mortalities and a 16% overall complication rate, with no complications deemed to be major. The cohort of study patients analyzed included a large proportion of treated tumors located near major vascular or biliary structures, and a 6% rate of thrombosis or stricture of these structures was observed. The study authors hypothesized that this may have been due to the development of heat adjacent to the electrodes, so they recommended against placing electrodes within 2 mm of central bile ducts or the intestines to avoid such complications. Other adverse events of IRE include dehydration, biliary stent occlusion, acute renal failure, neurogenic bladder, and abdominal/flank pain.

Procedural Approach
Although overall familiarity with these techniques has been expanding during the past 10 years, reliable interpretation of ultrasonographic imaging and the accurate placement of probes remain limitations of the technology. In addition, although effective ablation can be reliably achieved in small (<3 cm) tumors, ablation of larger tumors may be compromised by accurate targeting, the proximity of these tumors to vascular or biliary structures, or risk of postablation complications. Use of ablation via laparotomy may appear to result in few local recurrences, but no randomized trial has been performed to date that has compared approaches for ablation delivery; moreover, multiple factors (eg, lesion number, size, location) influence recurrence.

Percutaneous approaches are the least invasive and may be better suited for patients with small lesions deep in the hepatic parenchyma or those patients who are not candidates for surgical resection or surgical ablation. Instilling intraperitoneal fluid to create artificial ascites has been suggested as a means to protect adjacent structures and potentially allow for more aggressive patient selection for percutaneous ablation. A surgical approach, using either an open or laparoscopic technique, is better suited for patients with peripheral lesions or lesions located near the hepatic or portal veins; in the latter group, hepatic vascular inflow occlusion, via a Pringle maneuver, may help the clinician achieve adequate ablation temperatures.

Surgery vs Chemotherapy
Thus far, no randomized controlled trial has compared RFA with surgical resection for colorectal or other liver metastases, and only a single, phase 2 randomized controlled trial has compared systemic therapy with systemic therapy in combination with RFA for unresectable colorectal liver metastases. Ruers et al compared systemic therapy with or without RFA, but their study was slow to accrue and was closed early. Although the study met its primary end point of a 30-month OS rate of greater than 53% in the combined treatment arm, the OS rate for those assigned to the chemotherapy-alone arm was higher than anticipated, and the difference in rate of survival between the 2 arms was not significantly different at a median follow-up period of 4.4 years. However, the median progression-free survival rate (PFS) was 16.8 months in the combined treatment group compared with 9.9 months in the chemotherapy-alone arm, a difference that was statistically significant (95% confidence interval [CI], 9.3–13.7). Long-term follow-up data from this study have not yet been published, so whether a difference in OS will emerge remains to be seen. Data from prospective studies comparing microwave ablation or IRE with chemotherapy have not yet been published.

In 2008, Mulier et al published data from a phase 3, prospective, randomized controlled trial began in 2002 comparing liver resection and RFA for small colorectal metastases, but the study failed due to lack of accrual. Although no prospective, randomized controlled trial has compared RFA and surgical resection for colorectal metastases, the best available data...
A retrospective study — and found that liver resection resulted in superior rates of OS (relative ratio [RR] 5-year OS, 1.47; 95% CI, 1.28–1.69) and DFS (RR 5-year DFS, 2.23; 95% CI, 1.82–2.72) compared with those seen with RFA. Those results held up in a subset analysis limited to study patients with solitary tumors of less than 3 cm in size.

A Markov decision analysis compared hepatic resection with percutaneous or laparoscopic RFA and predicted a mean quality-adjusted life expectancy rate of 5.67 ± 0.71 years with a 5-year survival rate of 38.2% following liver resection compared with a mean quality-adjusted life expectancy rate of 3.61 ± 0.49 years with a 5-year survival rate of 27.2% for RFA. However, this study also reported on a single-institution, prospective database of patients treated with laparoscopic RFA for resectable tumors, and it predicted a mean quality-adjusted life expectancy rate of 5.72 ± 0.5 years for RFA — a rate that exceeded liver resection.

Tanis et al compared the results of local control among study participants assigned to RFA with study patients assigned to resection who had colorectal metastases and tumors at least 4 cm in size and were treated with perioperative folinic acid/fluorouracil/oxaliplat-in. When the analysis of results after RFA was limited to tumors of no more than 3 cm in size, the local recurrence rate (median follow-up time, 4.7 years) was 2.9% per lesion, which compared favorably with the local recurrence rate of 6.0% per lesion in the resection cohort.

A single, small, prospective, randomized trial compared the use of microwave ablation and resection for resectable colorectal liver metastases. The researchers reported equivalent 1-, 2-, and 3-year survival rates between the 2 therapies, as well as significantly less intraoperative blood loss with microwave ablation.

Based on these results, RFA and microwave ablation are reasonable alternatives to liver resection in patients with small colorectal metastases, and this is particularly true in patients for whom liver resection would carry a higher-than-average risk of morbidity or mortality.

**Colorectal Metastases**

**Radiofrequency Ablation**

Prospective data on the long-term outcomes after RFA for colorectal liver metastases are limited. A study conducted by Evrard et al was a multicenter, single arm, nonrandomized phase 2 trial that evaluated the effectiveness of RFA with or without resection for the treatment of unresectable metastases. After a median follow-up time of 2.9 years, the researchers observed a 3-year event-free survival rate of 10% and a 5-year OS rate of 43%. Ruers et al headed a phase 2 trial and reported a median OS rate of 45.3 months for study patients with unresectable colorectal liver metastases who were treated with RFA and chemotherapy. Retrospective studies have reported 3-year OS rates ranging from 32% to 84% and 5-year OS rates ranging from 17.9% to 49%, with 3-year DFS rates ranging from 0% to 76% and 5-year DFS rates ranging from 0% to 69.7%.

A report from Gillams et al advocated use of thermal ablation for unresectable lesions, predominantly in the form of RFA, for colorectal liver metastases, noting that the best outcomes occur in tumors smaller than 3 cm in size, located more than 1 cm away from bile ducts or major blood vessels, and have an ablation margin greater than 1 cm.

**Microwave Ablation**

A randomized trial comparing microwave ablation and liver resection for the treatment of resectable colorectal liver metastases found that study patients treated with microwave ablation had 1-, 2-, and 3-year OS rates of 71%, 57%, and 14%, respectively, and a mean survival time of 27 months; thus, the survival rates were comparable with the resection group.

A retrospective study evaluating the long-term outcomes following microwave ablation included patients treated with microwave ablation alone and in combination with resection — 48.3% of whom were also treated with hepatic arterial infusion chemotherapy. This study reported a 4-year OS rate of 58.3% for those participants with colorectal liver metastases. The researchers also noted that local recurrence rates were higher for lesions larger than 3 cm in diameter and in those located in close proximity to major vessels or in a subcapsular location.

An early experience with ultrasonographic-guided microwave ablation reported a clinical success ablation rate of 100%; however, local tumor progression was seen in 9.6% of treated metastases.

**Irreversible Electroporation**

Long-term outcome data after IRE for colorectal liver metastases are lacking. A prospective registry study reporting on IRE for tumors located adjacent to major biliary and vascular structures found a 12-month local recurrence-free survival rate of 59.5%. One retrospective study of IRE for colorectal metastases described a 2-year OS rate of 62%.

**Noncolorectal Metastases**

RFA has also been used for the treatment of noncolorectal liver metastases, particularly those without a dominant blood supply from the hepatic artery; those predominantly supplied by the hepatic artery are preferentially treated with chemoembolization. Taşçı et al
conducted a retrospective study of patients with liver metastases from breast cancer treated with laparoscopic RFA and reported a median OS rate of 47 months and a 5-year survival rate of 29% following a diagnosis of liver metastases. Pawlik et al reported on outcomes after RFA with or without resection for sarcoma liver metastases and observed 1-, 3-, and 5-year OS rates of 91.2%, 65.4%, and 27.1%, respectively. A prospective study of arterial infusion chemotherapy followed by RFA for liver metastases from gastric adenocarcinoma observed a median survival rate of 16.5 months.

In summary, these studies have shown the potential role and survival benefit for hepatic ablation in carefully selected patients with noncolorectal liver metastases.

**Conclusions**

The technology and techniques used for ablation to treat liver metastases have continued to evolve in the past several decades and now include thermal methods, such as radiofrequency ablation and microwave ablation, as well as nonthermal methods such as irreversible electroporation. Although it is commonly performed in conjunction with systemic therapy or other liver-directed treatment, ablation may offer a survival benefit for patients with metastatic disease in the liver. This is particularly true for small lesions not located adjacent to biliary or vascular structures.

Further development of techniques to enhance ablation and study ablative methods as part of multimodality treatment may help patients achieve survival rates that approach those of surgical resection, the current treatment of choice.

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**Conclusions**

The technology and techniques used for ablation to treat liver metastases have continued to evolve in the past several decades and now include thermal methods, such as radiofrequency ablation and microwave ablation, as well as nonthermal methods such as irreversible electroporation. Although it is commonly performed in conjunction with systemic therapy or other liver-directed treatment, ablation may offer a survival benefit for patients with metastatic disease in the liver. This is particularly true for small lesions not located adjacent to biliary or vascular structures.

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HIPEC is a treatment option that may be appropriate for select patients with peritoneal carcinomatosis.

Hyperthermic Intraperitoneal Chemotherapy and Cytoreductive Surgery in the Management of Peritoneal Carcinomatosis

Rahul Rajeev, MBBS, and Kiran K. Turaga, MD

Background: Malignant peritoneal disease can lead to significant debility due to bowel obstructions, ascites, and cancer cachexia. Moreover, inadequate imaging techniques can lead to the suboptimal detection of disease, and the poor vascularity of tumors can lead to a poor response to systemic chemotherapy. However, combination cytoreductive surgery/hyperthermic intraperitoneal chemotherapy (HIPEC) is a promising novel treatment for patients with this disease.

Methods: The medical literature focusing on diagnostic updates and the management of peritoneal disease was reviewed. The application principles of HIPEC for use in peritoneal disease were also summarized.

Results: Improvements in imaging and the application of laparoscopic techniques have significantly increased the rate of diagnosis of early peritoneal disease with consequently less morbid cytoreductive procedures. Appropriate patient selection based on prognostic scores along with complete cytoreduction can identify a cohort of patients likely to derive durable benefit from this combination treatment.

Conclusions: Advances in diagnostic and therapeutic techniques, including surgical cytoreductive techniques, have demonstrated significant survival gains in patients with peritoneal disease. Although HIPEC can be used for the management of various types of histologies, further development of high-level evidence is necessary to advance the field.

Introduction
Peritoneal metastases represent an advanced stage of abdominal cancers and often present with disseminated disease. The incidence of peritoneal metastases varies from 60% in ovarian cancers to 20% in gastric cancer and 8% to 17% colorectal cancer. An estimated 134,490 cases of colorectal cancer, 26,370 cases of gastric cancer, and 22,280 cases of ovarian cancer are expected to be diagnosed in 2016 in the United States alone, with estimated deaths from these cancers to reach 74,160.5

Many mechanisms of peritoneal spread have been proposed.4-7 Invasion of the luminal wall by invasive cancer or a perforation of the wall by a noninvasive tumor may lead to a direct spread to the peritoneum.4 Adherence molecules on free cancer cells may aid in peritoneal implantation.5 Iatrogenic spread during surgery, when tumor emboli are released from blood vessels and the lymphatics, is also a possible cause. When untreated, peritoneal carcinomatosis rapidly progresses to malignant ascites, acute bowel obstruction, and perforation requiring aggressive palliative chemother-
apy or surgery. Although patients with extraperitoneal metastases undergoing interventions of curative intent now have acceptable survival rates, peritoneal surface disease was historically considered an incurable entity and palliative therapy resulted in a median survival rate of a few months to less than 1 year.6,7

The magnitude of the burden of peritoneal metastasis is often underappreciated due to the low sensitivity rate of common imaging techniques for peritoneal disease.8 The rate of sensitivity of computed tomography in identifying peritoneal disease depends on the location in the abdomen, the size of individual nodules, and the morphology of the disease. The rate of sensitivity of computed tomography is 11% for visualizing peritoneal nodules less than 0.5 cm in size in the setting of colorectal disease, a rate that is unacceptable in clinical practice.6 However, use of advanced imaging for the detection of peritoneal carcinomatosis outside referral centers is infrequent, and many cases may go unreported.9 Consequently, peritoneal metastases are rarely included in clinical trials of systemic chemotherapy because the disease and its progression are often not detected by imaging standards set in the trials.2

Conventional antineoplastic strategies for visceral metastasis are not effective for peritoneal cancer. Although systemic chemotherapy has become more successful for use in visceral metastases, a secondary analysis of 2 phase 3 trials on systemic chemotherapy in metastatic colorectal cancer demonstrated that peritoneal metastases incur a 30% reduction in rate of overall survival (OS) than nonperitoneal metastases, even after adjusting for other prognostic factors (Fig 1).2 This lower rate of survival in peritoneal carcinomatosis suggests an inherent unfavorable biology.2

Cytoreductive surgeries in solid organ metastases have been useful as a curative therapy, whereas its use has been evolving over time for peritoneal disease. For patients with isolated metastasis to the peritoneum, cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy (HIPEC) yields improved rates of survival and quality of life.10,11 This multidisciplinary therapy is based on the concept of the peritoneum as an organ and hypothesizes that improved prognosis is achieved with complete removal of disease from the peritoneum.

Techniques of Peritonectomy

The oncological principles of peritonectomy surgeries were first described by Sugarbaker.12 The outcome of cytoreductive surgery is reliant on the extent of the removal of tumor deposits from visceral and parietal peritoneal surfaces, and surgical techniques vary depending on the site and volume of disease. Peritoneal staging is performed using well-established scoring systems (Peritoneal Cancer Index [PCI], Simplified PCI) that utilize tumor size and region of distribution to quantify disease burden. Techniques of peritonectomy require complete knowledge of embryology and anatomy to ensure successful extirpation of tumor.

Greater Omentectomy

The greater omentum is elevated off the transverse mesocolon by stripping the entire surface of the mesocolon. The dissection includes separation of the specimen from the gastroepiploic vessels (potential ligation) and division of the short gastric vessels. The omentum is elevated off the splenic hilum (splenectomy if necessary) and the anterior surface of the pancreas. Meticulous dissection of the omentum is essential for complete tumor removal.

Epigastric Peritonectomy

The falciform ligament is separated from the umbilicus along with the anterior peritoneum and resected flush with the liver surface to include the ligamentum teres hepatitis. The bridge of liver is often divided to access the left portal vein.

Right Hemidiaphragmatic Peritonectomy

Diaphragmatic muscle is stripped along its entirety after making a cruciate incision in the anterior peritoneum. The peritonectomy includes stripping the Gerota fascia, the right adrenal gland, and the Glisson capsule of the liver. Complete mobilization of the liver is essential and the retrohepatic inferior vena cava (IVC) is used as the medial border of the dissection.

Left Hemidiaphragmatic Peritonectomy

The upper left portion of the cruciate incision is used to initiate the left hemidiaphragmatic peritonectomy. Complete stripping of the diaphragmatic fibers with skeletonization (or ligation) of the phrenic vessels is...
undertaken. Dissection includes stripping the adrenal gland and Gerota fascia.

**Lesser Omentum Peritonectomy**
The hepatoduodenal ligament and the pars flaccida are dissected from the caudate lobe of the liver and the porta hepatitis. Careful dissection of the celiac axis branches and the right gastric arteries can elevate the tumor off of the lesser omentum. The IVC bursa is occasionally stripped, using the IVC, caudate lobe of the liver, and the left limb of the right crus as anatomical landmarks.

**Pelvic Peritonectomy**
Pelvic peritonectomy includes resection of the anterior peritoneum with the urachus and the medial umbilical ligaments. Skeletonization of the ureters, gonadal vessels, and resection of the upper rectum are often necessary to complete the peritonectomy. Visceral resections of the uterus and ovaries are performed as necessary.

**Anterior Peritonectomy**
Scar excision and resection of the anterior peritoneum is carefully undertaken with preservation of the rectus fascia at the initiation of the procedure. This becomes contiguous with the right hemidiaphragmatic peritonectomy performed in the right upper quadrant, the epigastric peritonectomy superiorly performed, the left hemidiaphragmatic peritonectomy performed in the left upper quadrant, the paracolic gutter peritonectomy laterally performed, and the pelvic peritonectomy inferiorly performed.

The completeness of cytoreduction is graded as CC-0 (no visible disease), CC-1 (<2.5 mm residual disease), CC-2 (2.5–25 mm), and CC-3 (>25 mm residual disease), or using the R score (R0 = complete resection, R1 = no gross disease with microscopic positive margins, R2 = macroscopic residual disease [R2a = <5 mm, R2b = 6–20 mm, R2c = >20 mm]).

**Hyperthermic Intraperitoneal Chemotherapy**
Early attempts at treatment for peritoneal carcinomatosis with surgery alone produced rapid rates of progression and treatment failure. The tumor cell entrapment theory proposed that intraperitoneal tumor emboli generated during surgery seeded on the peritoneal surface and aided by the growth factors associated with wound healing and peritoneal vasculature, forming metastases. Chemotherapy intraperitoneally delivered at the time of surgery can theoretically kill the tumor emboli, thus avoiding tumor progression.

Chemoperfusion has continued to progress since the HIPEC delivery system was first trialed. The benefits of the intraperitoneal delivery of antineoplastic drugs are manifold; hyperthermia aids in increased cellular penetration of the drug while synergistically enhancing cytotoxicity. Moreover, vascular stasis of microcirculation occurs in neoplastic cells at temperatures produced by HIPEC, while normal cells experience increased flow. This, combined with the selective accumulation of lactic acid and the resulting lowered level of pH in neoplastic tissue, results in cell death.

Drug concentrations achieved through this route cannot be replicated by intravenous chemotherapy and, to some extent, systemic toxicities are avoided.

Verwaal et al compared cytoreductive surgery/HIPEC followed by systemic chemotherapy vs systemic chemotherapy with or without palliative surgery in patients with colorectal cancer and peritoneal carcinomatosis (CRC-PC) and found that those in the HIPEC group had better survival rates than their counterparts. After 8 years of follow-up, the 5-year survival rate was 45% and significant differences in progression-free survival and disease-free survival rates between the HIPEC and control arms were also noted.

A phase 3 randomized trial comparing cytoreductive surgery/HIPEC with cytoreductive surgery alone for the management of gastric cancer reported improved rates of survival and acceptable rates of morbidity with cytoreduction/HIPEC. Another trial compared cytoreductive surgery/HIPEC plus systemic chemotherapy with systemic chemotherapy alone and demonstrated that the combined modality achieved modest rates of prolonged survival in carefully selected patients with gastric cancer.

The evidence continues to accumulate, with numerous observational and retrospective studies supporting the superiority of multimodality treatment with cytoreductive surgery/HIPEC in the setting of peritoneal carcinomatosis (Fig 2). Although this combination treatment began as experimental therapy, cytoreductive surgery/HIPEC is now the standard of care in peritoneal carcinomatosis, which is now being viewed as a treatable entity rather than a terminal condition. Appendiceal mucinous neoplasms, colorectal adenocarcinoma, mesothelioma, and epithelial ovarian carcinoma are histologies that most benefit from intraperitoneal chemotherapy.

For patients with high-grade disease or poor prognostic factors, multidisciplinary treatment using systemic chemotherapy is often employed (Table 1). Select ongoing and recently completed trials involving cytoreductive surgery and HIPEC are described in Table 2.

**Patient Selection Prognostication Scores**
Selecting patients for a potentially morbid surgery requires careful discussion on the possible benefits of the procedure. Tumor burden determines the completion of surgery as well as the long-term outcomes. Intraoperative staging systems, such as the PCI (13 regions, with scores ranging from 0 to 3) or...
Fig 2. — Selected evidence supporting use of cytoreductive surgery and HIPEC for the management of peritoneal carcinomatosis. CRS = cytoreductive surgery, DPAM = diffuse peritoneal adenomucinosis, EPIC = early postoperative intraperitoneal chemotherapy, HIPEC = hyperthermic intraperitoneal chemotherapy, OS = overall survival, PC = peritoneal carcinomatosis, PCI = Peritoneal Cancer Index, PMCA = peritoneal mucinous carcinoma, PMP = Pseudomyxoma peritonei infection, RCT = randomized controlled trial.

