

Unique Patterns of Metastases in Common and Rare Types of Malignancy

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This review on the unique patterns of metastases by common and rare types of cancer addresses regional lymphatic metastases but also demonstrates general principles by consideration of vital organ metastases. These general features of successfully treated metastases are relationships to basic biological behavior as illustrated by disease-free interval, organ-specific behavior, oligo-metastatic presentation, genetic control of the metastatic pattern, careful selection of patients for surgical resection, and the necessity of complete resection of the few patients eligible for long-term survival after resection of vital organ metastasis. Lymph node metastases, while illustrating these general features, are not related to overall survival because lymph node metastases themselves do not destroy a vital organ function, and therefore have no causal relationship to overall survival. When a cancer cell spreads to a regional lymph node, does it also simultaneously spread to the systemic site or sites? Alternatively, does the cancer spread to the regional lymph node first and then it subsequently spreads to the distant site(s) after an incubation period of growth in the lymph node? Of course, if the cancer is in its incubation stage in the lymph node, then removal of the lymph node in the majority of cases with cancer cells may be curative. The data from the sentinel lymph node era, particularly in melanoma and breast cancer, is consistent with the spectrum theory of cancer progression to the sentinel lymph node in the majority of cases prior to distant metastasis. Perhaps, different subsets of cancer may be better defined with relevant biomarkers so that mechanisms of metastasis can be more accurately defined on a molecular and genomic level.

J. Surg. Oncol. 2011;103:607–614. © 2011 Wiley-Liss, Inc.

KEY WORDS: metastases; patterns; cancer

INTRODUCTION

Blake Cady and Stanley Leong

This session of the 3rd International Symposium on Cancer Metastases and the Lymphovascular System included discussions of regional lymphatic metastases in pancreas, thyroid cancers, and sarcomas, as well as distant metastases in stage IV melanoma and colorectal cancers. The role of sentinel lymph nodes in solid cancers will be addressed in a separate review [1].

This array of discussions of metastatic disease illustrated and emphasized several generic aspects of the metastatic process and the results of therapy of metastases. The presence of regional lymph node metastases varies from extremely common in some cancers (low risk, young differential thyroid cancer) to infrequent or rare in others (soft tissue sarcomas: STS). In an overall sense considering survival, there is a disconnection between overall survival and lymph node metastases as illustrated by the two situations noted above: low-risk papillary cancers have regional lymph node metastases in up to 75% of cases, and up to 60% in incidental microscopic primary cancers, yet have a 98% disease-free 20-year survival, while soft tissue sarcomas seldom have regional lymph node metastases, and when they do, have only a 50% 5-year survival.

Literature reviews of prospective randomized trials and high quality retrospective studies indicate no survival differences comparing a variety of regional lymph node therapies, from extra radical surgical resections, to modified resections, to limited resections, to just sentinel node biopsies, to observation only, without any surgical resection [2].

PANCREATIC CANCER

Eric Nakakura

Eric Nakakura illustrated this finding in pancreatic cancers, where randomized trials comparing greater or lesser regional node resections failed to demonstrate any survival difference. Pancreatic cancer is the fourth leading cause of cancer deaths in the United States [3]. In 2009, there were an estimated 42,470 new patients diagnosed with pancreatic cancer and 35,240 deaths in the US [3]. Overall, the 5-year survival after diagnosis is <5% [4]. With such grim statistics, one cannot help but ask a few questions. Why is pancreatic cancer so deadly? Is long-term survival possible? What can be done to improve outcome?

For some insight, it is helpful to examine the anatomy of the pancreas. The pancreas is located in the retroperitoneum. Therefore,

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Received 15 November 2010; Accepted 15 November 2010

DOI 10.1002/jso.21841

Published online in Wiley Online Library (wileyonlinelibrary.com).

unless a patient develops painless jaundice from a neoplasm located in the pancreas head, most experience non-specific symptoms until they are diagnosed with advanced disease. Consequently, fewer than 20% of patients are diagnosed with localized disease amenable to potential curative resection [5].

The lymphatic, vascular, and nervous systems of the pancreas are especially rich and complex, providing many potential means of metastasis. Pancreatic cancer spreads to regional lymph nodes located near the gland but can also spread directly to lymph nodes further back in the retroperitoneum around the aorta and inferior vena cava [6]. Venous invasion via the portal vein leads to liver metastases. Spread along the pancreas results in invasion of adjacent organs and structures and often causes carcinomatosis. Perineural invasion causes spread to nerve plexi in the retroperitoneum, resulting in unremitting abdominal and back pain.