Information from references 18, 19, and 21 to 37.

Table 1. — Therapeutic Strategies in Peritoneal Surface Malignancies

<table>
<thead>
<tr>
<th>Histological Type</th>
<th>Effectiveness of Systemic Chemotherapy</th>
<th>Preferred Intraperitoneal Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appendiceal carcinoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-grade appendiceal neoplasm with high risk of recurrence</td>
<td>–</td>
<td>HIPEC (mitomycin)</td>
</tr>
<tr>
<td>Well-differentiated appendiceal neoplasm</td>
<td>±</td>
<td>HIPEC (mitomycin)</td>
</tr>
<tr>
<td>Moderately differentiated appendiceal neoplasm</td>
<td>±</td>
<td>HIPEC (mitomycin)</td>
</tr>
<tr>
<td>High-grade appendiceal neoplasm</td>
<td>++</td>
<td>HIPEC (mitomycin)</td>
</tr>
<tr>
<td>Gastric carcinoma</td>
<td>++</td>
<td>HIPEC (cisplatin/mitomycin) ± IP docetaxel/paclitaxel/IP docetaxel/paclitaxel ± HIPEC (cisplatin/mitomycin)</td>
</tr>
<tr>
<td>Colorectal carcinoma</td>
<td>++</td>
<td>HIPEC (mitomycin/oxaliplatin/irinotecan)</td>
</tr>
<tr>
<td>Ovarian carcinoma</td>
<td>+++</td>
<td>Intraperitoneal chemotherapy (cisplatin/paclitaxel) in optimally debulked patients</td>
</tr>
<tr>
<td>Peritoneal mesothelioma</td>
<td>+</td>
<td>HIPEC (cisplatin/doxorubicin/mitomycin)</td>
</tr>
</tbody>
</table>

Effectiveness of systemic chemotherapy is represented in an ordinal scale (–, ±, +, ++, +++), with – being least effective and +++ being maximally effective. HIPEC = hyperthermic intraperitoneal chemotherapy, IP = intraperitoneal.
Simplified PCI system (7 regions with 3 score groups), can be used to detect disease burden. Scoring systems, such as the Peritoneal Surface Disease Severity Score (PSDSS), are a noninvasive method of assigning disease burden in colon cancer. PSDSS designates weighted scores for severity of disease, extent of carcinomatosis, and aggressiveness of histology, summating the scores to stratify the disease into 4 prognostic categories. For example, moderately differentiated disease (3 points) with a PCI score of 12 (3 points) and mild symptoms (1 point) would be PSDSS stage 2 (4–7 cumulative points), whereas well-differentiated disease (1 point) with extensive intra-abdominal spread and a PCI score of 20 (7 points) and severe symptoms (6 points) would be PSDSS stage 4 (> 10 cumulative points).

PCI scores higher than 19 confer a poor prognosis despite cytoreductive surgery/HIPEC and are often a relative contraindication for peritoneal metastasis from CRC-PC. Use of the PSDSS system as a prognostic indicator for selecting patients has been validated in multiple studies.

Diagnostic laparoscopy, also called staging laparoscopy, is being increasingly used in the preoperative staging of peritoneal disease to identify patients with high peritoneal burden whose disease is considered inoperable in order to avoid unnecessary intervention. The procedure is technically feasible and associated with minimal operative morbidity and mortality. Patients excluded from cytoreductive surgery/HIPEC by diagnostic laparoscopy are often candidates for conversion chemotherapy (using systemic chemotherapy to shrink tumors and convert unresectable disease to resectable) and repeat diagnostic laparoscopy. Although port-site metastases are of concern, Valle et al detected no seeding at the entry sites in their series of 351 diagnostic laparoscopy procedures. We advocate wider use of diagnostic laparoscopy, especially in tumors at risk for peritoneal spread.

## Table 2. Selected Ongoing and Recently Completed Trials on HIPEC

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Colorectal Carcinoma</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| NCT02231086    | Patients who underwent intentionally curative resection for a T4N0–2M0 or intra-abdominally perforated colon cancer | Control: adjuvant systemic chemotherapy  
Experimental: adjuvant HIPEC followed by adjuvant systemic chemotherapy |
| NCT01226394    | Patients with a high risk of developing CRC-PC after resection of primary and 6 mo of adjuvant chemotherapy | Control: surveillance  
Experimental: laparotomy + HIPEC |
| **Appendiceal Carcinoma** |
| NCT01580410    | Patients with peritoneal surface malignancies from primary appendiceal tumors | Experimental arm 1: cytoreductive surgery + HIPEC (mitomycin)  
Experimental arm 2: cytoreductive surgery + HIPEC (oxaliplatin) |
| NCT01815359    | Patients with appendiceal or colorectal cancer with isolated peritoneal metastasis | Treatment arm 1: cytoreductive surgery + HIPEC (mitomycin)  
Treatment arm 2: cytoreductive surgery + early postoperative intraperitoneal chemotherapy (leucovorin/floxuridine) |
| **Ovarian Carcinoma** |
| NCT01539785    | Patients with platinum-sensitive first recurrence of ovarian cancer         | Control: cytoreductive surgery  
Experimental: cytoreductive surgery + HIPEC |
| NCT02124421    | Newly diagnosed, otherwise untreated, advanced-stage (stage 3/4) epithelial ovarian, fallopian tube, and primary peritoneal cancers | Treatment arm 1: cytoreductive surgery with HIPEC (carboplatin)  
followed by IV combination chemotherapy  
Treatment arm 2: cytoreductive surgery followed by adjuvant IV/IP chemotherapy |
| **Gastric Carcinoma** |
| NCT01882933    | Patients with locally advanced gastric carcinoma                             | Control arm: curative gastrectomy with D1 to D2 lymph-node dissection  
Experimental: curative gastrectomy with D1 to D2 lymph-node dissection + HIPEC (oxaliplatin) |
| NCT02158988    | Patients with peritoneally metastasized gastric cancer                       | Control: preoperative chemotherapy + cytoreductive surgery  
Experimental: preoperative chemotherapy + cytoreductive surgery + HIPEC |

CRC-PC = colorectal cancer/peritoneal carcinomatosis, HIPEC = hyperthermic intraperitoneal chemotherapy, IP = intraperitoneal, IV = intravenous.
Diffusion-Weighted Magnetic Resonance Imaging

Conventional imaging with computed tomography or magnetic resonance imaging has poor rates of per-lesion sensitivity in the setting of peritoneal carcinomatosis, which varies with site and size of the tumor deposits, which can result in falsely low PCI scores. In addition, patients with low-volume disease may not be detected, and the therapeutic window before they progress into high-volume disease may be lost.

Diffusion-weighted imaging better captures the contrast between normal and tumor tissue compared with conventional cross-sectional imaging modalities. Diffusion-weighted imaging translates the restrictive effect of tissue structure on the mobility of water molecules into visible signal intensity or contrast (Fig 3A–C). Integrating diffusion-weighted imaging with conventional imaging has been shown to increase the rate of accuracy in the staging of ovarian cancer and in patients with peritoneal disease; however, the value of this modality must be balanced with the time, resource utilization, and patient discomfort associated with it.

Anesthesia and Critical Care

Use of hyperthermia puts unique demands on patient physiology, and patients undergoing HIPEC are at increased risk for cardiac complications, particularly those with existing cardiovascular disease or associated risk factors. In addition, chemoperfusion creates changes in intra-abdominal pressure that may increase systemic vascular resistance and affect cardiac output. Chemotherapeutic drugs intraperitoneally administered possess their own toxicities (eg, nephrotoxicity with cisplatin) that may be accentuated by altered fluid dynamics. Fluid loss is also high during peritoneectomy procedures due to loss from denuded areas.

Modern anesthetic techniques and stringent patient selection has made cytoreductive surgery/HIPEC feasible for patients at both extremes of age. Advances in invasive and noninvasive fluid status monitoring and management, along with analgesic control with regional anesthesia, have contributed to reduced stays in the intensive care unit and improved perioperative outcomes.

Combined Resections

Concurrent extraperitoneal metastases have long been considered a contraindication for resection, although some studies indicate the feasibility and better outcomes for the combined resection of hepatic and peritoneal metastases. Similar radical resections, including other viscera (bladder, colon, diaphragm, pancreas, small intestine, spleen, stomach), have been reported with acceptable rates of morbidity. Advances in electrosurgical techniques, including ablative technologies and hemostasis, have made such advances possible.

Laparoscopy

Minimal-access cytoreductive surgery/HIPEC has been studied in patients with low tumor burden and with disease of less malignant potential. Diagnostic laparoscopy is performed prior to surgery and patients with a PCI score lower than 10 are enrolled into a laparoscopic strategy. With strict patient selection, laparoscopic cytoreductive surgery/HIPEC is a procedure associated with minimal morbidity and mortality and shorter operating times while achieving similar operative and oncological outcomes to the open procedure. Concerns regarding the ability to complete a thorough examination of the peritoneal cavity have precluded the widespread application of this technique until further evidence is gathered.

Perfusion Techniques

Drug delivery to the peritoneum is controlled by flow rate, temperature, dose, and duration of HIPEC. Experiments by Facy et al on animal models suggest that increasing the intraperitoneal pressure enhances the penetration of the chemotherapeutic drug into the peritoneum but not the depth of penetration. The effect is maximal when using an open technique at

Fig 3A–C. — (A) Low-attenuation material along the perihepatic, lesser sac, and perigastric regions suggestive of mucinous ascites seen in contrast-enhanced computed tomography, (B) axial, T2-weighted magnetic resonance imaging, and (C) diffusion-weighted imaging of the abdomen showing peritoneal metastases along the left lobe of the liver and anterior to the greater curvature of the stomach consistent with peritoneal implants.
25 cm H₂O more in the parietal peritoneum than vis-
ceral, and it is feasible and well tolerated. In animal
and in vitro models, higher flow rates were seen to
shorten the time to attain and maintain target tem-
peratures during HIPEC, thus resulting in higher
temperature gradients that may potentially enhance
the rate of cytotoxicity. Ceelen et al reported that
higher perfusion temperatures worsen the metabolic
changes associated with oxaliplatin HIPEC but they
do not affect surgical outcomes.

Despite the evolution of HIPEC as standard ther-
apy in peritoneal carcinomatosis, standardized pro-
tocols are not yet followed in the United States. Af-
after a Dutch cancer institute implemented an HIPEC
protocol in peritoneal surface malignancy centers in
the Netherlands, outcomes were improved compared
with the preimplementation phase. A standard
protocol decreased the length of procedures, rate of
blood loss, complications, and duration of hospital
stay. It should be noted that, with experience, the
corresponding improvement in patient selection led
to fewer numbers of patients with extensive disease
after the protocol was enforced. Attempts at stan-
dardizing the delivery of HIPEC in US centers special-
izing in peritoneal surface malignancy have resulted
in a recommendation of a closed technique using a
standardized dual dose of mitomycin in a 90-minute
cycle in patients with CRC-PC.

Medications

Oxaliplatin and mitomycin are the components of
common HIPEC regimens for CRC-PC and compa-
risons of their efficacy have yielded conflicting re-
sults. One multisite, retrospective review reported
that treatment with oxaliplatin and mitomycin con-
ferred better rates of OS than oxaliplatin alone in pa-


tients with CRC, favorable histologies, and low-tumor
burden, but a separate comparison cohort trial found
no difference in survival. Both of these studies were
restricted to patients with complete cytoreduction.

Glockzin et al compared bidirectional oxaliplatin-
and irinotecan-based HIPEC and found no differences
in rates of morbidity and toxicity but did suggest that
oxaliplatin may have clinical superiority on the basis of
a positive survival trend after 3 years of follow-up.

Studies on animal models of CRC have shown that
combination irinotecan/oxaliplatin/mitomycin/pani-
tumumab exceeds any survival benefit from any other
agent, alone or in combination.

Applications of newer antineoplastic agents such as
melphalan are being investigated for intraperito-
nal therapy. Sardi et al reported on the successful
intraperitoneal use of melphalan in patients with
peritoneal carcinomatosis who failed conventional
systemic chemotherapies and cytoreductive surgery/
HIPEC. Their rates of OS were promising, particularly
in the milieu of documented chemoresistance; how-
ever, significant postoperative myelosuppression was
a noted concern. Although the peritoneal–plasma
barrier prevents the systemic absorption of intraperi-
toneal chemotherapy, systemic accumulation does oc-
cur with peritoneal stripping in cytoreductive surgery
and through other routes, such as in the hepatic met-
abolism of oxaliplatin and mitomycin. Rates of he-
matological toxicity following treatment with oxalipla-
tin, mitomycin, and HIPEC ranges from 27% to 40%,
but grade 4 toxicity is minimal. Kemmel et al ad-
vocated the monitoring of plasma concentration with
oxaliplatin and mitomycin treatment at 30 minutes af-
fter starting HIPEC to identify patients at high risk for
developing neutropenia. However, neutropenia after
HIPEC has not been shown to increase rates of mortal-
ity, postoperative infection, or length of hospital stay.

Other Surgical Methods

New methods of delivering intraperitoneal che-
otherapy, such as early and sequential postoperative
intraperitoneal chemotherapy, have been attempt-
ed. Sequential postoperative intraperitoneal che-
motherapy was shown to be inferior in terms of sur-
vival outcomes while offering comparable morbidity
and mortality rates in a case-control study of patients
with CRC, whereas early postoperative intraperito-
nal chemotherapy was shown to have a higher rate
of complications when given alone or in combination
with HIPEC. HIPEC is a reliable method for admin-
istering intraperitoneal chemotherapy with acceptable
complications and survival outcomes.

Role of Systemic Chemotherapy

The effect of perioperative chemotherapy on out-
comes after cytoreductive surgery/HIPEC has been
retrospectively investigated at multiple centers. A
prospective, observational study of neoadjuvant che-
motherapy (folinic acid/fluorouracil/oxaliplatin
or capecitabine/oxaliplatin with or without bevaci-
zumab) in mucinous appendiceal neoplasms showed
low tumor burden, fewer numbers of resections, and
more completed surgeries with chemotherapy. Sur-
vival rates were similar to patients without systemic
chemotherapy but were better in patients who had
significant histological response. By contrast, Black-
ham et al reported no survival advantage in preop-
erative chemotherapy but reported an 8% response
rate (radiological/clinical) compared with a 29% rate
in the former study. Adjutant chemotherapy was as-
associated with better rates of progression-free survival
but only in high-grade disease. A retrospective re-
view of the effect of neoadjuvant chemotherapy on
high-grade appendiceal adenocarcinoma found no
statistically significant differences in operative details
or survival outcomes between the chemotherapy and
nonchemotherapy groups, whereas another retrospective review showed improved prognosis in mucinous tumors with signet ring cell histology.78,79

Although it is impossible to quantify the advantage of perioperative systemic chemotherapy without a randomized controlled trial, it may be safe to assume that this therapy provides benefit in carefully selected patients without adding to postoperative complications.

A retrospective review of perioperative systemic chemotherapy in study patients with lymph-node positive colorectal cancer showed improved rates of OS and progression-free survival in a chemotherapy cohort irrespective of the timing of chemotherapy; however, a large number of these study patients did not receive chemotherapy due to major postoperative complications.80 Therefore, these results should be interpreted with caution. The effect of adding bevacizumab to a neoadjuvant chemotherapy regimen in the setting of colorectal cancer was investigated in 2 retrospective reviews.81,82 Although Eveno et al81 reported that use of bevacizumab doubled the morbidity rates and need for additional surgery even after propensity score matching, Ceelen et al82 demonstrated that bevacizumab therapy was associated with improved rates of OS and no added morbidity.

Although response to neoadjuvant chemotherapy is an indicator of favorable tumor biology, the converse is not always true. Treatment failure, as determined by imaging, surgical data, and tumor makers, should not dissuade the surgeon from proceeding with cytoreductive surgery as clinical response does not correlate with long-term prognosis in the setting of colorectal cancer; however, use of chemotherapy does.83

**Preemptive Approaches**

**Repeat Combination Treatment for Peritoneal Recurrences**

Recurrences are not uncommon after initial intervention in peritoneal cancers, so iterative rounds of HIPEC have been reported.84-89 Iterative cytoreductive surgery/hipec offers reasonable rates of survival when compared with conservative treatment using palliative chemotherapy, particularly in appendiceal neoplasms and malignant peritoneal mesotheliomas.84-86 However, effectiveness of a second round of HIPEC in the setting of CRC-PC is questionable. Careful patient selection using factors such as performance status, tumor volume, and symptom severity may improve outcomes.87 Some authors advocate using rates of progression-free survival to determine outcomes, but it is rational to select those patients for whom a second complete resection is feasible as survival after a second procedure is based on the completeness of the resection.85,87 Certain presentations, such as peritoneal mucinous carcinoma of the appendix with positive lymph nodes, incur poor survival rates even after additional procedures.88 Morbidity and mortality rates do not differ between initial and subsequent procedures; however, attempts to ensure complete cytoreduction using intensive surgery may result in undesirable complications.89 We recommend a second round of HIPEC in patients with recurrent disease for whom complete cytoreduction is feasible and histology is favorable.

**Prophylactic Combination Treatment vs Routine Second-Look Surgery**

Patients with known risk factors for peritoneal recurrence present a clinical quandary, as current diagnostic techniques for peritoneal carcinomatosis can be inaccurate. The choice between watchful waiting vs early intervention can be difficult to resolve on current evidence. Two approaches being tested are second-look surgery and prophylactic HIPEC, both of which involve selecting patients without detectable peritoneal metastases or those with low-volume peritoneal disease but who harbor risk factors.90,91 In the former, patients without clinically or radiologically detectable disease undergo a second-look surgery at the end of a fixed period of follow-up. HIPEC is systemically performed in all patients, with cytoreductive surgery performed in those with macroscopic peritoneal disease alone. In a prospective trial of 41 patients with CRC, more than one-half of study patients had detectable peritoneal disease and the reported 5-year OS rate was 90%.90 In a similar study looking at routine second-look surgery in patients with CRC-PC who underwent complete initial resection, rates of peritoneal recurrence and 2-year OS were 71% and 91%, respectively, after the second intervention was reported.91 The morbidity and mortality rates were 7% to 9.7% and 0% to 2.4%, respectively.

Prophylactic HIPEC is offered to patients with advanced disease who are at high risk for peritoneal spread at the time of diagnosis. Risk is determined on the basis of histology and pathological stage. A prospective study of 25 patients with nonmetastatic CRC reported morbidity rates comparable to standard surgery.92 After 4 years of follow-up, the aggressive group exhibited significantly lower rates of peritoneal metastasis and higher rates of OS and disease-free survival.92 A similar study of gastric cancer in patients with either locally advanced disease or with positive peritoneal washings identified a potential role for prophylactic HIPEC.93 However, the sample sizes were small, so future research is warranted before definitive conclusions can be reached.93 Sloothaak et al94 reported the feasibility of delayed laparoscopic HIPEC in patients with CRC who had a high risk for peritoneal carcinomatosis.

Selection criteria vary across studies, so the advantage of one approach over another cannot be compared and quantified. In addition, adjuvant chemotherapy was administered in some of the studies,
confounding the interpretation of the results. Despite the successes of both techniques, the dilemma lies in identifying patients at high risk for peritoneal recurrence.

Future Directions

Improving methods for appropriate patient selection and advancing surgical techniques are disruptive changes that will alter the treatment landscape for peritoneal carcinomatosis. Use of telemedicine, robotic assistance, and immersive simulations for training and interventions are likely to optimize the field of surgery. Dynamic techniques for accurate, intraoperative, pathological diagnoses can prevent unnecessary interventions. Robotic-assisted cytoreductive surgery for peritoneal disease has already been successfully used in metastatic ovarian cancers. In addition, with the advent of telesurgery, HIPEC can be introduced to areas that lack trained surgical oncologists.

Conclusions

Advances in diagnostic and therapeutic techniques, including surgical cytoreductive techniques, have demonstrated significant survival gains in patients with peritoneal disease. Such improvements must happen within the changing landscape of cancer care. In addition, advances in immunotherapy and personalized therapies hold significant promise, as do techniques in genetic sequencing that may predict and potentially prevent disease rather than treat it. Collaboration and partnerships with the community and our patients are critically important for significant strides to be made. Although hyperthermic intraperitoneal chemotherapy can be used for the management of various types of histologies, high-level evidence is still lacking for its use in patients with peritoneal carcinomatosis.

References


Racial Disparity in Time Between First Diagnosis and Initial Treatment of Prostate Cancer

Ballington L. Kinlock, PhD, Roland J. Thorpe Jr, PhD, Daniel L. Howard, PhD, Janice V. Bowie, PhD, Louie E. Ross, PhD, David O. Fakunle, and Thomas A. LaVeist, PhD

Background: Disparities among patients with prostate cancer exist across the continuum of care. The interval of time that lapses between first diagnosis and treatment is another disparity that may exist but has not been fully explored.

Methods: Our study looked at the data of 749 men (353 black and 396 white) who were 40 to 81 years of age when they entered the North Carolina Central Cancer Registry during the years 2007 and 2008. Our dependent variable was the amount of months that had passed between first diagnosis and treatment. Our main independent variable was self-reported race. Covariates included age, income, level of education, insurance status, treatment received, Gleason score, and level of medical mistrust. We used negative binomial regression analysis to determine the association between the amount of time that lapsed between a diagnosis of prostate cancer and treatment by race.

Results: Compared with white men, black men were more likely to experience a longer wait time between diagnosis and treatment of prostate cancer (incidence rate ratio [IRR] 1.19; 95% confidence interval [CI], 1.04–1.36). Controls for demographical, clinical, and psychosocial variables (IRR 1.24; 95% CI, 1.04–1.43) did not explain this difference between the races.

Conclusions: These results suggest that the amount of time that lapses between first diagnosis and treatment of prostate cancer is longer for black men compared with white men. Our findings have identified an under-reported racial disparity in the disease continuum of prostate cancer.

Introduction

In the United States, an estimated 180,890 new cases of prostate cancer will be diagnosed and 26,120 men will die from the disease in 2016; indeed, prostate cancer is one of the most common forms of cancer affecting American men, with black men being disproportionately affected. From 2007 to 2011, black Americans represented 224 cases per 100,000 new cases of prostate cancer compared with 139.9 cases in white Americans. The death rate for prostate cancer is twice as high in black Americans than any other group, and blacks have a 70% higher risk of developing prostate cancer than their white counterparts. Although the etiology of prostate cancer remains elusive, age, ethnicity, and family history have all been identified as risk factors associated with the likelihood of developing prostate cancer. Prostate cancer is more likely to occur in older men and in men with a family history of prostate cancer. Moreover, men whose fathers or brothers have been diagnosed have twice the risk of developing prostate cancer.

Prostate cancer disparities are known to exist at multiple levels, including stage of presentation, diagnosis, treatment modality selected, quality of life, and mortality rate, with black men experiencing the greatest burden at all levels of the prostate cancer care continuum. Another disparity not yet well characterized is the amount of time that lapses between first diagnosis and treatment of prostate cancer. The literature focusing on the consequences of delayed treatment of prostate cancer are mixed, but some reports have linked a longer wait time from diagnosis to treatment to higher biochemical recurrence, disease upgrading, and mortality. Studies examining the length of time between diagnosis and initial treatment that have compared blacks and whites have also yielded varying results.
Abern et al\textsuperscript{6} reported that African American men might have a longer wait time until treatment once diagnosed with prostate cancer. However, using data from Veteran Affairs health care systems, Banez et al\textsuperscript{10} found no significant difference between racial groups. By contrast to the findings of Banez et al,\textsuperscript{10} Stokes et al\textsuperscript{11} concluded that black men did have longer wait times between diagnosis and treatment. However, their sample was obtained using data from Medicare and the Surveillance, Epidemiology, and End Results Program, which meant that the data the researchers used were from men 65 years of age or older.\textsuperscript{11} When considering men aged 45 years and older, Porter et al\textsuperscript{12} suggested that minimal racial/ethnic differences exist in the time to treatment. The study sample consisted of a cohort of men taking part in a southern California managed care, equal-access health care system; thus, these patient data are not indicative of the health care experience of most Americans.\textsuperscript{12}

Lack of consensus among previous reports as well as lack of the generalizability of previous studies served as an impetus for our study. Our objective was to determine if differences were present in the amount of time between first diagnosis and initial treatment among black and white men with prostate cancer using a sample that reflected the variety of care systems representing the range of experiences to which most Americans are exposed.

### Methods

The Diagnosis and Decisions in Prostate Cancer Treatment Outcomes trial is a cross-sectional study designed to examine factors that influence the selection of treatment modality for prostate cancer, to explore racial differences in disease burden, and to examine quality of life among men with prostate cancer.

Using a rapid case ascertainment procedure, we retrospectively recruited 877 men (415 black and 462 white) between the ages of 40 and 81 years who had entered the North Carolina Central Cancer Registry (NCCCR) between the years 2007 and 2008. Of the 877 men originally recruited, 749 men (353 black and 396 white) who provided complete information on time to treatment and treatment modality were eligible for this study. Eligibility criteria included age 35 years or older, a diagnosis of prostate cancer, treatment for prostate cancer, and self-identification as either white or black.

Recruitment began in October 2009 and ended in December 2011. On a monthly basis, NCCCR staff contacted the primary research network hospitals to request reports identifying patients meeting the eligibility criteria. The NCCCR mailed prospective study participants a pamphlet describing the study and informing them that they may be contacted in the future to participate in a study.

After our study team confirmed the eligibility of the patients, the NCCCR mailed the physician of record of each eligible patient a notification of intent to contact the prospective participant about enrolling in the study. Physicians were given 3 weeks to object to our request to contact their patients. If the physician did not refuse patient contact within 3 weeks, then we mailed the eligible patient a packet containing a recruitment letter describing the study, an NCCCR brochure, and a copy of the Institutional Review Board–approved consent and Health Insurance Portability and Accountability Act forms. In the letter, a phone number was provided that prospective participants could call for questions or to decline inclusion.

Interviewers then contacted prospective study participants by telephone, screened them for study eligibility, explained the study, answered questions, and sought their participation. If the candidate agreed to participate, then the interviewer reviewed the consent form, obtained verbal consent, and proceeded with the survey questionnaire.

The survey consisted of a series of questions related to prostate cancer, the process of care, and their quality of life following treatment. The study was approved by the Institutional Review Boards of the Johns Hopkins Bloomberg School of Public Health, US Department of Defense, and NCCCR.

### Measures

The dependent variable was the amount of months that passed between first diagnosis and initial treatment. Respondents were asked, “In what month and year were you diagnosed with prostate cancer?” They were also asked, “In what month and year did you receive your first treatment?” Subtracting the months that lapsed between the first diagnosis and initial treatment for each man created a continuous variable that represented the time between first diagnosis and initial treatment. The main independent variable was self-reported race. A dichotomous variable was derived, where 0 represented white men and 1 represented black men.

Covariates included demographic, psychosocial, and clinical variables. Demographic variables included age, marital status, level of education, annual household income, and health insurance coverage (no insurance, private health insurance, Medicare, Medicaid, Civilian Health and Medical Program of the Uniformed Services, or Civilian Health and Medical Program of the Department of Veterans Affairs). Those who responded “yes” to having any of the health insurances were considered insured, and those who did not have any health insurance were considered uninsured.

Our psychosocial measure was level of medi-
cal mistrust, which was assessed using the 7-item Medical Mistrust Index. The scale employs 1 to 4 Likert responses ranging from “strongly disagree” to “strongly agree.” Examples of items in the mistrust scale included: “Sometimes I wonder if hospital staffs really know what they are doing,” “Patients have sometimes been deceived or misled by hospitals,” and, “When dealing with hospitals, one better be cautious.” The mean across all the measures for each respondent is a reliable score of their trust in the health care system, with higher mean scores reflecting greater levels of medical mistrust.

Clinical variables included treatment modality and Gleason score. Gleason scores were obtained from the pathology reports and were separated into 3 different categories (low-, medium, and high-grade cancer).

**Analysis**

We used the student *t* test for continuous variables and the chi-square test for categorical variables to compare select demographic, clinical, and psychosocial variables by race. Negative binomial regression models were specified to examine the association between race and time between first diagnosis and initial treatment controlling for covariates. The negative binomial regression model, rather than the Poisson regression model, was selected to account for the overdispersion of the outcome variable.

Incidence rate ratios (IRRs) and corresponding 95% confidence intervals (CIs) were used to present findings from the 4 negative binomial models. The first model tested the bivariate association between race and the length of time between first diagnosis and initial treatment. The second model tested the association between race and length of time between first diagnosis and initial treatment after accounting for demographic variables. The third model tested the association after adjusting for both demographic and clinical variables. The fourth model tested the association after adjusting for demographic variables, clinical variables, and medical mistrust.

White men were the reference group for all of our analysis. *P* values less than .05 were considered to be significant. All statistical procedures were performed using Stata, version 13.1 (StataCorp LP, College Station, Texas).

**Results**

Table 1 shows the distribution of demographic, psychosocial, and clinical variables for the study participants by race.

Overall, 749 participants were included in our analyses; of those, 47.3% were black. Compared with white men, black men had significantly lower levels of income, were younger, were less likely to be insured, and were less likely to be married. In terms of level of education, a lower percentage of black men (15.0%) had a bachelor degree compared with white men (30.7%). Black men (2.7 ± 0.3) reported a higher level of medical mistrust on average compared with white men (2.4 ± 0.4). Although a higher percentage of white men (79.5%) underwent prostatectomy than black men (69.4%), prostatectomy was the most common form of treatment for men from both groups. A higher percentage of black men (22.6%) received radiotherapy compared with white men (16.1%). Although differences were seen in the treatments received, no differences were present in the prostate cancer grade as indicated by the Gleason scores (see Table 1).

### Table 1. — Distributions of Select Characteristics of Study Participants for Total Sample by Race

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (N = 749)</th>
<th>Black (n = 353)</th>
<th>White (n = 396)</th>
<th><em>P</em> Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Race, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>47.3</td>
<td>52.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (mean ± SD), y</td>
<td>62.8 ± 7.7</td>
<td>61.9 ± 7.5</td>
<td>63.6 ± 7.7</td>
<td>.003</td>
</tr>
<tr>
<td>Married, %</td>
<td>76.6</td>
<td>67.4</td>
<td>84.8</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Annual household income ($), %</td>
<td></td>
<td></td>
<td></td>
<td>&lt; .001</td>
</tr>
<tr>
<td>&lt; 25,000</td>
<td>5.4</td>
<td>11.0</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>25,000–49,999</td>
<td>36.3</td>
<td>49.0</td>
<td>25.0</td>
<td></td>
</tr>
<tr>
<td>50,000–74,999</td>
<td>17.6</td>
<td>17.2</td>
<td>17.9</td>
<td></td>
</tr>
<tr>
<td>≥ 75,000</td>
<td>40.5</td>
<td>22.6</td>
<td>56.5</td>
<td></td>
</tr>
<tr>
<td>Level of education, %</td>
<td></td>
<td></td>
<td></td>
<td>.001</td>
</tr>
<tr>
<td>Less than high school</td>
<td>11.9</td>
<td>18.9</td>
<td>5.6</td>
<td></td>
</tr>
<tr>
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<td>34.2</td>
<td>16.5</td>
<td></td>
</tr>
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<td>23.6</td>
<td></td>
</tr>
<tr>
<td>Bachelor degree</td>
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<td>15.0</td>
<td>30.7</td>
<td></td>
</tr>
<tr>
<td>Master/doctorate</td>
<td>16.6</td>
<td>9.0</td>
<td>23.4</td>
<td></td>
</tr>
<tr>
<td>Health insurance, %</td>
<td>93.7</td>
<td>89.8</td>
<td>97.1</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Medical mistrust (mean ± SD)</td>
<td>2.6 ± 0.1</td>
<td>2.7 ± 0.3</td>
<td>2.4 ± 0.4</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Gleason score, %</td>
<td></td>
<td></td>
<td></td>
<td>.532</td>
</tr>
<tr>
<td>Low-grade cancer (≤ 6)</td>
<td>49.0</td>
<td>47.4</td>
<td>50.5</td>
<td></td>
</tr>
<tr>
<td>Medium-grade cancer (7)</td>
<td>43.0</td>
<td>45.1</td>
<td>41.1</td>
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<tr>
<td>High-grade cancer (8–10)</td>
<td>7.8</td>
<td>7.3</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>Treatment received, %</td>
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<td></td>
<td></td>
<td>.005</td>
</tr>
<tr>
<td>Prostatectomy</td>
<td>74.7</td>
<td>69.4</td>
<td>79.5</td>
<td></td>
</tr>
<tr>
<td>Radiation</td>
<td>19.2</td>
<td>22.6</td>
<td>16.1</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>6.0</td>
<td>7.9</td>
<td>4.2</td>
<td></td>
</tr>
</tbody>
</table>

SD = standard deviation.
Table 2 contains data from the 4 negative binomial regression models designed to examine the association between race and time between first diagnosis and initial treatment of prostate cancer.

In model 1, the length of time between first diagnosis and initial treatment was regressed on race. The data revealed that black men (IRR 1.19; 95% CI, 1.04–1.36) had a higher likelihood of experiencing a longer wait time between diagnosis and treatment than their white counterparts. The second model showed that the likelihood of black men experiencing a longer wait time between first diagnosis and initial treatment remained, even after accounting for age, marital status, annual household income, level of education, and health insurance status (IRR 1.24; 95% CI, 1.06–1.45). After accounting for the demographic variables used in model 2 along with the Gleason scores and type of treatment received, model 3 revealed that black men had a higher likelihood of experiencing a longer wait time until treatment (IRR 1.19; 95% CI, 1.02–1.39).

Medical mistrust was introduced into the analysis in model 4. Black race remained a significant predictor of having a higher likelihood of experiencing a longer wait time between first diagnosis and initial treatment after controlling for demographic variables, clinical variables, and the medical mistrust psychosocial variable (IRR 1.22; 95% CI, 1.04–1.43).

Discussion

Racial disparities in prostate cancer exist at multiple levels across the treatment continuum and have been well documented. The results of our study provide additional evidence that demonstrates yet another level at which racial disparities exist in prostate cancer care for an outcome not well characterized in the literature. After being first diagnosed with prostate cancer, the amount of time that lapses until initial treatment is longer for black men compared with white men. Therefore, health care professionals should place special emphasis on educating black men about their treatment options and following-up with them to ensure that they are beginning treatment in a timely manner.

In general, prostate cancer is a slow, progressing tumor, so it is feasible that health care professionals might be hesitant to rush their patients into treatment, giving them time to contemplate the number of treatment options available to them.

Table 2. — Associations Between Race and Time Lapse After Diagnosis Until Treatment in Study Participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1 IRR (95% CI)</th>
<th>Model 2 IRR (95% CI)</th>
<th>Model 3 IRR (95% CI)</th>
<th>Model 4 IRR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Racea</td>
<td>1.19 (1.04–1.36)†</td>
<td>1.24 (1.06–1.45)†</td>
<td>1.19 (1.02–1.39)†</td>
<td>1.22 (1.04–1.43)†</td>
</tr>
<tr>
<td>Age</td>
<td>—</td>
<td>1.01 (1.00–1.02)†</td>
<td>1.01 (1.00–1.02)†</td>
<td>1.01 (1.00–1.02)†</td>
</tr>
<tr>
<td>Married</td>
<td>—</td>
<td>1.06 (0.89–1.26)</td>
<td>1.08 (0.91–1.29)</td>
<td>1.09 (0.91–1.30)</td>
</tr>
<tr>
<td>Annual household incomeb, $</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25,000–49,999</td>
<td>—</td>
<td>1.06 (0.72–1.54)</td>
<td>1.11 (0.76–1.62)</td>
<td>1.10 (0.75–1.63)</td>
</tr>
<tr>
<td>50,000–74,999</td>
<td>—</td>
<td>0.97 (0.64–1.47)</td>
<td>1.01 (0.66–1.52)</td>
<td>0.99 (0.64–1.52)</td>
</tr>
<tr>
<td>≥ 75,000</td>
<td>0.99 (0.65–1.52)</td>
<td>1.06 (0.69–1.63)</td>
<td>1.05 (0.67–1.62)</td>
<td></td>
</tr>
<tr>
<td>Level of educationc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High school/general educational development degree</td>
<td>—</td>
<td>1.47 (1.11–1.94)†</td>
<td>1.48 (1.12–1.95)†</td>
<td>1.47 (1.11–1.98)†</td>
</tr>
<tr>
<td>Some college/associate degree</td>
<td>—</td>
<td>1.32 (0.99–1.77)</td>
<td>1.31 (0.99–1.75)</td>
<td>1.31 (0.98–1.76)</td>
</tr>
<tr>
<td>Bachelor degree</td>
<td>1.35 (0.99–1.77)</td>
<td>1.33 (0.98–1.81)</td>
<td>1.30 (0.95–1.78)</td>
<td></td>
</tr>
<tr>
<td>Master/doctorate degree</td>
<td>1.59 (1.16–2.19)†</td>
<td>1.55 (1.12–2.12)†</td>
<td>1.53 (1.10–2.12)†</td>
<td></td>
</tr>
<tr>
<td>Health insurance, %</td>
<td>—</td>
<td>1.11 (0.83–1.14)</td>
<td>1.18 (0.89–1.14)</td>
<td>1.19 (0.88–1.15)</td>
</tr>
<tr>
<td>Gleason scored</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium-grade cancer (7)</td>
<td>—</td>
<td>—</td>
<td>0.92 (0.80–1.07)</td>
<td>0.92 (0.79–1.06)</td>
</tr>
<tr>
<td>High-grade cancer (8–10)</td>
<td>—</td>
<td>—</td>
<td>0.68 (0.54–0.91)†</td>
<td>0.67 (0.50–0.89)†</td>
</tr>
<tr>
<td>Treatment receivede</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radiation</td>
<td>—</td>
<td>—</td>
<td>1.19 (0.99–1.44)</td>
<td>1.20 (0.99–1.46)</td>
</tr>
<tr>
<td>Other</td>
<td>—</td>
<td>—</td>
<td>0.86 (0.61–1.20)</td>
<td>0.85 (0.60–1.20)</td>
</tr>
<tr>
<td>Medical mistrust</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.92 (0.78–1.09)</td>
</tr>
</tbody>
</table>

aWhite men. b< $25,000. cLess than high school. dLow-grade cancer (≤ 6). eProstatectomy. †Statistically significant. CI = confidence interval, IRR = incidence rate ratio.
ment options available to them after being diagnosed. However, extended treatment delays for prostate cancer in black men may have subsequent consequences and, thus, contribute to higher rates of mortality and worse quality of life following treatment. Some reports have linked a longer wait time from first diagnosis to initial treatment to higher biochemical recurrence, disease upgrading, and mortality, while others fail to find that association. Biochemical recurrence and mortality rates notwithstanding, reports have suggested that a delay in radical prostatectomy could lead to erectile dysfunction and urinary incontinence, both of which can affect patient quality of life.

Gleason scores also served as a predictor of the length of time between first diagnosis and initial treatment (see Table 2). The length of time that lapsed between first diagnosis and initial treatment decreases as one goes from a low to a high Gleason score, meaning those with more aggressive tumors are treated the fastest (see Table 2). This finding is not surprising as one can imagine that a more severe diagnosis might create a sense of urgency.

An obvious limitation to our study is the omission of other racial/ethnic groups. Another shortcoming is that men who received orchietomy, hormone therapy, cryotherapy, chemotherapy, and other treatments were all placed into the “other” category due to the small sample size for each; thus, we were unable to analyze wait time based on each individual treatment choice. We were also not able to determine if the delays were due to the physician, patient, or other factors, nor were we able to determine with certainty whether there was mutual agreement on the delays.