How common are these modes of spread? Insight into the frequency of the various means by which pancreatic cancer spreads can be gleaned from a large study from The Johns Hopkins Hospital. Examination of over 1,400 patients who underwent pancreaticoduodenectomy for pancreatic cancer revealed that 78% had lymph node metastases [7]. Approximately 50% of patients had vascular invasion, and 90% had perineural invasion. Over 40% of patients had a positive resection margin, which reflects, in part, continuous local spread. Strikingly, this large series shows that even in a carefully selected group of patients who underwent potentially curative surgery for pancreatic cancer, the majority exhibit multiple routes of spread. This underscores the unique anatomy of the pancreas and also the aggressive biology of pancreatic cancer.

Given these findings, is long-term survival after resection of pancreatic cancer possible? Several large series have provided some insight into this question. At MD Anderson, where most patients receive adjuvant therapy, often in a neo-adjuvant fashion, a 27% rate of actual 5-year survivors was recently reported [8]. This is not actuarial data but actual 5-year survival data. Other high-volume institutions have also reported actual 5-year survival rates ranging from 12 to 17% [9–11]. It is important to note that 5-year survival does not mean cure. Approximately half of patients who survive 5-years will be alive 10-years after surgery [8–11].

Because pancreatic cancer frequently spreads to regional lymph nodes, can we improve outcome by performing a more extensive surgical resection? Numerous groups have compared a standard pancreaticoduodenectomy with one in which a more extensive lymphadenectomy is performed—removing the lymph nodes deep in the retroperitoneum. The results from four randomized controlled trials found no difference in survival in patients who underwent an extended lymphadenectomy versus standard lymphadenectomy [12–16]. Moreover, patients may experience an impaired quality of life following extended lymphadenectomy. Therefore, a standard pancreaticoduodenectomy is recommended for patients with pancreatic cancer located in the head of the gland [17].

Is there a role for resection of pancreatic cancer liver metastases? An analysis of over 1,450 patients who underwent hepatic resection for non-colorectal non-endocrine liver metastases included 41 patients with an exocrine pancreatic primary [18]. Surprisingly, patients with pancreatic adenocarcinoma had a 20% 5-year survival after hepatic resection. For the entire study cohort, factors associated with a poor prognosis included non-breast origin, disease-free interval <12 months, and age >60 years. The results of this study must be interpreted cautiously. From this study, it is unclear whether hepatic resection influenced the outcome or whether this highly select group of patients had favorable biology and would have had the same outcome regardless of hepatectomy. Another study evaluated the outcome, following resection of synchronous pancreatic cancer liver metastases in 17 patients [19]. Median survival was only 5.9 months. Therefore, resection of synchronous liver metastases from pancreatic cancer does not appear to be

warranted. The role of resection of metachronous pancreatic cancer liver metastases remains ill defined and might only be considered after careful multidisciplinary assessment and when the disease is responding to systemic therapy [18].

What are important prognostic factors for patients after pancreaticoduodenectomy for pancreatic cancer? On multivariate analysis, important predictors of survival include tumor size <3 cm, negative lymph node status, negative resection margin, and well- or moderately-differentiated histology [7]. In a report from Johns Hopkins, only 4% of patients had all four favorable features, and the median survival for this select group of patients was 44 months, with an estimated 43% 5-year survival [7]. Clearly, we would like to diagnose patients earlier with these favorable features because they appear to do the best after surgery.

How many lymph nodes need to be assessed in order to be certain that a patient has no lymph node involvement? An analysis of the SEER national cancer registry revealed that a lymph node cut-point of 15 was associated with the most significant survival difference [20]. Analyzed in another way, the SEER data showed that after evaluation of 15 lymph nodes, approximately 90% of patients with single-positive-node disease would be identified. Therefore, it appears that 15 lymph nodes should be evaluated to accurately stage patients after pancreaticoduodenectomy for pancreatic cancer.

The overall outcome for patients with pancreatic cancer is dismal because most are diagnosed with advanced disease. Long-term survival is possible in patients after resection of pancreatic cancer in select patients with favorable pathological features. Patients with early-stage disease need proper evaluation, so that they can receive appropriate treatment that can extend their survival. Improvements in the understanding of pancreatic cancer biology are needed to develop more effective systemic therapies, which are needed to improve the outcomes for patients with advanced disease and to extend the lives of patients after pancreatic resection.