However, our study has several strengths. First, our participants comprised men with prostate cancer of differing ages (< and ≥ 65 years), a study cohort not typical of other prior studies involving samples of Medicare recipients alone (≥ 65 years of age). Our study also looked at data from men with varying forms of health insurance, which is representative of the general population. An additional strength to this study is that we incorporated the Medical Mistrust Index to determine whether the longer wait time until initial treatment among blacks is a response to their well-documented mistrust of the health care system.

Conclusions

All factors associated with disparities in the prostate cancer care continuum, from time of first diagnosis to treatment, must be identified to make a better attempt at addressing and eliminating the disparities. Our work demonstrates that a racial disparity exists in the time interval between first diagnosis and initial treatment of men with prostate cancer. However, more work is warranted to pinpoint the exact proximal as well as distal factors that may be responsible for this disparity.
Background: The incidence of anal cancer is on the rise among HIV-infected men who have sex with men (MSM). Given the increasing availability of screening, this study explored anal cancer screening awareness and behaviors among MSM infected with HIV.

Methods: In-depth interviews were conducted with 58 MSM infected with HIV.

Results: Other than 2 participants treated for anal cancer and 3 treated for precancerous anal lesions, the majority of participants had never heard of anal cancer. Men reported lack of awareness and recommendations from their health care professionals as the greatest barriers to screening. Upon learning about their risk for anal cancer and the availability of screening, the men were eager to discuss screening with their physicians. Participants provided numerous recommendations for future interventions, including training health care professionals to promote screening, disseminating information pertaining to anal cancer through social networks, and creating media campaigns to raise awareness about the need to screen for this type of cancer.

Conclusions: Future intervention work should focus on ensuring that health care professionals, particularly among HIV/primary care specialists, promote screening for anal dysplasia. It is critical that intervention methods use a community-based approach to raise awareness about the need to screen for anal cancer, especially among MSM infected with HIV.

Introduction
The incidence of squamous cell carcinoma of the anus, more commonly known as anal cancer, is on the rise. Persistent infection of high-risk strains of human papillomavirus (HPV) is the most common cause of anal cancer. Whereas the majority of individuals infected with HPV spontaneously clear the virus without ever showing symptoms, individuals with compromised immune systems, such as those infected with HIV, are at increased risk of persistent HPV infection. Two low-risk strains of HPV (types 6 and 11) cause condylomas, also known as genital warts, which, although they are non-cancerous, indicate HPV exposure and infection at the site where the warts develop. Persistent infection of the anal canal by high-risk strains of HPV (types 16 and 18) can lead to the development of high-grade anal dysplasia, known as high-grade intraepithelial lesion (HSIL). If HSILs are left untreated, then these lesions are thought to develop into anal cancer. Men who have sex with men (MSM) and who are also infected with HIV are at greatest risk for developing HSIL and anal cancer. This population is 52 times more likely to develop anal cancer as compared with the general, non–HIV-infected population, a disparity that can be potentially reduced through timely screening of and treatment for HSIL.

Although no national screening guidelines for detecting anal dysplasia are available, the anal Pap smears and Papanicolaou (Pap) smear have been proposed as a tool for the initial screening of anal dysplasia in populations infected with HIV. The goal of screening is to identify patients with HSIL, which is a presumptive precancerous lesion. If the patient has no symptoms and the anal Pap smear shows no abnormalities, then further screening can be offered annually. If the results from the anal Pap smear are abnormal, the patient is symptomatic, or both are present, then further evaluation can be performed with high-resolution anoscopy (HRA), also called colposcopy of the anal canal. Most HSILs are not visible to the bare eyes, so HRA allows the health care professional to perform a magnified examination and evaluation of the anal canal. A digital anorectal examination is also recommended at the time of screening. If HSIL is detected, then the lesion can be treated using different techniques, including trichloroacetic acid, a hyfrecator, and infrared coagulation. Some disease may be too advanced to be treated with HRA and may require referral for colorectal surgery.
Although some HIV primary care clinics are beginning to integrate anal dysplasia screening into the routine examination, the screening rate for anal dysplasia is still lower than ideal, and the incidence of anal cancer continues to rise among MSM infected with HIV.18,19 Thus, with the increased availability of screening, it is critical to explore the level of understanding among MSM infected with HIV about anal cancer and their need for screening. This research study explored the level of understanding, current behaviors, facilitators, and barriers to anal dysplasia screening, as well as the preferences of MSM infected with HIV for future health education efforts among this population.

Methods
Institutional Review Board approval was obtained to conduct in-depth interviews with MSM infected with HIV to explore their understanding, attitudes, and current behaviors related to anal dysplasia screening and prevention. The study team created an in-depth interview guide to explore participant perceptions related to HPV infection, anal cancer, screening for anal dysplasia, and preferences for future educational interventions. The interview consisted of open-ended questions and probes organized to enhance the flow of conversation and to elicit the clearest responses about HPV infection, anal cancer, screening for anal dysplasia, and preferences for upcoming educational interventions.20

The study team created recruitment fliers that included the topic of the research study, information about incentive for participation, a phone number for study screening and enrollment, and the following participant inclusion criteria: previously tested positive for HIV infection; self-identification as a gay or bisexual man; age 21 years or older; ability to fluently speak English; resident of Miami-Dade County; and willingness to provide informed consent. The fliers were disseminated at a local health department meeting that focused on MSM health. At this meeting, representatives of MSM-serving, community-based organizations agreed to pass out study fliers to their clients. Many of the study participants were referred to the trial via snowball sampling when past participants recommended that friends call the study number and screen for eligibility.

Individuals interested in research participation were screened by phone, and interviews were scheduled with men who met the inclusion criteria. Participants recommended a date, time, and location for the interview. Table 1 contains demographic information about the study participants.

### Conducting the Interviews
On the day of the in-depth interview, the interviewer reviewed the informed consent documents with participants and, upon obtaining consent, began the audio-recorded, in-depth interview. The interviewer used the in-depth interview guide to steer the conversation, varying the questions and probes based on participant responses and the direction of the conversation.20 Upon completing the interview, the research

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td></td>
</tr>
<tr>
<td>21–30</td>
<td>7</td>
</tr>
<tr>
<td>31–36</td>
<td>7</td>
</tr>
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<td>37–40</td>
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<td>41–45</td>
<td>10</td>
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<td>46–50</td>
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<td>51–55</td>
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<td>56–60</td>
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<td>61–65</td>
<td>4</td>
</tr>
<tr>
<td>≥ 66</td>
<td>2</td>
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<td>Race</td>
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<td>White, non-Hispanic</td>
<td>12</td>
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<tr>
<td>African American</td>
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<td>Unemployed and looking for work</td>
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</tr>
<tr>
<td>Unable to work (disability)</td>
<td>21</td>
</tr>
<tr>
<td>Retired</td>
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</tr>
<tr>
<td>Annual Income, $</td>
<td></td>
</tr>
<tr>
<td>&lt; 20,000</td>
<td>36</td>
</tr>
<tr>
<td>20,000–40,000</td>
<td>12</td>
</tr>
<tr>
<td>&gt; 40,000–70,000</td>
<td>9</td>
</tr>
<tr>
<td>&gt; 70,000</td>
<td>1</td>
</tr>
</tbody>
</table>
team member provided the participant with a monetary incentive ($60) and conducted a short health education lesson about HPV infection, anal dysplasia screening, and anal cancer.

All digitally recorded interviews were transcribed by a local transcription company. The researchers created an initial codebook of a priori themes based on the in-depth interview guide to direct their qualitative content analysis.21,22 The team then coded a single, in-depth interview together to ensure that the information was similarly coded. Three additional transcripts were coded separately and the team then met to compare codes, resolve any coding discrepancies, and discuss the codebook.23 The researchers then coded an additional interview together to test the updated codebook. The team then split the remaining transcripts and separately hand-coded the interviews and organized all coded data on Atlas.ti software (ATLAS.ti, Berlin, Germany).

Results
A total of 60 participants were enrolled in the study. During the interview process, 2 participants did not meet inclusion criteria, so only 58 participant interviews were analyzed. The level of understanding and the perceptions of HPV infection and anal cancer among the participants varied based on whether or not they had been affected by HPV infection, anal dysplasia, or anal cancer.

Experiences
Of the 58 participants, 3 had been treated for HSIL and 2 were treated or were in treatment for anal cancer. Individuals treated for HSIL described how treatment was mildly uncomfortable. Table 2 details the responses from participants who were diagnosed and treated for HSIL or anal cancer. One participant recalled feeling itchiness around his anal canal and sought care to determine the cause of the itching. His HIV/AIDS specialist conducted an anal Pap smear followed by HRA, and the participant was diagnosed with and treated for HSIL.

Another participant had been diagnosed with HIV infection 3 months prior to the interview. He described the treatment he received during his hospital stay for multiple sores and warts (low-grade, benign growths) around his anal canal and on his upper thighs. He was currently receiving treatment for the warts, but the warts were responding poorly to treatment. He also reported having blood in his stool, so his health care team performed colonoscopy. Outcomes of the colonoscopy did not reveal polyps or risk for colorectal cancer, but his physicians were still unsure about the cause of the blood in his stool. To his knowledge, no one had ever screened him for anal cancer.

The participants diagnosed with anal cancer had also been diagnosed with AIDS. One partici-

<table>
<thead>
<tr>
<th>Theme Subtheme</th>
<th>Participant Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Received treatment for HSIL (n = 3)</td>
<td>Positive experience</td>
</tr>
<tr>
<td>Painful experience</td>
<td>“They removed everything [lesions] in the rectum. I stayed in the hospital a total of 3 or 4 days because I lost a lot of blood during the surgery. That was a very bad experience because it was very painful.”</td>
</tr>
<tr>
<td>Experience with anal cancer (n = 2)</td>
<td>Self-misdiagnosis</td>
</tr>
<tr>
<td>Symptoms</td>
<td>“I was limping real hard because I couldn’t really walk. It hurt. I couldn’t even sit. I couldn’t close my legs. I had to shuffle.”</td>
</tr>
<tr>
<td>Cancer treatment</td>
<td>“And when you go to the bathroom you feel like you’re peeing fire. It just hurts all of the time. And this went on; I couldn’t sleep at night. All my skin came off my body, and all of it was just raw.”</td>
</tr>
</tbody>
</table>

HSIL = high-grade intraepithelial lesion.
body where he had received chemotherapy injections.

Another participant was in his early 20s when he was diagnosed with anal cancer. He had tested positive for HIV infection a few years prior but did not immediately seek treatment. Within a few years, his HIV infection progressed to AIDS, and he periodically returned to the hospital to treat AIDS-related comorbidities. One day at work, a coworker who saw him limping forced him to go to the hospital. At the hospital, he was treated for anal warts. He returned home but was rushed to the hospital a few months later for rectal pain. On a subsequent visit, he was tested for anal dysplasia and diagnosed with anal cancer. His treatment included various trips to the hospital, each time resulting in aggressive tissue removal around the anal canal and antibiotics to treat infection. At 25 years of age, his anal canal collapsed, and he began using a colostomy bag. At the time of the interview, he was free of disease, but his overall quality of life had been diminished by treatments aimed at stopping the spread of cancer.

**Level of Knowledge**

Unless directly affected by the disease, most participants had never heard of anal cancer or the need to screen for this disease. Table 3 details the responses from participants never diagnosed with HSIL or anal cancer. Throughout the interview, they drew conclusions about the disease and how it affected the body. One participant joked, “I had no idea my butt could rot off.” However, some participants confused anal cancer with prostate cancer and, more often, colorectal cancer. For example, when asked about whether or not they had ever received screening for anal cancer, participants discussed having completed a digital rectal examination, prostate-specific antigen testing, or, more commonly, colonoscopy. They also interchangeably used the terms “polyp,” “anal warts,” and “lesion.”

Those who had heard of HPV infection learned about the virus through advertisements for the HPV vaccine, but many believed that HPV affected women alone. Another participant sought medical attention after experiencing hoarseness that had lasted for more than 1 year. He was later diagnosed with condylomas, which are HPV-related noncancerous growths, on his vocal chords. Although he was aware that HPV infection caused these warts, he believed that HPV infection only caused cervical cancer as opposed to other types of cancers.

Table 3. — Experiences Among Participants Without Symptoms or a Diagnosis of Anal Dysplasia, HSIL, or Anal Cancer

<table>
<thead>
<tr>
<th>Theme</th>
<th>Subtheme</th>
<th>Participant Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awareness of anal cancer</td>
<td>Lack of awareness</td>
<td>“And the anal cancer. I’ve never heard — it’s never really heard of. Not in that — other than, again, like I say, prostate cancer or something like that. I don’t even know what it is. I haven’t really heard anybody talk about it in my support groups. We talk about different things, but that has never come up.”</td>
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<tr>
<td>Facilitators to screening for anal</td>
<td>Media</td>
<td>“If I knew there was a likelihood of getting it … You know, nobody got colonoscopies before Katie Couric talked about it on the Today Show. So you do hear it [the need to screen for colon cancer] through the media. If there was something similar in the news or media [about anal cancer], or if my doctor strongly recommended I get screened for anal cancer.”</td>
</tr>
<tr>
<td>dysplasia</td>
<td>Physician recommendations</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Willingness to screen</td>
<td>“So, when I go back to my doctor, I will ask him to do it [screen for anal cancer] again since it has been a couple of years now and because I had this interview with you. I am really going to do that, and I am probably going to recommend it [screening] to a few friends I know.”</td>
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<tr>
<td>Barriers to screening for anal</td>
<td>Lack of physician recommendations</td>
<td>“Yes, he basically said you should talk to your doctor and get screened. But, I’ve never had my doctor talk to me about it, and I’ve never brought it up. I don’t know that I’ve ever seen any media articles or anything about getting screened for anal cancer.”</td>
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<tr>
<td>cancer</td>
<td></td>
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<tr>
<td></td>
<td>Fear of cancer</td>
<td>“It’s confirming [the anal cancer diagnosis]. It’s a psychological thing. I do find this more with the people that I meet. I don’t tell everybody about my virus, but only those that need to know because there are those that are struggling with the same things — but it’s just the fear of actually knowing it.”</td>
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<td></td>
<td>Embarrassment of testing for</td>
<td>“Just the embarrassment of knowing someone is going to put something up your butt…Most people are not going to let a stranger do that, even if it is your doctor.”</td>
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<tr>
<td>anal dysplasia</td>
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<td></td>
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<tr>
<td></td>
<td>Health care professional</td>
<td>“In my opinion, the doctors should recommend to do it [screen for anal cancer], and they should also work together with the case manager and peer educators to explain to people they have to do it.”</td>
</tr>
<tr>
<td></td>
<td>Media</td>
<td>“Anything you put out there [in the media], advertisement or news or anything, is better than coming from a doctor. Not everybody wants to go to a doctor. Not everybody goes to a doctor.”</td>
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<td></td>
<td>Print media</td>
<td>“There are HIV posters on the bus stops; I see them all the time. I think a poster campaign in the areas where gay men are going. But in the back of whatever the magazine is — I mean, people do look at those, and they do read them.”</td>
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<td></td>
<td></td>
<td>“You should put posters or flyers on buses. You know how they have a lot of flyers inside the bus, and the buses actually have on the outside, and maybe some billboards along the highways or something, I mean — maybe even have some people near health clinics pass out pamphlets or something.”</td>
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*HSIL = high-grade intraepithelial lesion.*
Facilitators and Barriers: According to the participants, the 2 greatest factors that influenced screening for anal dysplasia screening were awareness about anal dysplasia screening and recommendations from a health care professional to obtain screening. The participants described how the current interview alone would prompt them to ask their HIV/AIDS specialist to screen or refer them for screening at their next health care visit.

Similarly, the participants reported that the greatest barrier to anal dysplasia screening was lack of awareness about anal cancer and the need to screen. Participants repeated their intentions to screen and prevent diseases, especially if prompted by a health care professional (more often their physicians). One participant shared an emotional story about a former partner who survived anal cancer. After describing his partner’s medical treatment, he questioned why he had never screened for that same cancer. Further, he explained how no one had ever spoken with him about the need to screen for anal dysplasia.

One participant reported being unwilling to screen for anal dysplasia. He had stopped taking his antiretroviral therapy, believing that his HIV infection could be controlled without medication, and his health had rapidly declined. His rationale for not wanting to screen for anal dysplasia was based on his need to focus on controlling his HIV infection before testing and potentially detecting another health concern.

When the participants were asked about what they believed may stop other men with HIV infection from screening for anal dysplasia, they reported fear of cancer as being a barrier. Fear of knowing about cancer and having to confront the diagnosis also served as a barrier to screening. Another participant described embarrassment of anal dysplasia screening as a potential barrier. Although few participants described potential barriers for screening, all of them wanted to learn more about anal cancer and recommended various methods for interpersonal and community health education.

Preferences for Methods of Health Education

When asked about the best ways to increase awareness among men infected with HIV regarding the need to screen for anal dysplasia, participants most often recommended interpersonal health education methods to increase anal cancer awareness. Many participants described how the current interview followed by the one-on-one discussion about HPV infection and anal cancer was the best way to teach men infected with HIV about anal dysplasia screening. Participants also recommended working with HIV/AIDS specialists and case managers to ensure they discuss anal cancer with their patients and recommend screening. Some recommended creating and disseminating a screening checklist to use during their primary care visits. This would empower the participant with the information necessary to have informed conversations with their physicians related to the screenings they need.

Participants also recommended training local leaders and health care advocates to disseminate information about anal dysplasia screening through their peer networks. Health care professionals could train peer health educators, community champions, or other community members to rapidly spread anal dysplasia screening messages to social networks. This method could also reach individuals not currently receiving HIV primary care.

Another recommendation included the creation of print media such as educational brochures, running health education messages in newspapers and magazines for HIV-infected populations, and using posters to increase community awareness about anal cancer. Specifically, participants recommended leaving educational brochures in HIV primary care clinic waiting and examination rooms for patients to read. Many participants also recommended posting similar informational fliers at bus stops and on buses to reach individuals who may rely on public transportation and who are currently not seeking HIV primary care.

Overall, participants were eager to receive any recommendation possible to improve their health and were therefore eager to learn more about anal dysplasia screening. They noted that increasing awareness about anal cancer is critical to ensuring the health of patients infected with HIV. Saturating their physical and social environments would enhance general awareness and also acceptance for anal dysplasia screening. They also needed clear descriptions of the different screening tests and information about how often to screen.

Discussion

Similar to prior research, most of the MSM infected with HIV participating in this study did not know about HPV infection and its connection to anal cancer. Among those who were aware of HPV infection, most knew only about the connection between...
HPV infection and cervical cancer in women. These findings highlight the need to correct this perception and inform communities that HPV infection is a universal health issue and can cause various cancers that affect both men and women.

Roughly one-half of the study participants regularly received regular care at HIV primary care clinics that perform anal Pap smears as part of a routine annual examination. Among those who did not screen for anal dysplasia, their reported barriers were lack of awareness about anal dysplasia, HSIL, and anal cancer, as well as lack of health care professional recommendations to screen. Although some participants mentioned that the anal Pap smear was slightly uncomfortable, most did not report this as a barrier to screening.

The few participants who reported hesitancy to screen noted that embarrassment about screening procedures may stop them from screening. Similar to home-based, self-sampling for precancerous colorectal polyps, individuals could use anal dysplasia self-screening kits to avoid the embarrassment of having a health care professional conduct the screening. However, most MSM infected with HIV described their willingness to screen for anal dysplasia. Among those who had never been screened, they said they were willing to undergo screening once they learned more about the procedure for the anal Pap smear.

Patients with anal symptoms may not be receiving appropriate screening, diagnostic testing, or both for anal dysplasia and cancer. One participant had symptoms concerning for anal cancer, but, rather than undergoing a digital rectal examination or anoscopy, he had undergone colonoscopy for colorectal cancer. Although this participant had not been diagnosed with HPV infection or anal cancer, his symptoms — particularly the presence of genital warts and sores — led us to believe that he could have benefited from a detailed anal examination, including a digital anorectal examination, HRA, or both. This participant's experience may point to underscreening for anal dysplasia and cancer or even misuse of colonoscopy. However, more information about the patient's health status is needed to support this conclusion. No studies, to our knowledge, have reported the incorrect use of colonoscopy after detecting and treating genital warts and lesions.

**Future Research**
Future research should explore the processes, policies, barriers, and facilitators of HIV primary care clinics to screen for anal dysplasia as part of the routine examination of HIV-infected populations. Further, given our participants' emphasis on a physician recommendation as the greatest influence on screening, future studies should explore awareness and the behaviors related to screening for anal dysplasia among HIV/AIDS specialists and primary care physicians, because this information could help identify what is needed to ensure this screening is offered as a part of standard care among patients infected with HIV.

Participants wanted to learn more about anal cancer, and they wanted this information to come from both health care professionals and the local media, highlighting the need for health education about anal dysplasia screening at the community level. Future intervention research studies should utilize community-based, multilevel interventional designs that target doctor–patient, clinical, and community health education to enhance anal dysplasia screening among HIV-infected populations.

**Study Limitations**
Our data come from a convenience sample of men infected with HIV recruited from HIV primary care clinics who were adherent to their antiretroviral therapy. Thus, generalizations to other HIV-infected individuals should be made with caution. Another limitation is that Spanish-language interviews were not conducted. However, these interviews will be conducted in the near future.

**Conclusions**
Anal cancer is a disease that can potentially be prevented with timely anal dysplasia screening that should be promoted using a 3-prong approach. To improve anal dysplasia prevention, gay and bisexual men infected with HIV must be empowered to prevent cancer by being knowledgeable about anal dysplasia screening and be aware of how to access that screening. More training is needed for health care professionals, particularly among HIV/AIDS specialists, to perform this test or refer patients to other clinics that conduct the test. National guidelines are needed to promote anal dysplasia screening among HIV-infected populations to standardize this procedure as part of HIV primary care. Such guidelines will increase the visibility of this disease among patients and health care professionals, possibly leading to increased early detection and treatment of precancerous lesions.

This study also indicates the need to use various modes of communication (eg, in-clinic print media, community-wide health education, doctor–patient communication) to inform HIV-infected populations about HPV infection and the possible benefits of screening for anal dysplasia. Such public health communication is warranted to increase screening efforts for anal dysplasia and, thus, reduce the incidence of this potentially preventable cancer.
References

Hyperuricemia in 2 Patients Receiving Palbociclib for Breast Cancer

David J. Bromberg, MD, Mauricio Valenzuela, MD, Sowmya Nanjappa, MD, and Smitha Pabbathi, MD

Summary: The authors reviewed retrospective cases of 2 women — one aged 78 years and the other aged 86 years — with estrogen receptor (ER)-positive, human epidermal growth factor receptor 2 (HER2)-negative breast cancer treated with combination palbociclib/letrozole who presented with hyperuricemia. In both cases, the patients experienced hyperuricemia and neutropenia that required palbociclib to be temporarily discontinued and its dose to be subsequently reduced. Although study data have demonstrated that combination palbociclib/letrozole is safe and effective as a first-line treatment option for patients with advanced ER-positive, HER2-negative breast cancer, the efficacy and safety of cyclin-dependent kinase inhibitors, including their adverse events, still remains an active area of research. The authors postulate that hyperuricemia may be a potential adverse event of palbociclib not yet reported in randomized control studies or in clinical practice.

Background
Palbociclib is a selective inhibitor of cyclin-dependent kinases (CDK) 4 and 6 approved as treatment for women with advanced or metastatic estrogen receptor (ER)-positive, human epidermal growth factor receptor 2 (HER2)-negative breast cancer with manageable adverse events. Data indicate that palbociclib improved the response rate and duration in postmenopausal women with locally advanced or metastatic ER-positive/HER2-negative breast cancer.1 Thus, targeting CDK4/6 may overcome acquired resistance to conventional endocrine therapy. Randomized phase 2 and 3 studies have demonstrated that palbociclib/letrozole as a first-line treatment option for patients with advanced ER-positive, HER2-negative breast cancer is safe and effective, and those data enabled palbociclib to receive expedited approval from the US Food and Drug Administration.2 However, the efficacy and safety of CDK inhibitors, including their adverse events, still remains an active area of research.3

Case Reports
Case 1
A woman aged 78 years presented with lethargy and dehydration. Her chemistry panel revealed hyperuricemia (uric acid, 11.4 mg/dL), hypercalcemia (corrected calcium, 10.9 mg/dL), and acute kidney injury (serum creatinine, 1.5 mg/dL). Seven days prior to this presentation, she had received treatment with letrozole and palbociclib for hormone-receptor (HR)-positive breast cancer metastatic to the bones and bone marrow.

Her past medical history was positive for stage 3 papillary thyroid cancer for which she underwent total thyroidectomy and radioactive iodide therapy. Four years prior to this presentation, she was diagnosed with ER-positive, HER2-negative stage 2 invasive ductal breast cancer for which she underwent partial mastectomy and a single fraction of intraoperative radiation. She was briefly treated with exemestane, but it was stopped due to severe arthralgia, a drug-related adverse event.

Four years after the initial diagnosis of breast cancer, she presented with pancytopenia. Position emission tomography (PET) was obtained that revealed diffuse bone metastases. Subsequent bone marrow biopsy was obtained and the results were consistent with ER/PR-positive, HER2-negative metastatic breast adenocarcinoma involving 60% of the hypercellular bone marrow.

During a 2-day hospitalization, she was treated with aggressive intravenous fluids and allopurinol 300 mg daily. Complete resolution of the acute kidney injury was achieved and her uric acid level was normalized to 3.8 mg/dL. On discharge, she was continued on letrozole, but palbociclib was held for 14 days due to neutropenia. After the neutropenia resolved, her dose of palbociclib was restarted and reduced to 100 mg, and no evidence of recurrent hyperuricemia was seen on treatment day 21. She tolerated this new dose, and, at 8 weeks after the treatment was initiated, her cancer antigen (CA) 15-3 level had declined from 500 to 11 U/mL.
Case 2
A woman aged 86 years presented with a past medical history of right-sided, ER-positive, HER2-negative invasive ductal breast carcinoma. Biopsy was performed and the results confirmed metastasis to the liver. Her right adrenal gland was initially treated with lumpectomy, postoperative radiation, and paclitaxel.

Over the course of 8 years, the adrenal mass and all of the liver masses decreased in size; however, due to a slow increase in CA 15-3 level, she was treated with exemestane and fulvestrant and underwent 2 hepatic artery chemoembolizations. Within 6 months of therapy, results on PET revealed new and increasing liver lesions. Exemestane and fulvestrant were discontinued and palbociclib 125 mg daily and letrozole 2.5 mg daily were started. She developed hyperuricemia within 10 days after therapy was initiated and had a uric acid level of 9.3 mg/dL. Allopurinol 300 mg daily was started and she was instructed to increase her oral fluid intake.

A repeat uric acid test on day 25 of treatment showed a decreased level of 3.6 mg/dL, but palbociclib was held due to neutropenia. After the neutropenia resolved, she was restarted on a reduced dose of palbociclib at 100 mg daily. She completed a total treatment course of 28 days. Her CA 15-3 level declined from 142 U/mL to 86 U/mL.

Discussion
To our knowledge, this is the first time that hyperuricemia has been reported as a possible complication of palbociclib treatment in combination with letrozole since palbociclib was approved by the US Food and Drug Administration. Given the rapid tumor response in the first case, it is reasonable to postulate that hyperuricemia could have resulted from rapid tumor lysis, as confirmed by the rapid decline in tumor marker level.

The safety and efficacy of palbociclib in combination with letrozole as a first-line treatment for patients with advanced ER-positive, HER2-negative breast cancer was demonstrated in a randomized phase 2 study. Neutropenia, leukopenia, and fatigue were the most common adverse events. In addition, a double-blind, randomized phase 3 trial compared fulvestrant and palbociclib with fulvestrant and placebo for the treatment of metastatic, hormone-responsive breast cancer.

Results of that phase 3 study revealed that fulvestrant/palbociclib increased the rate of progression-free survival among women with ER-positive, HER2-negative cancer, irrespective of menopausal status. In that trial, the most common adverse events were neutropenia, leukopenia, fatigue, and nausea. However, the study was stopped early because it met its primary end point of demonstrating an improvement in the rate of progression-free survival. Clinically significant hyperuricemia was not noted in either study.

Aromatase inhibitors such as letrozole have an active role in the treatment of ER-positive, postmenopausal breast cancer. However, because 30% to 50% of patients do not respond to aromatase inhibitors, ongoing research suggests that more individualized treatment should be developed.

Conclusion
In the absence of a biomarker or other tests to identify those at higher risk for developing hyperuricemia with palbociclib and aromatase inhibitor treatment, the findings of these 2 cases suggest that close monitoring of uric acid levels might be prudent in patients with a high-disease burden who are receiving palbociclib. In patients who develop clinically significant hyperuricemia, reducing the palbociclib dose may be necessary and allopurinol treatment should be timely instituted.

References
Genetic Investigation of Uterine Carcinosarcoma: Case Report and Cohort Analysis

Timothy N. Hembree, DO, PhD, Jamie K. Teer, PhD, Ardeshir Hakam, MD, and Alberto A. Chiappori, MD

Background: Uterine carcinosarcoma, a rare gynecological malignancy, often presents at the advanced stage with a poor prognosis because current therapies have not improved rates of survival. Genetic characterization of this tumor may lead to novel, specifically targeted drug targets to provide better treatment options for patients with this malignancy.

Methods: We present a case of a woman aged 61 years with uterine carcinosarcoma and retrospectively analyzed 100 study patients with uterine carcinosarcoma. From this group, 9 study patients underwent targeted sequencing of 1,321 genes.

Results: All 9 study patients had at least 1 mutation in \( \text{JAK2}, \text{KRAS}, \text{PIK3CA}, \text{CTNNB1}, \text{PTEN}, \text{FBXW7}, \text{TP53}, \) and \( \text{ERBB2}; \) of these, \( \text{TP53} \) was the most frequently mutated gene (6/9). In addition, \( \text{ARID1A} \) and \( \text{KMT2C} \), which have been described and identified as part of a set of chromatin-remodeling genes, were also found in our analyses. From our 100-person cohort clinical analyses, study patients with stage 1 cancer had a median survival rate of 33 months (95% confidence interval, 19–109) compared with a median survival rate of 6 months (95% confidence interval, 3–12) in those with stage 4 disease.

Conclusions: Disease stage alone predicted the rate of clinical survival. Up to 50% in the study group were identified at having early stage disease (stage 1/2), indicating improved rates of overall detection compared with previously reported data. Our mutational analysis findings add to the number of tumors in which these mutations have been found and suggest that chromatin-remodeling dysregulation may play a role in the tumorigenesis of carcinosarcoma.

Introduction

Uterine carcinosarcomas are rare gynecological tumors that make up fewer than 5% of all uterine malignancies; however rare, these tumors account for a disproportionate percentage of associated mortality.\(^1,2\) Their classic presentation has been described as a triad of symptoms: pain, severe vaginal bleeding, and the passage of necrotic tissue through the vagina.\(^1\) More than one-half of patients (53%) present with advanced stage disease, and 20% of patients with localized disease at presentation are upstaged during laparotomy.\(^3,4\)

Staging is the most significant prognostic indicator and reflects a poor overall, 5-year, disease-specific survival rate (36.4% for all stages; 62.3% for stage 1 and 0% for stages 2 to 4).\(^5\) Risk factors are similar to those seen in endometrial carcinoma and include nulliparity, advanced age, obesity, exposure to exogenous estrogens, history of pelvic irradiation, and the long-term use of tamoxifen.\(^2,6\)

The histology of the tumor is mixed with epithelial and mesenchymal elements, thus designating it a carcinosarcoma. The tumor is classified based on the sarcomatous portion as either homologous (consisting of mesenchymal elements found in the female genital tract) or heterologous (consisting of mesenchymal elements foreign to the female genital tract). Within the uterus, carcinosarcomas commonly arise from the posterior wall of the uterine body near the fundus. The mass usually grows to fill and distend the uterus, and most carcinosarcomas are visualized as exophytic lesions. The extent of myometrial invasion of these tumors is sometimes controversial, but it is generally accepted that the degree of myometrial invasion is a poor prognostic indicator and likely a predictor of metastasis.\(^7\)

The origin of the tumor is thought to follow the embryological development path of the Müllerian ducts and is most likely derived from a pluripotent stem cell that differentiates into both epithelial and mesenchymal cell types. Further characterization of the tumor histol-
ogy supports the “conversion” theory, which states that an epithelial-to-mesenchymal transition occurs because the sarcomatous portion of the tumor exhibits markers consistent with an epithelial origin.

Historically, this tumor was considered to be uterine sarcoma, and treatment was directed at the sarcomatous element. However, the carcinoma portion of the tumor is now favored as the primary determinant of tumor aggressiveness, with recent advances in treatment directed toward those elements. Regardless, standards of therapy (surgical debulking, lymphadenectomy, and adjuvant chemotherapy) have not improved overall survival rates, particularly in those with advanced disease. Therefore, improving our understanding of tumor biology (molecular profile and oncogenic drivers) should be a priority.

In that regard, somatic mutations (eg, KRAS, PI3KCA) known to be carcinogenic drivers in other tumors (eg, lung adenocarcinomas, colon adenocarcinomas) have been demonstrated in carcinomas. This increased our curiosity on a more detailed molecular analysis of these tumors. Thus, we present a case patient with uterine carcinomas and report on our clinical and molecular analysis findings for a cohort of patients with this disease.

**Case Report**

**Presentation**

A 61-year-old postmenopausal, gravida 3, para 3 woman with uncontrolled type 2 diabetes mellitus, hypertension, and a 32-pack-year smoking history (quit 12 years prior) but no history of gynecological cancer or exogenous estrogen exposure presented with urinary retention for 2 days and weight loss of 24 pounds during the previous 2 to 3 months. She denied abdominal pain, hematuria, abnormal vaginal bleeding, changes in her bowel pattern, night sweats, shortness of breath, and cough. She had undergone tubal ligation after her last pregnancy and also reported abnormal results on a Papanicolaou test 6 months before her presentation. Findings on physical examination were benign, except for a palpable but nontender mass above the pubic symphysis. Serum levels of cancer antigen 125, cancer antigen 19-9, and carcinoembryonic antigen were all within normal limits.

Computed tomography (CT) revealed a large, 16-cm heterogeneous intrapelvic mass with no invasion into the surrounding structures, no mesenteric or retroperitoneal lymphadenopathy, and no ascites. Secondary to the large pelvic mass, external compression of the bilateral distal ureters was present that resulted in severe bilateral hydroureteronephrosis and urethral obstruction. Based on these findings, the diagnosis of uterine malignancy was suggested and surgical debulking was recommended for diagnostic and therapeutic purposes to relieve the urethral obstruction and bilateral hydroureteronephrosis.

Exploratory laparotomy, modified radical hysterectomy, bilateral salpingo-oophorectomy, and tumor debulking were performed. Physical examination under anesthesia revealed a fixed mass involving the entire uterus and gross tumor spillage in the vagina. Midline laparotomy confirmed a grossly necrotic tumor in the cul-de-sac, arising from the uterus, and all visible tumors were excised and submitted for pathology.

**Results**

Histopathology with uterine carcinomas was consistent with the diagnosis of high-grade primary uterine heterologous carcinomas. The carcinoma component was endometrioid adenocarcinoma and the sarcomatous component was rhabdomyosarcoma (Fig 1).

Fluorodeoxyglucose positron emission tomography/CT was obtained of the skull base to the midthigh and revealed at least 5 metabolically active pulmonary nodules (2 nodules on the right and 3 nodules on the left; Fig 2). CT angiography of the chest showed pulmonary thromboembolism in the left lower lobe and also confirmed bilateral pulmonary metastases (Fig 3). The patient was started...
Fig 2. — Postoperative positive emission tomography obtained for the case patient.

Fig 3. — Postoperative computed tomography obtained for the case patient.
on carboplatin and paclitaxel therapy but did not respond. Subsequently, she died from complications of progressive disease.

**Cohort Analysis**

**Clinical**

We accessed clinical and tissue data from the Total Cancer Care protocol, which was approved by the Institutional Review Board of the H. Lee Moffitt Cancer Center & Research Institute (Tampa, Florida); patients also provided prospectively written informed consent. Analysis of our study patient cohort included staging by either clinical or pathological staging, with the composite stage being the higher of the 2 scores. We used the composite stage to describe our cohort.

Deidentified clinical data, including age, race, sex, smoking status, stage of disease, histology, and type of treatment, were obtained through an “honest broker” web-based system designed by Moffitt Cancer Center to protect confidential patient information following the regulations set forth by the Health Insurance Portability and Accountability Act. Tissue samples had been collected using common protocols for tissue preservation, processing, and data annotation.

**Molecular**

Tumor samples from Total Cancer Care were subjected to genomic capture and massively parallel sequencing. Sequences were aligned to the hs37d5 human reference with the Burrows-Wheeler Alignment tool. Insertion/deletion realignment, quality score recalibration, and variant identification were performed with the Genome Analysis ToolKit (Broad Institute, Cambridge, Massachusetts). Sequence variants were annotated with ANNOVAR (http://annovar.openbioinformatics.org). Additional contextual information was incorporated, including allele frequency in other studies (eg, 1000 Genomes, National Heart, Lung, and Blood Institute’s “Grand Opportunity” Exome Sequence Project), in silico function impact predictions, and observed impacts from select databases such as ClinVar (www.ncbi.nlm.nih.gov/clinvar) and the Collection of Somatic Mutations in Cancer (COSMIC; version 61; Wellcome Trust Sanger Institute, Cambridge, UK). Mutation frequencies were compared with data from The Cancer Genome Atlas (TCGA) using the cBioPortal for Cancer Genomics tool (Memorial Sloan Kettering Cancer Center, New York, New York).

To enrich for somatic mutations, we excluded any positions observed in 1,000 genomes or at a frequency larger than 5% in a local normal population. We eliminated any variants with a variant quality score recalibration tranche level equal to 100 and required a genotype quality of at least 10 to include the variant of a particular sample.

**Results**

**Clinical Analysis:** The Total Cancer Care cohort included 100 study patients diagnosed with uterine carcinosarcoma seen at Moffitt Cancer Center or an affiliated hospital between 1996 and 2012. Most study patients at presentation were between the ages of 60 and 79 years, female, white, and diagnosed in an early composite stage (1/2). Tumor grade was most often poorly differentiated. The first course of treatment was distributed between surgery alone, surgery and chemotherapy, and combination surgery, chemotherapy, and radiotherapy. The strongest predictor of survival was stage at presentation, with stage 1 having a median survival rate of 33 months (95% confidence interval, 19–109) and stage 4 having a median survival rate of 6 months (95% confidence interval, 3–12; Fig 4).

**Genetic Mutation Analysis:** Nine of the 100 study patients were part of a targeted sequencing study covering 1,321 genes. We examined their mutation profiles, comparing observed frequencies to 31 patients with carcinosarcoma in a previous study of 15 cancer genes. We focused on those positions seen more than 5 times in COSMIC. Mutations in JAK2, KRAS, PIK3CA, CTNNB1, PTEN, FBXW7, TP53, ARID1A, and ERBB2 were identified (Fig 5; Supplemental Table). All 9 samples had at least 1 mutation in these genes, with TP53 being the most commonly mutated (6 of 9). If TP53 is removed, then 8 of the 9 samples had at least 1 mutation in the remaining genes, suggesting that these pathways play an important role in carcinosarcoma. Many of these genes are mutated at recurrent positions, including PIK3CA (R88Q, H1047R/Y), FBXW7 (R465H, R658X), ERBB2 (V842D), JAK2 (V617F), and CTNNB1 (S37F, T41A). Truncating mutations in PTEN (R233X, Q97X), TP53 (2 different nonsense mutations, 1 splice-altering mutation), KMT2C (1 nonsense mutation), and ARID1A (2 nonsense mutation) were also observed, consistent with previous observations of truncating mutations in these genes in different tumor types (TCGA via cBio Portal). A single KRAS G12D mutation was also identified.