COMMENT BY BLAKE CADY

How these data and observations can be reconciled to the near universal acceptance of surgical resection of adjacent regional lymph nodes, with or without metastases, was one unspoken underlying question of this session.

THYROID CANCER

Orlo Clark

Orlo Clark reviewed the extensive literature in thyroid cancer [21] and noted not only the extremely high incidence of lymph node metastases in differentiated papillary cancer of young patients, but also cited ongoing controversies regarding their surgical management. Follicular thyroid cancers have few lymph node metastases, but a worse prognosis than papillary cancers, again illustrating a disconnection between regional nodal metastases and survival. In high risk thyroid cancer patients, particularly older patients, the usual poorer prognosis and outcome in the presence of increasing numbers of node metastases is noted, as also occurs in medullary thyroid cancers (of "C" cell, not follicular cell origin). But in the unique papillary cancers of young patients, including children, where node metastases are common and usually multiple, frequently more than 10, yet death is rare (1%), that expected prognostic relationship between numbers of positive nodes and survival is absent. In almost all of the established risk groups in differentiated thyroid cancer (AGES, AMES, MACIS, UICOC, TNM/AJCC) lymph node metastases are not found to be a prognostic factor, and emphasizes this unique feature; only age, size, extent of primary cancer, and a few uncommon special pathologic varieties are controlling features for survival. Even in low risk patients who present with recurrences (only about 10% recur), two-thirds of all recurrences are regional

nodal metastases, which have a minimal adverse effect on overall survival. However, such patients have a more uncertain long-term course.

SOFT TISSUE SARCOMAS

Raphael Pollock

Raphael Pollock described the unique molecular aspects of STS and nodal metastases, noting their relationship to VEGF-C and the blocking of VEGF-C effect by other molecular factors in STS that are the probable mechanism of the lack of regional nodal metastases, and the differences in VEGF-C function from breast cancers where regional nodal metastases are common. One of the intriguing features of sarcoma biology is the reality that these malignancies strongly favor hematogenous as compared to lymphatic pathways of dissemination, the latter only infrequently occurring in several soft tissue sarcoma histologies including epithelioid sarcoma, clear cell sarcoma, angiosarcoma, rhabdomyosarcoma, and synovial sarcoma. In these histologic subtypes lymphatic metastasis has a reported incidence of 10–40%, whereas overall lymphatic spread accounts for only approximately 5% of all sarcoma metastases. Of interest, the question remains open as to why hematogenous dissemination is a much more common means of sarcoma dissemination as compared to the much more frequent lymphatic pattern of spread observed in epithelial-origin malignancy.

It is known that lymphatic metastasis is a multi-step process requiring initial peri-tumoral lymphangiogenesis, invasion, transport, tumor homing, and subsequent proliferation of tumor within lymph nodes. Recent evidence suggests that this cascade of events depends on tumor-stroma crosstalk; within this context it is known that VEGF-C, a ligand of the VEGFr-3 receptor, is the major promoter of intra- and peri-tumoral lymphangiogenesis and also drives proliferation and migration of cultured lymphatic cells. The key role of VEGF-C in lymphangiogenesis is also suggested by the finding that VEGF-C null mouse embryos lack lymphatic vasculature, whereas genetically engineered murine preparations over-expressing VEGF-C exhibit lymphatic vessel hyperplasia. In addition, there is a known correlation between the concentration of primary tumor VEGF-C, lymphatic vessel number, and regional lymphatic metastasis in breast, gastric, colon, prostate, pancreas, head and neck, lung, and esophageal carcinomas as well as malignant melanoma.

Given the central role of VEGF-C and VEGFr-3 in lymphangiogenesis and lymphatic metastasis, we have been interested in determining whether or not VEGF-C expression and function might be decreased in soft tissue sarcoma as a possible explanation for the infrequency of soft tissue sarcoma spread via the lymphatic system. To address this question, soft tissue sarcoma cell lines and tumor tissues were assembled and then used in a series of experiments directed at answering this essential query. We began by screening a large panel of soft tissue sarcoma cell lines and tumor tissues and were able to demonstrate that these soft tissue sarcoma biologicals both express VEGF-C mRNA and VEGF-C protein relative to either control cell lines or autologous normal tissue. In addition, it was possible to inhibit soft tissue sarcoma VEGF-C mRNA and protein production in sarcoma cell lines using anti-VEGF-C siRNA knock down.