Many of these genes (PIK3CA, KRAS, TP53, PTEN, and CTNNB1) were observed in an earlier study and were part of the 15-gene panel used by Penson et al. ARID1A, KMT2C, and FBXW7 were identified in a recent study by Jones et al. Two of these genes were observed to be commonly mutated in uterine carcinosarcoma (TCGA via cBio Portal): ARID1A at 18% and FBXW7 at 39%. However, although we observed 1 mutation in PPP2R1A (the most significantly mutated gene in uterine carcinosarcoma via the cBio Portal, as of publication), this exact mutation was not observed in TCGA data. Furthermore, CTNNB1 mutations were observed in our study as well as in Penson et al but not in TCGA data.

A single sample, DS–90250, had many mutations.
across these commonly mutated driver genes (Fig 5; see Supplemental Table). Upon further examination, we identified a POLE proofreading-domain mutation, V411L, in this sample. After reviewing uterine carcinosarcoma data from TCGA, we identified a single sample with a POLE mutation in the same region, P286R; the sample also had a high number of mutations.16 Thus, although these mutations are uncommon (ie, less common than those reported in uterine corpus endometrial carcinoma19), our findings suggest that POLE mutations can be observed in uterine carcinosarcoma.

Discussion
The patient discussed in our case report presented in the sixth decade of life with stage 4 disease and more than 50% myometrial invasion, so her prognosis was poor. She was referred to our gynecological oncology clinic and received chemotherapy with carboplatin and paclitaxel. She tolerated 2 cycles of chemotherapy before she died from her disease.

Most women in our cohort were white and their stages of presentation were equally distributed throughout the group. This is in contrast to other epidemiological studies, which have included higher percentages of black or non–white women and those likely to present with advanced-stage disease.20,21

In our examination of mutations in 1,321 genes thought to be associated with cancer in a small cohort of individuals diagnosed with uterine carcinosarcoma (n = 9), we reproduced some earlier mutations. Direct comparisons of frequency between studies are limited by small sample size, but general similarities exist. Therefore, we can conclude that mutations in PIK3CA, KRAS, PTEN, TP53, ARID1A, KMT2C, and FBXW7 are important drivers of uterine carcinosarcoma. However, some differences in mutation frequencies are apparent, perhaps reflecting the pathological heterogeneity

Fig 4. — Characteristics of the 100-person cohort. TNM = tumor, node, metastasis.

Fig 5. — Mutations in commonly mutated cancer-related genes. Mutation matrix shows missense (blue) and truncating (black) mutations in each study patient (n = 9) for the listed genes.
of this disease. The large number of potential driver genes suggests that uterine carcinosarcoma is a genetically heterogeneous disease.

Genetic investigation of additional samples will be needed to more accurately determine incidence, to identify lower-frequency genetic events, and to associate status with clinical phenotype. However, we observed a mutation in at least 1 well-characterized oncological gene for every sample analyzed, suggesting that, although uterine carcinosarcoma may be driven by heterogeneous events, these drivers are already known to be important for the development of other cancer types.

Given poor overall survival rates for uterine carcinosarcoma, genetic characterization offers the potential to improve our understanding and treatment of this rare type of cancer. Our preliminary investigation demonstrated that pathways altered in other cancer types are also altered here, suggesting that therapeutic options (including targeted therapies) may also be effective in uterine carcinosarcoma. We also confirmed the presence of mutations in chromatin-remodeling genes in this disease, suggesting a possible therapeutic strategy via histone deacetylase inhibitors.

In addition, we observed a POLE proofreading-domain mutation resulting in a large mutational load in uterine carcinosarcoma. Large mutational load has been linked to durable response to immune checkpoint–inhibitor therapy in non–small-cell lung cancer and melanoma, suggesting another possible strategy for this disease. Future studies will also need to incorporate clinical outcome information to further understand the progression and prognosis of this malignant disease.

Conclusions

Given the genetic heterogeneity reported here, knowledge of the mutational profile of any given tumor could be linked to a differential prognosis or therapeutic response, and mutation profiles described here and elsewhere suggest new therapeutic options. Potential exists for personalized understanding and treatment options for patients with uterine carcinosarcoma; however, given the rarity of the disease, collaborative efforts will be critical to understanding the relationships between molecular alterations and clinical features.

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References

Background: Barrett esophagus (BE) continues to be a major risk factor for developing esophageal adenocarcinoma.

Methods: We review the risk factors, diagnosis, and management of BE, with an emphasis on the most current endoscopic diagnostic modalities for BE.

Results: Novel diagnostic modalities have emerged to address the inadequacies of standard, untargeted biopsies, such as dye-based and virtual chromoendoscopy, endoscopic mucosal resection, molecular biomarkers, optical coherence tomography, confocal laser endomicroscopy, volumetric laser endomicroscopy, and endocytoscopy. Treatment of BE depends on the presence of intramucosal cancer or dysplasia, particularly high-grade dysplasia with or without visible mucosal lesions.

Conclusions: Recent advances in endoscopic diagnostic tools demonstrate promising results and help to mitigate the shortcomings of the Seattle protocol. Future research as well as refining these tools may help aid them in replacing standard untargeted biopsies.

Introduction
Barrett esophagus (BE) is a condition that has been controversial ever since Barrett first described it in 1950 — from the definition to its appropriate surveillance, treatment, and management.1 As defined by the American Gastroenterological Association, BE involves any extent of metaplastic columnar epithelium predisposed to developing cancer that then replaces the stratified squamous epithelium normally lining the distal esophagus.2 Intestinal metaplasia is required for the diagnosis of BE because intestinal metaplasia is the only type of esophageal columnar epithelium that predisposes a person to malignancy.2 DNA content abnormalities may occur with equal frequency and extent in the metaplastic columnar epithelium regardless of the presence of goblet cells.3 BE is a major risk factor for developing esophageal adenocarcinoma, and the incidence of esophageal adenocarcinoma continues to rise in Western countries.4

The underlying etiology of BE is not well characterized; however, in the majority of cases, it is associated with a combination of acid and bile reflux, even in the absence of symptoms. Esophagitis secondary to gastroesophageal reflux disease (GERD) is a common medical condition in Western countries, with up to 30% of adults experiencing heartburn at least once per month; of those, one-third has evidence of esophagitis during endoscopic evaluation.5 Approximately 10% of patients have esophagitis that progresses to BE.5,6 Addressing the pathological changes in the mucosa and the underlying etiology are the basis of BE treatment.

Predicting the Disease Evolution
A large, prospective, multicenter study of GERD with more than 6,000 participants showed that age, male sex, Caucasian race, increased body mass index, long-standing reflux disease and smoking were consistently and independently related to GERD.7 A large body of literature indicates that GERD is also an independent factor for the development of BE.8-11 Use of statins, aspirin, or nonsteroidal anti-inflammatory drugs may be associated with a decreased risk of BE.12,13 BE is one of the most common premalignant lesions affecting more than 2% of the adult population, strongly predisposing them to esophageal adenocarcinoma.14,15 Esophageal cancer has a 5-year survival rate of less than 15%, making it a deadly cancer.16 In clinical practice, the presence of dysplasia is the most important useful factor for identifying patients at increased risk for developing esophageal adenocarcinoma.17

Metaplasia–Dysplasia–Carcinoma Sequence
The formation of intestinal metaplasia appears to start with an injury to the esophageal mucosa due to gastric refluxate, which causes the desquamation process
of squamous cells (Fig 1). This action causes stem cells to migrate from the epithelial-mesenchymal junction toward the luminal surface. Here, the behavior of the stem cells is modified by the refluxate, which causes a selected lineage of resistant cells that then populate the neoesophageal mucosa.\(^{18}\) Barrett segments may also develop from the upward progression of the cardia mucosa, in part because Barrett glands replicate the organization of the gastric glands — an observation made when analyzing the architecture of the stem cells and the pattern of the gene expression.\(^{19}\)

In cases of low-grade dysplasia (LGD) in BE, the epithelium reveals enlarged nuclei with an increased nucleus-to-cytoplasm ratio, stratification of the nuclei, mucin depletion, partial loss of nuclear polarity, and lack of surface maturation (Fig 2). This becomes markedly increased in high-grade dysplasia (HGD) in BE, with an increased nucleus-to-cytoplasm ratio and increased nuclear pleomorphisms with prominent nucleoli, full-thickness nuclear stratification, and loss of polarity (Fig 3).

BE is relatively common, but the development of dysplasia and adenocarcinoma occurs in a small number of those affected.\(^{18}\) Once intestinal metaplasia develops, persons with BE are at increased risk for developing dysplasia and adenocarcinoma when compared with the general population.\(^{18}\) This underscores the principal clinical importance of BE, particularly because the prevalence of adenocarcinoma has increased in the United States and Europe during the past 20 years.\(^{18}\) BE is estimated to carry a risk of cancer 30 to 125 times greater than that for an age-matched population.\(^{20}\) In a meta-analysis by Yousef et al,\(^{21}\) the overall estimate of cancer incidence in BE was 4.1 cases per 1,000 person-years (3.9 per 1,000 person-years using high-quality study data), and the incidence of combined cancer and HGD was 9.1 per 1,000 person-years (7.7 per 1,000 person-years using high-quality study data).

Based on these data,\(^{18,21}\) the risk of developing or dying from esophageal carcinoma from BE is relatively low, but the diagnosis of BE can have psychological and financial implications on the affected patients, in part due to the limited treatment options available and poor survival rates once the cancer is diagnosed.\(^{2}\) Patients with BE report a worse quality of life than the general population, and receiving a diagnosis of BE can cause psychological distress and increase life and health care insurance premiums.\(^{2}\)

**Diagnosis**

**White Light Endoscopy**

Standard of care for the diagnosis of BE is endoscopic evaluation performed with white light endoscopy (WLE) with 4-quadrant biopsy specimens taken every 2 cm as

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**Fig 1.** — Mucosal biopsy specimen of the squamocolumnar junction showing intestinal metaplasia (mucinous columnar cells with scattered goblet cells) without dysplasia, consistent with Barrett esophagus. Hematoxylin and eosin stain, ×20.

**Fig 2.** — Low-grade dysplasia in Barrett esophagus. Epithelium shows enlarged nuclei with an increased nucleus-to-cytoplasm ratio, stratification of the nuclei, mucin depletion, partial loss of nuclear polarity, and lack of surface maturation. Hematoxylin and eosin stain, ×20.

**Fig 3.** — High-grade dysplasia in Barrett esophagus. Epithelium shows a markedly increased nucleus-to-cytoplasm ratio, marked nuclear pleomorphism with prominent nucleoli, full thickness nuclear stratification, and loss of polarity. Hematoxylin and eosin stain, ×40.
described by the Seattle protocol (Fig 4; Table). The American Gastroenterological Association also recommends that 4-quadrant biopsy specimens be obtained every 1 cm in patients with known or suspected dysplasia; however, doing so may require additional biopsy procedures, longer procedure times, and increased cost. This technique is also subject to sampling error, thus resulting in a low diagnostic uncertainty rate between 1% and 10%, suboptimal disease management, and decreased adherence to practice guidelines.

The mosaic of BE dysplasia is widely recognized (Fig 5). Areas of HGD and microscopic carcinoma are often small, making differentiation between these lesions difficult on biopsy. Therefore, other diagnostic tools have been sought to solve the shortcomings of standard, untargeted biopsies for diagnosing BE.

**Chromoendoscopy**

Chromoendoscopy is a technique that utilizes dyes in

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**Table. — Endoscopic Diagnosis of Barrett Esophagus**

<table>
<thead>
<tr>
<th>Endoscopic Technique</th>
<th>Method</th>
<th>Comment</th>
<th>Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>WLE with random biopsies</td>
<td>4-quadrant biopsy specimens obtained every 1–2 cm in patients with known or suspected dysplasia (Seattle protocol)</td>
<td>Wide availability Low cost Ease of use and integration into standard endoscopy with no additional risks</td>
<td>May require larger number of biopsies, longer procedures times, and increased cost Sampling error with low diagnostic uncertainty</td>
</tr>
<tr>
<td>Chromoendoscopy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dye</td>
<td>Utilizes dyes (methylene blue or acetic acid) with WLE to enhance visibility of high-grade dysplasia, intestinal metaplasia, and cancer</td>
<td>Increased diagnostic yield compared with WLE Well tolerated Low cost Ease of use and integration into standard endoscopy with no additional risks</td>
<td>Larger, prospective studies warranted Dysplasia can still be missed</td>
</tr>
<tr>
<td>Virtual</td>
<td>Enhances the endoscope through special filter inside scope, narrowing bandwidth of light that illuminates tissue with light at specific wavelengths, thus allowing enhancement of the underlying vasculature Various modalities used (eg, narrow band imaging)</td>
<td>Increased diagnostic yield compared with WLE Fewer biopsies and more effective at targeting biopsies Ease of use and integration into standard endoscopy with no additional risks Same efficiency as dye-based method, but more decreased time expenditure No studies show that one modality is better over another</td>
<td>Larger, prospective studies warranted Dysplasia can still be missed</td>
</tr>
<tr>
<td>Endoscopic mucosal resection</td>
<td>Local snare excision of lesion down to the submucosal level allows depth of tumor invasion to be histologically identified</td>
<td>Useful as “giant biopsy” given large amount of surface area it can resect in presence of raised mucosal lesions May also serve as tool for staging purposes</td>
<td>Differentiating depth of invasion is important, as inaccurate diagnosis may lead to inappropriate treatment</td>
</tr>
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*continued on next page*
combination with WLE to better visualize the mucosal surface of the gastrointestinal tract (see Table). The most commonly used dye is methylene blue, which is actively absorbed by the mucosa (peak absorption, 670 nm) by intestinal metaplasia. It is topically applied to the mucosal surface to enhance visibility for HGD, intestinal metaplasia, and cancer. Results from early studies evaluating the diagnostic advantage of methylene blue have shown that it is similar to conventional biopsy in its detection of specialized intestinal metaplasia and indefinite/LGD, while others have shown that methylene blue may be superior to random biopsy for identifying intestinal metaplasia, but not dysplasia or carcinoma. A meta-analysis of 450 participants in 9 studies did not significantly increase the detection of specialized intestinal metaplasia and dysplasia with methylene blue compared with WLE by the Seattle protocol.

Acetic acid has also been used to produce a transient whitening effect caused by protein acetylation.

### Table. — Endoscopic Diagnosis of Barrett Esophagus (continued)

<table>
<thead>
<tr>
<th>Endoscopic Technique</th>
<th>Method</th>
<th>Comment</th>
<th>Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Autofluorescence and molecular biomarkers</strong></td>
<td>Autofluorescence endoscopy incorporates real-time, wide-angle view allowing back-and-forth switch between standard white light imaging and autofluorescence endoscopy. Molecular biomarkers may be objectively used or used as indicator of normal biological processes, pathological processes, or response to therapeutic intervention.</td>
<td>Provides equivalent diagnostic rates of accuracy for dysplasia compared with current gold standard therapy with reduction in number of biopsies.</td>
<td>Low-quality evidence for their use cannot be used to confirm diagnosis or predict which patients are at risk for progression.</td>
</tr>
<tr>
<td><strong>OCT</strong></td>
<td>High-resolution, cross-sectional imaging technique that provides visualization of internal microstructure of tissues using measurements of optical backscattering or back reflection.</td>
<td>Useful in guiding targeted biopsies in areas with higher probability of dysplasia.</td>
<td>Imaging depth limited by optical attenuation from tissue scattering and absorption, allowing ≤ 3 mm of depth to be achieved in most tissues. Low-contrast images. High interobserver and intraobserver variations between image interpretation and prediction of pathology.</td>
</tr>
<tr>
<td><strong>CLE</strong></td>
<td>Combination of endoscopy with microscopic imaging of gastrointestinal mucosa. Uses argon-ion laser delivered with a wavelength of 488 nm to generate optical histological slices of 7 μm and lateral resolution of 0.7 μm.</td>
<td>Real-time assessment of gastrointestinal mucosa. Improves diagnostic yield and helps guide therapeutic treatment through targeting of lesions. Requires fewer biopsies with better diagnostic yield.</td>
<td>Requires specialized equipment and training. Only available at large academic institutions.</td>
</tr>
<tr>
<td><strong>Volumetric laser endomicroscopy</strong></td>
<td>Variant of OCT. Allows visualization of comprehensive, simultaneous images of distal esophagus using laser instead of sound waves. OCT laser beam helically scanned across long length of esophagus (~6 cm in 1–2 min) using miniature optics in center of 2.5-cm diameter, transparent balloon-centering catheter.</td>
<td>Real-time assessment with comprehensive, simultaneous images of distal esophagus. Fills the gap between endoscopic ultrasonography and CLE in resolution and imaging depth. Has 10-μm resolution and 3-dimensional imaging to 3.5 mm in muscularis propria. Performed at faster frame rate of 100× than OCT.</td>
<td>Biopsies cannot be excised during the scan because the dataset is continuously acquired through inflated balloon, but new targeting system may improve localization for biopsy site. Larger, multicenter prospective trials warranted. Head-to-head comparisons needed with other endoscopic modalities.</td>
</tr>
<tr>
<td><strong>Endocytoscopy</strong></td>
<td>Involves real-time visualization of superficial mucosa using high-level ≤ ×1400, necessitates use of mucosal staining to assess cytological and architectural features of superficial mucosa.</td>
<td>May be useful as adjunctive technique for targeted assessment of already identified lesions.</td>
<td>Not practical for wide-field screening of mucosa given restrictive sampling area. Uses optical lens alone, limited to visualization of superficial mucosa.</td>
</tr>
</tbody>
</table>

CLE = confocal laser endomicroscopy, OCT = optical coherence tomography, WLE = white light endoscopy.
and tissue edema, with dysplasia retaining color faster than BE and squamous mucosa. Studies have shown that acetic acid detects neoplasia better and more often than standard random biopsy, requiring 15 times fewer biopsies per neoplasia detected and equivalent rates of sensitivity and specificity to that of histological analysis. However, a high false-positive rate of 19.6% was noted in 1 study, and the other study was not a randomized or blinded-controlled trial. In another meta-analysis, dye-based chromoendoscopy increased the diagnostic yield of dysplasia by 35%, and testing for difference in yields of detection of dysplasia between virtual chromoendoscopy and chromoendoscopy failed to detect any significant differences between the 2 modalities. However, the possibility of inadequate or uneven surface application of either methylene blue or acetic acid creating inconsistent results is a limitation of the method.

**Virtual Chromoendoscopy**

Virtual chromoendoscopy encompasses a set of digitally enhanced imaging techniques achieved with the use of optical filters or selective wavelengths of light to highlight vessel and mucosal patterns. Narrow band imaging is a type of virtual chromoendoscopy that enhances the diagnostic capability of the endoscope through a special filter inside of the scope, thus narrowing the bandwidth of light that illuminates tissue with light at specific wavelengths. This technique creates blue light of wavelengths corresponding to the hemoglobin absorption band (415 nm), and, because the light does not deeply penetrate, the underlying vasculature is enhanced, producing contrast between the vessels and surrounding mucosa (Fig 6). Use of narrow band imaging has been evaluated in BE and compared with WLE, and these results have showed that it is superior to WLE. Narrow band imaging detected significantly more patients with dysplasia and higher grades of dysplasia with fewer biopsy samples than WLE. A meta-analysis and systematic review, which included 7 studies on virtual chromoendoscopy, found that virtual chromoendoscopy increased the diagnostic yield for identifying dysplasia or cancer in patients with BE by 34% compared with WLE with random biopsies. Narrow band imaging is not better at visually diagnosing dysplasia, but this technique has proven to be more effective at targeting biopsies, thus reducing the number of biopsy passes. This is the main practical application of narrow band imaging — namely, allowing the health care professional to focus on the tissue, find suspicious lesions, perform target biopsies, and avoid performing 4-quadrant biopsies. Overall, narrow band imaging increases diagnostic yield and requires fewer biopsies than WLE.

**Other Imaging Modalities**

Proprietary models, including i-Scan imaging (Pentax, Tokyo, Japan), Fuji Intelligent Chromo Endoscopy (Fujifilm, Tokyo), and the Storz Professional Image Enhancement System (Karl Storz, Tuttingen, Germany), are imaging modalities that use software-based processing to alter the wavelength ranges of reflected light to obtain different effects on surfaces, tissues, and vessel enhancement. By contrast to narrow band imaging, these proprietary modalities offer different filter options that allow the health care professional to visualize tissue and surface structures in a selective and accentuated manner.

For example, Hoffman et al found that i-Scan has significantly higher diagnostic yield for identifying specialized columnar epithelium, thus resulting in fewer biopsies than the random biopsy protocol. It also provides the precise detection of lesions associated with erosive reflux disease via esophagogastroduodenoscopy, and it is superior to standard-resolution colonoscopy and equivalent to chromoendoscopy using methylene blue when detecting colorectal neoplasia. These virtual imaging modalities may eventually replace dye-based chromoendoscopy due to their decreased time expenditure and equivalent effectiveness.

**Endoscopic Mucosal Resection**

Endoscopic mucosal resection (EMR) may be used as a diagnostic tool and a therapeutic treatment option for patients with HGD or intramucosal cancer (see Table). It entails the local snare excision of a lesion down to the submucosal level, allowing the depth of tumor invasion to be histologically iden-
Differentiating the depth of invasion is important, as an inaccurate diagnosis could have severe consequences. Patients with neoplasia limited to the mucosa are at a 0% to 3% risk of lymph-node metastasis, while those with submucosal infiltration have an increased risk of lymph-node metastasis of 20% to 30% and require surgical evaluation instead of mucosal resection. An accurate diagnosis depends on an interpretation of the depth of invasion and also on an accurate diagnosis of BE, LGD, and HGD, and should require 2 expert pathologists to read the specimen.

Curvers et al evaluated histopathology reports of 147 study patients with a prior diagnosis of LGD. After pathology review, 85% of study patients were downstaged to nondysplastic BE or to indefinite for dysplasia, highlighting that most cases of dysplasia in BE are overdiagnosed. However, once LGD was confirmed, a high risk of progression to HGD or carcinoma was seen.

In a multicenter cohort study by Wani et al., 138 patients underwent EMR (10.9% LGD; 63% HGD; 26.1% esophageal adenocarcinoma). EMR resulted in a change of the diagnosis in 31.1% of study patients (10.1% upgrade and 21.0% downgrade). Although EMR can be used as a diagnostic tool in cases of malignancy or HGD, it is also useful as therapy in HGD and early cancer by acting as a “giant biopsy” sample and, in cases where it does not resect HDG or early cancer, it serves for staging purposes. In the presence of raised mucosal lesions, which are often signs of inflammation but sometimes contain LGD or HGD, or even early cancer, EMR can be a useful diagnostic tool.

Endoscopic ultrasonography (EUS) has also been evaluated for its role in staging. Although EUS is used for staging other types of gastrointestinal malignancy, its use for BE staging is suboptimal and carries the risk of overstaging. Its role in distinguishing mucosal from submucosal lesions is limited and, in 1 systematic review, was shown to carry a 65% concordance rate with surgical or EMR staging. Thus, EUS has no clinical impact and diagnostic EMR is superior. Detectable lesions should be staged with EMR to determine the risk of lymph-node metastasis to help guide treatment options.

**Autofluorescence and Molecular Biomarkers**

Several studies have evaluated the combination of autofluorescence endoscopy and molecular biomarkers as a novel diagnostic tool in BE. di Pietro et al created a 3-biomarker panel to include p53 immunohistochemistry, cyclin A, and aneuploidy, and they demonstrated a strong association with prevalent dysplasia. They concluded that the 3-biomarker panel, in combination with autofluorescence imaging-targeted biopsies, provided an equivalent diagnostic accuracy rate for dysplasia when compared with the gold standard of therapy; a significant reduction in the number of required biopsies was also seen.

Glycosylation patterns may also be candidate biomarkers for detecting disease progression in BE through the use of fluorescence endoscopes and fluorescent-labeled lectins sprayed onto the mucosal surface of tissue. Sturm et al showed that fluorescent-labeled peptides with specific binding for esophageal neoplasia could be topically and safely administered using real-time confocal laser endomicroscopy, in addition to showing strong fluorescence in HGD and esophageal adenocarcinoma. However, although biomarkers show promise in assisting with the diagnosis of BE, the quality of evidence for their use is low and cannot be used to confirm the diagnosis or predict which patients will be at risk of disease progression.

**Cross-Sectional Imaging Techniques**

**Optical Coherence Tomography (OCT):** Optical coherence tomography (OCT) uses a low-coherence laser to help the health care professional visualize the internal microstructure of tissues by measuring differences in time delay between light that backscatters from below the tissue surface and a reference beam (see Table). It
was initially developed for ophthalmology, is noninvasive, and analogous to EUS in that it uses a laser instead of sound waves, with air not producing artifacts. Imaging depth is limited by optical attenuation from tissue scattering and absorption, allowing up to 3 mm of depth to be achieved in most tissues. Other limitations include low-contrast imaging and high interobserver and intraobserver variation between image interpretation and prediction of pathology. Imaging of HGD and intramucosal cancer by OCT exhibits more heterogeneous structures that correspond to irregular, heterogenous tissue morphology from distorted and cribriform or villiform glandular architectures.

Two prospective studies evaluated OCT, with 1 trial demonstrating a 68% sensitivity rate and an 82% specificity rate for detecting Barrett neoplasia, and the other detecting an 83% sensitivity rate and a 73% specificity rate for detecting HGD and esophageal adenocarcinoma. Overall, OCT can be used to target areas for biopsy with a higher probability of the presence of dysplasia.

OCT has also been used to predict responses to radiofrequency ablation (RFA) for which the BE mucosa was significantly thinner in patients who achieved complete eradication of intestinal metaplasia compared with those without complete eradication at follow up, corresponding to rates of 92.3% sensitivity, 85% specificity, and 87.9% accuracy.

Confocal Laser Endomicroscopy: Confocal laser endomicroscopy (CLE) is a novel, endoscopic imaging modality that combines endoscopy with microscopic imaging of the gastrointestinal mucosa (see Table). First described for the diagnosis of BE in 2006 by Kiesslich et al., this fluorescence-aided endomicroscopy of BE uses an argon-ion laser delivered with a wavelength of 488 nm to generate optical, histological slices of 7 µm with a lateral resolution of 0.7 µm. This action allows for real-time, in vivo histology of mucosal layers during endoscopy, with the aim of improving the effectiveness of the surveillance of dysplasia and esophageal adenocarcinoma in BE. Although CLE cannot replace histopathology, this method may improve diagnostic information and guide therapeutic management because of its ability to localize pathology and specifically target lesions in patients with BE.

Select retrospective reports found that CLE was an effective tool for guiding endoscopic therapy in the setting of BE. A multicenter, randomized controlled trial conducted by Canto et al. that included 192 study participants with BE compared high-definition WLE alone with random biopsy and high-definition WLE with CLE and targeted biopsy. Adding CLE to high-definition WLE increased the rate of sensitivity from 40% to 96% without significantly compromising the rate of specificity (92%); it also tripled the diagnostic yield for neoplasia and obviated the need for biopsy in 65% of study participants. CLE also changed the treatment plan in 36% of these study patients. Overall, the trial results demonstrated significantly enhanced rates of diagnostic yield and accuracy, leading to an 80% reduction in mucosal biopsy specimens by enabling more selective tissue sampling.

A systematic review and meta-analysis by Gupta et al. compared the diagnostic accuracy of CLE with random biopsy and included 7 prospective studies (N = 345 participants; N = 3,080 lesions). Although CLE had good rates of diagnostic accuracy for detecting HGD and esophageal adenocarcinoma, the researchers concluded that CLE might not replace the standard of care, given its relatively low sensitivity rate and negative predictive value. However, this study was performed prior to the study by Canto et al., so those study data were not included in their meta-analysis.

The majority of these studies also included a higher overall prevalence of HGD and esophageal adenocarcinoma than is seen in clinical practice, and they were focused on academic centers not otherwise generalizable to community-based physicians.

As a tool, CLE can be used to decrease unnecessary mucosal biopsies, improve targeting of HGD and neoplasia, and to potentially alter the diagnosis and management of BE. However, because this modality requires specialized equipment and training, and because it is mainly used in large academic centers, CLE has not yet been deemed sufficient to replace the Seattle protocol. Thus, additional, larger prospective studies are warranted.

Volumetric Laser Endomicroscopy: Volumetric laser endomicroscopy (VLE) is an emerging endoscopic tool that is a variant of OCT, a cross-sectional imaging technique, that can be used to simultaneously view comprehensive images of the distal esophagus (see Table). An OCT laser beam is helically scanned over a long length of esophagus (~ 6 cm) in 1 to 2 minutes using miniature optics in the center of a 2.5-cm diameter, transparent balloon-centering catheter. VLE attempts to fill the need between EUS and CLE in resolution and imaging depth: It has 10-µm resolution and 3-dimensional imaging to 3.5 mm in the muscularis propria. It is also performed at a faster frame rate of 100 times than OCT. One limitation is that biopsy samples cannot be excised during the scan because the dataset is continuously acquired through an inflated balloon; however, this limitation can be resolved by placing visible marks on the esophagus to delineate tissues corresponding to regions of interest.

A pilot feasibility study performed with 22 participants looked at the effectiveness of VLE for image-guided biopsy in the setting of BE. This modality uses a laser with a different wave length to create 2 burning marks, with the abnormality seen on VLE in the middle, and doing so helps the health care
professional target biopsy/EMR the area. When compared with histopathological interpretations, the rates of accuracy for diagnosing tissue between cautery marks were 67% for independent readers, 93% for VLE intent-to-biopsy, and 100% for corrected VLE post-marking images. No adverse events from VLE and laser marking were seen. Thus, preliminary data in BE are promising and could be useful for “buried” BE. However, larger, multicenter, prospective trials are warranted and head-to-head comparisons are needed with other endoscopic modalities.

Endocytoscopy
Endocytoscopy involves real-time visualization of the superficial mucosa using high-level magnification (≥ 1400; see Table). Unlike CLE, endocytoscopy uses an optical lens alone and is limited to visualization of the superficial mucosa. Endoscopic visualization of subcellular structures, such as nuclei, necessitates the use of mucosal staining with either methylene or toluidine blue, which is then washed off prior to imaging. Diagnosis involves assessing several cytological and architectural features such as cell density, size, and arrangement, as well as the size and shape of nuclei and the nucleus-to-cytoplasm ratio. Neoplastic features typically involve an increase in cellular density and marked heterogeneity in nuclear staining and size in comparison with orderly cellular arrangement and homogenous staining of the normal squamous epithelium.

When Pohl et al assessed the rate of accuracy of endocytoscopy in patients presenting for BE surveillance, they found that endocytoscopy resulted in a high proportion of unusable imaging due to suboptimal image quality, fair interobserver agreement, and a poor diagnostic specificity rate. Overall, endocytoscopy lacked sufficient image quality to assist with the identification of neoplastic areas.

Treatment for Mucosal Pathology Changes
Nondysplastic
BE is a complication of uncontrolled GERD, so patients with BE must be given medications that effectively treat GERD. Not all patients with GERD require endoscopy to screen for BE, but the presence of multiple, well-established risk factors can be used to identify individuals with increased risk of developing BE. These factors include age (≥ 50 years), male sex, white race, chronic GERD, hiatal hernia, elevated body mass index, and an intra-abdominal distribution of fat.

Once the diagnosis of BE has been confirmed, the health care professional must take several factors into consideration to determine the appropriate management of BE (beyond controlling GERD). The most accepted approach is based on the presence of dysplasia and the degree of that finding (LGD and HDG): Endoscopic surveillance is recommended by most of the gastroenterology societies worldwide, even though no data support the reduction of esophageal adenocarcinoma due to this practice. The management of nondysplastic BE has been controversial, and guidelines recommend continued surveillance endoscopy every 3 to 5 years. Controlled trials are lacking to show that endoscopic eradication therapy of BE without dysplasia is more effective at reducing esophageal adenocarcinoma or more cost effective than surveillance.

In our opinion, patients with numerous clinical and demographic risk factors for neoplastic progression of BE, including age, sex, ethnicity, obesity, alcohol use, smoking, and long-segment BE, may need to be considered on a case-by-case basis given the lack of controlled trials, but these patients likely do have an increased risk of progression to HGD or adenocarcinoma.

Dysplastic
When the presence of dysplasia has been confirmed by 2 pathologists (preferably 1 is an expert in gastrointestinal pathology), the management plan can vary based on the degree of dysplasia.

For patients with LGD, endoscopic surveillance can be continued in 6 to 12 months as recommended by societal guidelines. In addition, endoscopic eradication therapy can be considered with photodynamic therapy, cryoablation, or RFA. A large body of literature is dedicated to ablation therapy for BE, and, given its high rate of effectiveness, its low adverse-event profile, and its easier treatment protocol (for physicians and patients alike), RFA appears to be the preferred modality of treatment. In 1 randomized control trial, RFA resulted in reduced risk of neoplastic progression in patients with BE who had a confirmed diagnosis of LGD during 3 years of follow-up.

Patients with BE and HGD represent a challenging group due to their risk of malignancy. Although esophagectomy has been the gold standard treatment for patients with HGD or intramucosal cancer, a paradigm shift is taking place to using endoscopic procedures given the higher morbidity and mortality rates associated with surgical resection. Most patients with BE also have significant comorbidities, which medically preclude them from undergoing such surgical interventions. Therefore, endoscopic ablative procedures have been advocated as alternative and minimally invasive treatment modalities.

With the presence of HGD, it is important for the health care professional to evaluate the BE mucosa for any abnormalities. If irregularities are detected in the area with BE, then abnormal mucosa should be resected by means of EMR. EMR can aid in the histological diagnosis and is often used to treat patients with HGD. However, a limitation to focal resection is its
high rate of synchronous and recurrent lesions, ranging from 14% to 47%, which tends to increase with longer durations of observation.79

Therefore, complete Barrett eradication EMR (CBE-EMR) was developed at select centers with the curative intent of removing all HGD or intramucosal cancer in patients with BE to reduce such risks.79 A long-term follow-up study of 24 patients for 28 months evaluated CBE-EMR for the treatment of HGD and intramucosal cancer and found the method to be a safe and effective, long-term treatment option.80 CBE-EMR with close endoscopic surveillance has also been demonstrated to be an effective treatment modality for HGD and intramucosal cancer.79 However, a limitation to CBE-EMR is its increased risk for esophageal strictures: The post-EMR stricture rate has been reported to range from 4% to 70%.81 Risk factors for stricture formation depend on the size of the lesion excised and the number of lesions, with larger lesions and removal of several lesions associated with a higher risk of esophageal strictures.81 Although CBE-EMR carries a significant rate of stricture development, strictures can be treated by endoscopic dilation.

Following endoscopic resection, argon-plasma coagulation may significantly reduce the neoplasia recurrence rate compared with a surveillance-alone strategy for the management of residual Barrett epithelium. In a study by Manner et al,82 63 study patients with HGD or intramucosal cancer curatively resected by endoscopy had a significantly high recurrence-free survival rate with ablation of residual Barrett segment by argon-plasma coagulation during a follow-up of 2 years in a randomized control trial.82 However, a longer follow-up duration is warranted.

If a patient has BE with HGD and no mucosal abnormalities are present, then RFA may be considered an appropriate treatment option. RFA was first described in 2004 by Ganz et al,83 and it involves a balloon-based, bipolar electrode that creates a circumferential, thin-layer epithelial ablation zone within the esophagus. The principle is to deliver high power at about 300 W in a short period of time (<300 milliseconds). RFA typically extends into depths of 700 µm, with the concept that Barrett tissue will not extend into the submucosa; this also decreases a patient’s risk of bleeding, fibrosis, and strictureting.84

RFA, which is a relatively conservative approach, has been advocated by some investigators given its lower rates of morbidity and mortality when compared with more invasive treatment options such as surgical resection.85 RFA has also been found to be more effective and less costly than endoscopic surveillance in HGD.86

One multicenter, randomized, sham-controlled study showed a high rate of complete eradication of dysplasia and intestinal metaplasia with decreased disease progression.87 In HGD, complete eradication of dysplasia was seen in 81% of study patients with RFA compared with 19% in the control group.87 A total of 2.4% study patients with RFA progressed to esophageal cancer compared with 19% in the control group.87 A meta-analysis and systematic review found that ablation may be associated with a reduced incidence of cancer, with the greatest benefit observed in patients with BE with HGD.88 In our opinion, use of RFA should be advocated in patients with HGD without mucosal abnormalities given the effectiveness of RFA in treating this patient population.

Studies evaluating RFA preceded by EMR have shown this combination method to also be a safe and effective treatment option, including in patients with BE (≥10 cm); however, given the longer-length BE, the combination method was found to be more challenging in this patient population.89,90 Thus, it should be performed in centers with staff members who have experience in BE imaging and therapy.

We consider cryoablation to be an alternative treatment option for HGD or intramucosal cancer. It uses low-pressure liquid nitrogen to eradicate precancerous or cancerous tissue. In our opinion, the procedure is easier to perform when compared with other treatment modalities, and it relatively lacks any patient discomfort.

Data are limited, but current studies show promising results for either the downgrading of pathology or complete eradication.91,92 A prospective, pilot study revealed the reversal of BE in all 11 study patients at 6 months of follow-up.93 Longer-term results have demonstrated complete eradication of HGD at 100% after 2 years of follow-up in 32 participants.94 However, additional long-term studies are warranted; in addition, lack of uniformity for applying liquid nitrogen to esophageal mucosa and its effectiveness as a treatment option are still existing concerns.

Conclusions
Barrett esophagus is a major risk factor for developing esophageal adenocarcinoma, a cancer with increasing incidence and a dismal 5-year survival rate.16 Significant advances have been made in endoscopic modalities to improve diagnostic yield, rates of accuracy, and better guidance for therapeutic management. Based on preliminary studies, confocal and volumetric laser endomicroscopy have both shown promising results for endoscopic diagnosis and therapeutic guidance of Barrett esophagus. Endoscopic mucosal resection has also evolved as a diagnostic and therapeutic tool, thus allowing for more accurate staging to help guide treatment options and better select patients for esophagectomy. These endoscopic tools help to mitigate the shortcomings of the Seattle protocol, which is the current standard of care. With additional future studies and increasing use, they may
replace standard, untargeted biopsies.

Given the high rates of morbidity and mortality associated with surgery, a paradigm shift has taken place to focus on endoscopic therapies for the treatment of high-grade dysplasia and intramucosal cancer. Treatment of Barrett esophagus depends on the presence of dysplasia, which is further categorized as low- or high-grade dysplasia with or without intramucosal cancer. Thus, the presence of mucosal lesions in high-grade dysplasia further delineates the method of treatment.

References


**Association of microRNA 21 With Biological Features and Prognosis of Neuroblastoma**

Yaodong Zhou, MD, and Bo Sheng, MD

**Background:** The aim of this study was to assess the differences in microRNA 21 expression among neuroblastoma (NB), embryonic tissue, and normal adrenal tissue and to identify correlations between microRNA 21 expression, the biological features of the tumor, and prognosis.

**Methods:** A total of 70 patients with NB were selected from December 2005 and December 2007. Real-time polymerase chain reaction was used to assess microRNA 21 expression. All patients were followed-up for 5 years.

**Results:** Significant differences in microRNA 21 expression were found between the 3 groups, with the highest expression in the NB samples \((P < .001)\). The expression of microRNA 21 was highest in the high-risk group compared with the moderate- and low-risk groups \((P < .001)\). The microRNA 21 expression in the MYCN amplification group was higher than in the group without amplification \((P = .001)\). The 5-year overall survival rate of patients with NB was 71.4%.

**Conclusions:** The higher expression of microRNA 21 in NB samples compared with embryonic and normal tissue samples predicted a close correlation between microRNA 21 expression and the biological features of NB. In patients with NB, higher microRNA 21 expression correlated with lower rates of overall survival. Therefore, microRNA 21 expression may represent a novel risk factor for determining the prognosis of patients with NB.