These findings suggest that the lack of sarcoma lymphatic spread could not be due to the lack of VEGF-C production in that soft tissue sarcomas express VEGF-C mRNA and produce VEGF-C protein, a process that could be selectively and specifically inhibited using siRNA. It was therefore pertinent to next consider whether or not soft tissue sarcoma-secreted VEGF-C protein was fully functional. This was shown to be the case as demonstrated by the ability of sarcoma-generated VEGF-C to support the growth of human lung-origin as well as human umbilical vein-origin endothelial cells. Likewise, conditioned medium from soft tissue sarcoma cells after VEGF-C siRNA

knockdown abrogated the growth of both types of endothelial cells. Moreover, soft tissue sarcoma-secreted VEGF-C protein was also demonstrably functional in its support of endothelial cell migration, whereas conditioned medium from sarcoma cells after VEGF-C siRNA knock down inhibited endothelial cell migration relative to untreated controls.

Next we considered the possible impact of functional sarcoma-secreted VEGF-C protein on lymph-angiogenesis as assessed by tabulating peri-tumoral lymphatic vascular structure formation. In comparison to breast carcinomas there was significantly decreased number and concentration of peri-tumoral lymphatic vascular structures observable in soft tissue sarcoma tumor tissues. Moreover, lymph nodes derived from sarcomas without lymphatic metastasis compared to sarcomas that were positive for nodal spread had fewer and less concentrated peri-tumoral lymphatic vascular structures. However, many of the lymph node metastasis negative sarcomas had lymphatic vessels counts and concentrations well above the median value observed in lymph node metastasis positive sarcomas, suggesting that lymphatic structure number or concentration per se was not associated with the presence of lymphatic dissemination.

While the VEGF-C/VEGFr-3 axis appears to be the major driver of epithelial-origin tumor lymphangiogenesis, a different mechanism appears to be at play in mesenchymal-origin soft tissue sarcoma. In addition, while both soft tissue sarcoma tumors and cell lines express VEGF-C mRNA and produce functionally active VEGF-C protein, and while soft tissue sarcoma tumors demonstrate intra- and peri-tumoral lymphatic vessels, the presence of this lymphangiogenesis machinery may be necessary but is clearly not sufficient to facilitate the soft tissue sarcoma lymphatic metastatic process when it occurs [22]. In the future, it may be possible to identify specific chemokine receptors such as CCR-7, a chemokine receptor that participates in melanoma lymph node lymphatic homing, as being involved in soft tissue sarcoma metastatic biology, especially in histologic subtypes prone to lymphatic dissemination. Such chemokine receptors, if relevant, might be targetable using yet to be developed “cutting edge” molecular therapeutics, hopefully to aid in tumor control for those sarcoma patients most likely to benefit by such novel molecular biological-based approaches.

COMMENT BY BLAKE CADY

Thus, Pollock implied that through specific molecular factors, there is organ specificity of nodal metastases, and suggested the genetically controlled processes that lead to the frequency, or infrequency of this particular organ-specific phenomena.

Blake Cady pointed out the emerging clinical and laboratory evidence for patterns of organ specificity of all metastatic disease, and thus the rationale for the lack of causal relationship between regional nodal metastases therapies and overall survival. Since it is clear that varieties of therapy of regional nodal metastases do not alter overall survival, the conclusion is inescapable that lymph node metastases fill a role different from vital organ metastases, for instance, yet may have similar mechanisms of metastatic cell behavior. Since regional nodal metastases, or any nodal metastases, do not cause a vital organ failure, as do liver, lung or brain metastases, variations of therapy do not control outcome; they may be statistically related as prognostic indicators in most cancers, but do not have a causal relationship to survival. That point has been illustrated by the differences between STS and low-risk thyroid cancers.

METASTATIC CANCER TO THE LIVER

Michael Choti

This theme was continued by Michael Choti who described liver metastases from colorectal cancers, and noted the good survival rate

following liver resection. Patients with improved outcomes following resection are marked by a longer disease-free interval, limited number of metastatic sites (oligo-metastatic disease), low CEA levels (another biological indicator of aggressiveness), and lack of non-hepatic metastases (evidence of liver organ-specific behavior). In addition, patients whose tumors respond to pre-operative systemic chemotherapy have an improved long-term outcome following resection.