**Introduction**

Neuroblastoma (NB) is the most frequently occurring solid tumor in children, with an incidence of 1.3 cases per 100,000 children aged 14 years and younger.\(^1\) In addition, it is the most common extracranial tumor in children. Clinically, NB is characterized by rapid growth, susceptibility to multidrug resistance, and metastasis. As is characteristic of embryonic tumors, neuroblasts are histologically indistinguishable from developing neuroblastic cells in the embryo. Despite many advances made during the last 30 years, NB has remained a challenge to clinical and basic research scientists.

To date, several approaches for determining the prognosis of NB have been described, including assessment of \(MYCN\) status or the Shimada classification. \(MYCN\) was originally found to be amplified in NB, and since then research has focused on the search for other genetic markers.\(^2\) The International Neuroblastoma Risk Group, which represents the major cooperative groups on pediatric cancer, met in 2005 to review data collected on 11,000 patients studied between 1974 and 2002.\(^3\) Consensus was reached to consider age (\(>\) or \(<\) 18 months), image-defined risk factors for surgery, International Neuroblastoma Staging System, and \(MYCN\) status as basic tools in the risk group schema, which included high-, moderate-, and low-risk groups.\(^3\) Elucidating the exact molecular signature of NB will allow for analysis of how specific markers, alone or in combination, can help to stratify disease in prospective studies. Currently, stratification is based on age, tumor stage, \(MYCN\) status, and Shimada pathology.

NB may be one of the first examples of a disease for which genetic tumor markers are used as a tool to define tumor behavior and to aid in clinical staging. microRNAs can act as oncopgenes by posttranscriptionally repressing the expression of target tumor suppressor genes, or as tumor suppressors by repressing the expression of target oncogenes. microRNA 21 is up-regulated in many solid tumors, including lung, breast, prostate, and stomach carcinomas, as well as pancreatic endocrine tumors, hepatocellular carcinoma, and glioblastoma.\(^4,5\) Growing evidence suggests that microRNA 21 has an antiapoptotic function and promotes growth and chemosensitivity in tumor cells.\(^4,6,7\) However, these data were mainly derived from experiments on cell lines in vitro or in xenograft animal models. Thus, we previously used a microRNA array to screen for genes involved in NB and found that microRNA 21 was up-regulated,
and our previous data suggest a correlation between microRNA 21 expression and the biological features and prognosis of NB (Fig 1A–C).

The aim of this study was to assess the differences in microRNA 21 expression among NB, embryonic tissue, and normal adrenal tissue samples and to determine the correlation between microRNA 21 expression, the biological features of the tumor, and prognosis.

**Materials and Methods**

**Patients and Specimens**

A total of 70 patients with NB were selected from the Children's Hospital of Fudan University (Shanghai, China) between December 2005 and December 2007 (Table 1). Tumor samples were collected from each study participant during surgical resection, frozen in liquid nitrogen, and stored at –80 °C. Sections from each specimen were examined by a pathologist and histologically graded. Patients who had received neoadjuvant chemotherapy or radiation therapy prior to surgery were excluded from this study. Staging was retrospectively determined and based on surgery and pathology. A total of 120 samples were obtained; 60 samples of normal adrenal tissue were retrieved from patients with nephroblastoma and 60 samples of embryonic tissue were obtained from deceased donors.

This study was approved by the Institutional Review Board and Ethics Committee of the Children's Hospital of Fudan University. The parents or guardians of patients provided written informed consent.

**Tissue RNA Isolation**

Total RNA was isolated from the tissues using Trizol reagent (Invitrogen, Grand Island, New York). Some of the total RNA specimens were further purified using a Qiagen midi column (Qiagen, Shanghai, China).

**Real-Time Polymerase Chain Reaction**

Primers for the analysis of microRNA 21 expression were designed as F: 5'-TAGCTTATCAGACTGATGTTGA-3' and R: 5'-TGCGTGTCGTGGAGTC-3'. Mixtures of 1 μg of total RNA together with 50 nM reverse primer, 2 U of an RNAase inhibitor (Promega, Madison, Wisconsin), 5 U of M-MLV reverse transcriptase (TaKaRa Bio, Shiga, Japan), and 0.5 μM dNTP were used for each reverse transcription (RT) reaction. The expression of microRNA precursors was determined using a real-time quantitative polymerase chain reaction (PCR) assay as described, with the ex-

![Fig 1A–C. — (A) Gene screening results in tumor tissue, (B) embryonic adrenal tissue, and (C) normal adrenal tissue.](image-url)
ception that 35 cycles of PCR were used. The re-
action parameters were as follows: incubation at
16 °C for 30 minutes, 42 °C for 42 minutes, and 85 °C
for 5 minutes. Fluorescence was then measured.

To generate the complementary DNA (cDNA)
template for the endogenous-control PCR reactions,
first-strand cDNA was synthesized using 1 μg of RNA
from the same samples for stem-loop RT and oligo
d(T) as the primer. The reaction parameters were as
follows: 95 °C for 5 minutes followed by 35 cycles of
95 °C for 10 seconds, 56 °C for 15 seconds, and 72 °C
for 20 seconds. Fluorescence was then measured.

Real-time PCR was performed on an Applied Bio-
systems 7500 detection system (Applied Biosystems,
Foster City, California) in a 15-μL reaction volume. All
reactions were performed in triplicate. For quantita-
tion of microRNA 21 expression, a 15-μL PCR reaction
mixture was used that included 1 μL of the microRNA
21 RT product, 1X SYBR-Green I Mastermix (Toyobo
USA, New York, New York), and 0.5 μM each of the
forward and reverse primers. For the endogenous
control (U6), 1 μL of cDNA synthesized using oligo
d(T) was used as a template. The housekeeping gene
primer was F: 5’ GCTTCGGCACGCACTATACAAAT
3’ and R: 5’ CGTTCAGATTTGCTGTCAT 3’. The
reaction parameters were 95 °C for 5 minutes followed
by 35 cycles of 95 °C for 10 seconds, 56 °C for 15 sec-
onds, and 72 °C for 20 seconds. The cycle threshold
(Ct) was defined as the cycle number at which fluo-
rescence passed a predetermined threshold.

For expression analysis, the experiment was de-
signed to use the matched normal adrenal tissue and
embryonic adrenal tissue as the control, so the rela-
tive quantification of microRNA 21 in tumor tissue
was calculated using the equation:

\[
\text{Amount of target} = 2^{-\Delta\Delta\text{Ct}} \quad (17), \quad \Delta\Delta\text{Ct} = (C_{\text{tumor}} - C_{\text{U6}}) - (C_{\text{matched nontumor}} - C_{\text{U6}})
\]

For the matched, normal adrenal tissue control
samples, ΔΔCt was 0 and 2^ΔΔCt was 1. We set the nor-
mal adrenal value as 1 and calculated the value of em-
bryonic adrenal and NB tissue. Melting curves were
generated, and 8% polyacrylamide gel electrophoresis
was performed for each real-time PCR to verify the
amplification of the desired product alone.

**MYCN Detection**

Amplified MYCN was used as a major prognostic fac-
tor of localized NB. Fluorescence in situ hybridiza-
tion was applied to identify MYCN, as previously de-
scribed.

**Pathological Categorization**

Pathological classification of all tumor tissues was
performed according to the modified Shimada clas-
sification. After confirming the histological diagnosis,
2 morphological features were considered for prog-
nostic categorization: degree of differentiation of the
neuroblasts and proportion of mitotic and karyor-
rhectic cells (MKCs) to determine the mitotic karyor-
rhectic index (MKI). The proportion of tumor cells
showing mitosis and karyorrhexis was used to clas-
sify whether the NB tissue had a low (< 2% MKCs),
intermediate (2%–4% MKCs), or high (> 4% MKCs)
MKI. The average number of MKCs was assessed in
approximately 10 hpf, depending on the cell density
of the NB tissue (total number of tumor cells in the
chosen hpf ≤ 5,000).

All pathological sections were stained with he-
matoxylin and eosin and observed under an optical
microscope. Samples were classified as unfavorable
or favorable histology based on a classification sys-
tem according to the amount of Schwannian stroma,
degree of differentiation, MKI, and age at diagnosis.

**Clinical Follow-Up**

All patients were followed for up to 5 years. We col-
clected and reviewed clinical data of patients from our
center during a 5-year period to evaluate the correla-
tion between microRNA 21 expression and NB prog-
nosis. We combined the data obtained from regular
visits to the hospital every 3 months and follow-up
phone calls.

**Data Analysis**

Statistical analyses were performed using SPSS 15.0
(SPSS, Chicago, Illinois). The results are expressed as
mean ± standard error, and less than .05 was con-
sidered statistically significant. The Wilcoxon test was
used to assess the difference in the microRNA levels
among NB, embryonic adrenal, and normal adrenal
tissues. The Mann-Whitney test or the Tukey-Kramer
test was selected to assess the associations between
mature microRNA levels and clinicopathological pa-
rameters.

**Results**

**Groups Analyzed in the microRNA Array**

Three groups were assessed in this study, including
a NB group (4 samples), an embryonic adrenal tis-
sue group (4 samples), and an adrenal tissue group
(4 samples; see Fig 1).

**Expression of microRNA 21 in Tumor and
Matched Nontumor Samples**

All tissues were assessed by real-time PCR. As shown
in the representative amplification curves in Fig 2,
the Ct value of microRNA 21 in tumor tissue was
lower than that of microRNA 21 in nontumor tis-
sue, suggesting that the expression level of mi-
croRNA 21 in tumor samples was higher than that
in the controls. Of the 70 study patients, MYCN amplification was observed in 16 cases (Fig 3). In addition, 28 cases had favorable histology and 42 cases had unfavorable histology (Fig 4A–B). The expression of microRNA 21 in NB, normal adrenal, and embryonic adrenal tissues significantly differed, with NB samples having the highest expression followed by normal embryonic tissue and then adrenal tissue ($P < .001$; Table 2, Fig 5).

Groups with a more advanced tumor stage and greater risk had high expression of microRNA 21 (see Table 2 and Fig 5). Of the 70 study patients, MYCN amplification was detected in 16 tumors. In addition, microRNA 21 expression in amplification cases was higher than in nonamplification cases ($P = .001$). In the 70 study patients, expression of microRNA 21 in samples with unfavorable histology was higher than in those with favorable histology ($P = .008$).

**Patient Follow-Up**
The median follow-up for all patients was 5 years and was
conducted from December 2005 to December 2012. The 5-year overall survival (OS) rate of patients with NB was 71.4%. Twenty participants died during the study, among whom 12 had stage 4 (OS, 55.6%), 5 had stage 3 (OS, 75%), and 3 had either stage 1 or 2 NB (OS, 87.0%). The 5-year OS rates were 48.0% in the high-risk group, 78.3% in the moderate-risk group, and 90.9% in the low-risk group. Among the 20 study patients who died, MYCN amplification was present in 9 cases (45%), and 12 cases (60%) had unfavorable histology. The 5-year OS rate in the MYCN amplification group was lower than that in the group without amplification (43.5% vs 82.9%, respectively; \( P = .001 \)). The 5-year OS rate for patients with unfavorable histology was 57.1% compared with 81.0% for patients with favorable histology (\( P = .02 \)). In addition, microRNA 21 expression was higher in participants who died compared with those who were alive at the end of the study (70.5 ± 4.16 vs 25.3 ± 1.24, respectively; \( P = .03 \)). In addition, we found that higher Ct values for microRNA 21 correlated with a shorter rate of OS (Fig 6).

### Table 2. — microRNA 21 Expression in Various Tissues, Tumor Stages, and Risk Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>2-ΔΔCt Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue Type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>70</td>
<td>40.34 ± 3.16</td>
</tr>
<tr>
<td>Embryonic adrenal tissue</td>
<td>60</td>
<td>3.13 ± 0.52</td>
</tr>
<tr>
<td>Normal adrenal tissue</td>
<td>60</td>
<td>1.09 ± 0.10</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>23</td>
<td>12.66 ± 1.78</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>24.89 ± 2.77</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>60.35 ± 5.23</td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low risk</td>
<td>22</td>
<td>10.66 ± 1.12</td>
</tr>
<tr>
<td>Moderate risk</td>
<td>23</td>
<td>30.53 ± 6.45</td>
</tr>
<tr>
<td>High risk</td>
<td>25</td>
<td>61.95 ± 11.16</td>
</tr>
</tbody>
</table>

\( P = .001 \).  
Ct = cycle threshold.
Discussion

To the best of our knowledge, this is the first study to assess microRNA expression patterns in patients with NB using microRNA microarrays. microRNA 21 seems to play an important oncocgenic role in malignant tumors, and it has generated more research interest than any other microRNA given its involvement in various cancers, cardiac hypertrophy, and neointimal formation.\textsuperscript{10-12} Widespread overexpression of MIR21 in cancer has led to the hypothesis that it is oncocgenic.\textsuperscript{5} Similar to many other malignant tumors, the development, progression, invasion, and metastasis of NB are caused by multiple genetic alterations, but the mechanism has not been fully elucidated until now. It has been hypothesized that 1 microRNA may simultaneously down-regulate multiple target genes; therefore, microRNAs may act as efficient regulators of tumor-related genes.\textsuperscript{6} To reduce error caused by gene-expression differences between individuals, we used matched nontumor tissue as a control and used 2\textsuperscript{-ΔΔCt} to represent the level of microRNA 21 expression in tumor samples relative to matched nontumor samples. Unfavorable and favorable histology classification is a common method for evaluating the prognosis of NB.

The average number of MKCs was assessed in approximately 10 hpf depending on the cell density of NB. Expression of microRNA 21 in unfavorable histology samples was higher than that in nontumor tissue samples. In our study, the 5-year OS rate in the MYCN amplification group was lower than that in the group without amplification. MYCN amplification indicates poor prognosis, and the expression of microRNA 21 in amplification cases was higher than that in non-amplification cases, indicating that microRNA 21 expression may be related to NB prognosis. In NB, MIR17/92 expression has been shown to correlate with MYCN amplification and adverse outcomes, a finding also confirmed by Schulte et al.\textsuperscript{13,14} Chen and Stallings\textsuperscript{8} further suggested that MYCN may mediate a tumorigenic effect, in part through directly or indirectly regulating the expression of microRNAs involved in neural cell differentiation, apoptosis, or both. Thus, these findings warrant further study of microRNAs as potential therapeutic targets.

In our study, microRNA 21 was up-regulated in patients with MYCN amplification, unfavorable histology, and stages 3/4 NB, as well as those in high- and moderate-risk groups, which predicted a close correlation between microRNA 21 expression and the biological features of NB. However, further research is needed to understand the mechanistic link between MYCN and microRNA 21.

Significant differences were found in the expression of microRNA 21 in NB, normal adrenal, and embryonic adrenal tissues, with NB samples having the highest microRNA 21 expression. These results indicate that microRNA 21 may play a role in the function and development of NB, but this hypothesis requires additional data for verification.

We found that the 5-year OS of all patients with NB was 71.4%. In addition, expression of microRNA 21 was higher in the patients who died than in those who remained alive at the end of the study. Several risk factors can affect the prognosis of NB, such as MYCN status, unfavorable histology, and favorable histology. However, these factors have a binary classification index that cannot definitively determine risk. Therefore, microRNA 21 expression analysis could potentially solve this problem. In our study, the relative expression value in the high-risk group was approximately 60, whereas it was 30 and 10 in the moderate- and low-risk groups, respectively. Thus, the expression values of microRNA 21 may be useful in determining risk in patients with NB. An expression level close to 60 may indicate poor prognosis, whereas a value less than 10 may not indicate poor prognosis. Thus, the predictive value of microRNA 21 expression may be advantageous in the clinical setting, but further studies are needed for clarification.

High expression of microRNA 21 in NB suggests that microRNA 21 may be an effective therapeutic target, and numerous studies have confirmed that inhibiting microRNA 21 expression blocks tumor growth in various tumor types.\textsuperscript{6,8} Other reports have shown that inhibiting the expression of microRNA 21 can increase the sensitivity of tumors to chemotherapy.\textsuperscript{10,15} Therefore, future studies should focus on targeting microRNA 21 as a cancer treatment modality.\textsuperscript{16} Nevertheless, the utility of microRNA 21 expression as a prognostic factor or molecular target in the treatment of NB will require further clarification with more samples and patients from multicenter studies.

Conclusions

The higher expression of microRNA 21 in neuroblastoma samples compared with embryonic and normal tissue samples predicted a close correlation between microRNA 21 expression and the biological features of neuroblastoma. In patients with neuroblastoma, higher rates of microRNA 21 expression correlated with lower rates of overall survival. Therefore, microRNA 21 expression may represent a novel risk factor for determining the prognosis of patients with neuroblastoma.

Acknowledgment: We thank Reginald C. Tsang, MD, for his help in editing this manuscript.
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Ten Best Readings Relating to Regional Therapies


This is the first randomized study on the efficacy of radiofrequency ablation (RFA) and its potential benefits in the setting of nonresectable colorectal liver metastases. The primary end point of the study was an overall survival rate of 30 months. RFA combined with systemic treatment resulted in significantly longer progression-free survival, but the effect of RFA on overall survival was uncertain.


Use of stereotactic body radiotherapy was analyzed in patients with limited metastases. Rates of long-term survival and tumor control following treatment were then reviewed.


Researchers investigated whether metastasis-directed radiotherapy might benefit select patients with metastatic cancer in limited organs. They discovered that carefully selected patients with no more than 5 areas of metastases can safely receive radiotherapy to multiple body sites.


Researchers sought to determine the optimum fractionation of stereotactic body radiotherapy (SBRT) for locally recurrent, previously irradiated head and neck cancers. They also looked at methods of patient selection. They found that SBRT with or without cetuximab was promising with regard to rates of survival and tumor control. Low rates of acute and late toxicities were seen, even for sites of recurrence larger than 25 cc in size. However, prolonging treatment may decrease the rate of late toxicity at the expense of disease control.


The shortest non-toxic course of stereotactic body radiotherapy (SBRT) was studied to determine whether it was possible to achieve the same rate of local control seen with longer protocols. The researchers found that short-course SBRT with cetuximab was an effective salvage treatment and had a good response rate in select patients. They also observed that the rate of acute toxicities was acceptable and the treatment was feasible if appropriate care was taken to limiting the amount of radiotherapy to critical structures.


Treating peritoneal carcinomatosis due to colorectal cancer is controversial, so researchers evaluated long-term outcomes following intraperitoneal chemotherapy (IPC) and cytoreductive surgery (CRS) to determine the prognostic factors related to cure. Following complete CRS of colorectal peritoneal carcinomatosis, followed by IPC, a 16% cure rate was observed in select patients, a rate similar to that seen in resection of colorectal liver metastases.


Talimogene laherparepvec was compared with granulocyte macrophage colony-stimulating factor in study patients with unresected stage 3B to 4 melanoma. Study patients assigned to talimogene laherparepvec tolerated it well and had longer rates of median overall survival; this finding was particularly true for untreated patients or those with stage 3B, 3C, or IV M1a disease.

Thompson JF, Agarwala SS, Smithers BM, et al. Phase 2 study of intralesional PV-10 in refrac-
The safety and efficacy of intralesional rose bengal were studied in 80 patients with refractory cutaneous or subcutaneous metastatic melanoma. Patients treated with intralesional rose bengal were observed to have durable local control and high rates of complete response. Therefore, the researchers concluded that this intralesional approach for local disease control could be used to complement investigational and currently used treatments for melanoma.


Researchers reviewed the data from retrospective clinical trials to determine the effect of liver resection and radiofrequency ablation on colorectal cancer liver metastases. They found that liver resection was superior to open surgery, laparoscopic approach, or radiofrequency ablation. This finding still held true when the tumor size was smaller than 3 cm.


Whenever feasible, salvage surgery is the curative method of choice for head and neck squamous cell carcinomas. Physicians should advise patients at high risk for local recurrence that reirradiation following surgery may increase locoregional control, but that this will come with higher rates of toxicity and no survival advantage when compared with salvage surgery without reirradiation. Patients should also be counseled that an increased risk of serious toxicity and impaired quality of life are associated with retreatment efforts.
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