Cancer of the colon and rectum is the third most commonly diagnosed cancer in the United States [23]. In spite of an increasing emphasis on diagnosis and therapeutics, more than 40% of patients with colorectal cancer eventually die from their cancer [23,24]. Among those patients with advanced disease, more than half will develop liver metastases, more than any other organ, and many will have disease recognizably confined to this organ [25].

Although it is now uncommon not to treat this form of metastatic colorectal cancer, several earlier studies have reported the natural history of patients untreated for liver metastases [26–28]. Although the survival of such patients was quite variable, only about 20% survived 3 years, and few lived beyond 5 years from the time of the detection of the liver metastases. It is clear from these reports that survival time in these patients is related to the extent of liver involvement. The median survival of untreated patients with a solitary hepatic metastasis was approximately 17 months compared to <6 months in those with multiple site, bilateral disease [27].

A multitude of studies have demonstrated that complete resection in patients with hepatic colorectal metastases is associated with dramatically improved survival compared with patients not undergoing surgical therapy [29–33]. Advances in imaging technology, surgical techniques, and systemic chemotherapy have brought steady improvements in long-term outcome in patients undergoing resection, with 5-year overall survival currently exceeding 50% [34–37]. In addition, other surgically delivered locoregional strategies offer promising directions in improving outcomes in these patients, including ablation, and intrarterial therapies [34–39].

The reason for the propensity for colon cancer metastases to develop in the liver is unclear. In the past, many postulated that mechanical and anatomic factors related to direct portal seeding from colon to liver as well as the unique features of hepatic blood flow contribute to this metastatic pattern [40,41]. In fact, attempts have been made to capitalize on the unique feature of direct portal blood flow from the large bowel to the liver by designing clinical trials treat the liver through direct intraportal delivery of adjuvant chemotherapy [42–44]. To date, such approaches have not been shown to be advantageous over intravenous or oral drug delivery, nor was the pattern of recurrence within the liver altered [43].

Other factors, including features of the host micro-environment, microcirculation [45–48], organ-specific growth factors, [49] and host immunologic factors [50,51] have been implicated in the mechanism for liver-predominant spread. More recently, molecular studies have shown that the circulating cancer cells may predispose to the development of liver metastases [52–54]. These include processes related to tumor cell invasion [55,56], migration, adhesion [57], extravasation [57–59], angiogenesis [60,61], growth, and survival [50] within the liver environment.

Understanding the mechanisms by which metastases develop within the liver is clinically important. Elucidating of the biology of metastatic phenotype may provide useful insights into targeted drug development and more patient-specific therapies. Predicting the pattern of recurrence in specific patients may allow the identification of tumors at high risk for the development of hepatic metastases, perhaps identifying those who may benefit from liver directed therapy of metastatic disease. Liver-directed adjuvant therapies and organ-specific screening/surveillance might be considered in those identified to have high risk of developing recurrence specifically within the liver.

COMMENT BY BLAKE CADY

These selection criteria point out the necessity of careful selection of appropriate biological and technical factors, which in turn emphasize the distant organ site specificity of metastatic disease in the “pure culture” of favorable features of vital organ metastases that allow survival after resection. Certain subgroup of melanoma patients with distant metastasis may be benefitted by surgical resections with an average 5-year overall survival of about 20% [62–75]. This theme of a highly selective approach to distant metastases was again emphasized by Don Morton in describing his trial of surgical therapy of stage IV melanoma, to be compared to non-surgical therapies. His previous trial of surgical resection plus Bacille Calmette-Guerin (BCG) vaccine revealed an impressive 40% 5-year survival utilizing strict selection criteria for surgical resection plus BCG and either vaccine or placebo.

SURGICAL VERSUS MEDICAL APPROACHES FOR INITIAL TREATMENT OF METASTATIC MELANOMA

Donald Morton

In 2009, an estimated 8,650 Americans died from melanoma. Most of these patients had distant metastases, for which there is no standard, highly effective treatment regimen. Unfortunately, the roles of surgical versus medical therapies remain controversial. Resection is considered efficacious for selected patients with solitary lesions, [62–75] but some oncologists prefer initial systemic therapy because many patients will develop multiple additional metastatic sites shortly after initial surgical resection. Our ongoing phase III comparative efficacy trial will determine the best initial management approach for distant melanoma metastases.

This multicenter trial compares surgery plus BCG immunotherapy versus best medical therapy for patients whose stage IV melanoma is judged to be completely resectable. Because there is no consensus among medical oncologists as to optimum therapy for melanoma metastatic to a distant site, best medical therapy for stage IV melanoma is defined by the treating physician and may include clinical trial protocols of new agents or standard non-protocol treatments.

As initially designed, this trial included a surgery-alone arm, but the two-arm design is easier for patients to understand and statistically more efficient. BCG adjuvant therapy is included in the surgery arm because of the very favorable overall survival in our recent phase III trial of postoperative adjuvant immunotherapy for resected stage IV melanoma (5-year rate >40%, as compared with <5% for the general stage IV population). All patients in this trial received either BCG plus placebo or BCG plus melanoma vaccine. The current phase III trial will test our hypothesis that surgical resection plus BCG immunotherapy is a clinically important initial option for distant metastatic melanoma. The study begins with screening to determine which patients with stage IV melanoma are candidates for complete surgical resection. These patients are then randomized to one of the two treatment arms. Although the study is not a cross-over design, patients whose melanoma progresses on best medical therapy may switch to a different medical therapy or, if appropriate, undergo delayed surgical resection. Similarly, patients whose melanoma recurs after resection may undergo additional resection or medical therapy.

A total of 330 patients with resectable stage IV melanoma will be enrolled; an estimated 10% dropout rate will leave 300 evaluable patients. The study is open to patients with metastases in no more than three visceral organs and with no more than six separate metastases. Patients with brain or bone metastases are not eligible. All patients will be followed for clinical and correlative endpoints, including overall survival (primary endpoint), melanoma-specific survival, progression-

free survival in regard to the initial metastatic sites and time to the development of additional metastatic sites. More than 30 sites in the United States and abroad are planning to participate. Accrual is proceeding as planned and the study is expected to last 4–5 years.

COMMENT BY BLAKE CADY

David Henner, Anita Lal, and Raji Pillai described a unique microarray gene-expression test to define the likely primary source of distant metastases in patients without a clinically discernible organ origin.

METASTATIC CANCER WITHOUT A KNOWN PRIMARY

David Henner, Anita Lal, and Raji Pillai

Metastatic cancer patients without an identifiable primary tumor constitute 3–5% of all new cancer cases [76]. These patients frequently undergo an exhaustive clinical workup to determine the tissue of origin of their cancers including diagnostic imaging tests, serum tumor marker assays and immunohistochemical (IHC) stains for specific tumor sites. Knowledge of the primary site is important because it significantly impacts prognosis and clinical management of patients, anticancer drug choices, and entry criteria for clinical trials [77,78]. Most oncologists use the primary site as the basis for standard-of-care patient management. Patients with unknown primaries receive generic platinum and/or taxane-based chemotherapy. Response rates to this treatment range from 13 to 39% and prognosis remains poor with median survival ranging from 7 to 10 months [76,79]. Clinical trials with newer targeted chemotherapies such as bevacizumab and erlotinib have shown only modest increases in survival for these patients [80].

The emergence of a variety of targeted tissue-specific therapies that are highly efficacious suggests that an accurate diagnosis of the primary site would lead to improved clinical outcomes. In addition, it would result in less exposure to toxic/ineffective chemotherapies, and increase the quality of life for these patients. Consistent with this hypothesis are the observations that carcinoma of unknown primary (CUP) patients with colon identified as the most likely primary site had better response to colon cancer-specific treatment regimens than they did to empiric therapy and, consequently, improved clinical outcomes [81,82]. IHC is often successful in discriminating between two or three tissue types [77,83] and can narrow the range of diagnostic possibilities [84,85]. However, antibody panels are less useful when a wide range of potential primary sites are being considered lacking the required range, sensitivity and specificity [83,86,87]. Validation of antibodies is expensive, time consuming and often performed inconsistently. Interpretation and reporting of IHC results are also highly subjective. For metastatic samples, IHC panels are able to correctly identify the primary tissue in only about two-thirds of blinded specimens [88]. Thus, there is an increasing need to identify the correct primary site of metastatic tumors in order to select the optimal targeted therapy and current methods are insufficient. Molecular assays that can accurately identify the tissue of origin of metastatic cancers have the potential to fill this unmet need.

The feasibility of using gene expression profiling with DNA microarray to classify cancers according to their primary sites has been established by several studies [89–92]. In addition, advances in gene annotation and array design along with the use of standardized protocols and array platforms across laboratories have made microarray-based gene expression profiling reproducible and reliable [93–96]. These studies and advances have allowed the development of commercial molecular diagnostic tests that identify the tissue of origin of cancers of unknown primary.

The Pathwork Tissue of Origin Test (Pathwork Diagnostics, Redwood City, CA) is a microarray-based gene expression test that aids in the diagnosis of primary site for metastatic, poorly differentiated,

and undifferentiated tumors. The Tissue of Origin Test measures the gene expression of 2,000 genes in formalin-fixed paraffin embedded (FFPE) specimens and assesses the degree of similarity between the tumor's expression pattern and a panel of 15 different tissue types. The tissue types represented are: bladder, breast, colorectal, gastric, testicular germ cell, hepatocellular, kidney, non-small cell lung, non-Hodgkin's lymphoma, melanoma, ovarian, pancreatic, prostate, sarcoma, and thyroid. This panel represents approximately 90% of all solid tumors and 58 tumor morphologies overall.

The Tissue of Origin Test Result is a set of similarity scores for each of the 15 tissue types included in the Tissue of Origin test panel. The similarity score is a measure of the similarity of the gene expression profile of the tumor specimen to the profile of the indicated tissue, ranging from 0 (very low similarity) to 100 (very high similarity). Similarity scores for all 15 tissues sum to 100. In the validation studies for the Tissue of Origin Test performance of the test was measured as the percent agreement of the tissue with the highest similarity score with the known primary site.

Rigorous validation studies on metastatic and poorly differentiated primary tumor specimens have proven the Tissue of Origin Test to be robust and highly accurate. In 462 FFPE metastatic or poorly differentiated specimens with known primary sites, the test demonstrated 88.5% positive percent agreement (akin to sensitivity) with available diagnosis, and >99% negative percent agreement (akin to specificity) [97]. Although not a direct comparison, this rate of agreement of the Tissue of Origin Test is superior to the 66% agreement seen in similar blinded studies using IHC to identify the primary site [88]. In addition, any tissue with a similarity score of ≤ 5 has a 99.8% probability of not being the tissue of origin and is excluded as a potential primary site. The Tissue of Origin Test also has high reproducibility, showing an overall laboratory to laboratory concordance of 89.3% in three different laboratory settings. A version of the Tissue of Origin Test that utilizes frozen tumor specimens as the starting material had an accuracy of 87.8% in a set of 547 frozen specimens and delivered reproducible results (93.8% concordance) in four different laboratory settings [98,99]. Thus, the Pathwork Tissue of Origin Test is an independent and highly accurate method to assess the most likely tissue of origin of metastatic tumors. It can be particularly useful in situations in which the diagnosis of tissue of origin is highly uncertain or even after the workup may have still have two or more diagnoses on the differential. The Tissue of Origin Test can be used as an independent method to resolve these cases, allowing the oncologist to select therapy optimal for that tissue type. As the number and efficacy of highly targeted and tissue-specific therapies increase, the importance of correct diagnosis of tissue of origin for metastatic tumors will also increase. In summary, the Pathwork Tissue of Origin Test predicts a tumor's identity through its gene expression profile. This information is complementary to IHC and may facilitate use of standard-of-care, tumor-specific treatments.

COMMENT BY BLAKE CADY AND STANLEY LEONG

The above-described test lent support to the concept of organ-specific metastases as well as the role of oligometastases, that are associated with better survival by the resultant selection of appropriate systemic therapy. The linkage of specific genetic patterns and occult primary cancers illustrate, in a different way, the session theme of understanding metastatic behavior, and their organ site specificity.

This session on "Unique Patterns of Metastases by Common and Rare Types of Malignancy" thus particularly addressed regional lymphatic metastases but also illustrated general principles by consideration of vital organ metastases. These general features of successfully treated metastases are relationships to basic biological behavior as illustrated by disease-free interval, organ-specific behavior, oligo metastatic presentation, genetic control of the metastatic pattern, careful selection of

patients for surgical resection, and the necessity of complete resection of the few patients eligible for long-term survival after vital organ metastasis resection. Lymph node metastases, while illustrating these general features, are not related to overall survival because lymph node metastases themselves do not destroy a vital organ function, and therefore have no causal relationship to overall survival. On the other hand, nodal status is an excellent prognosticator for patient outcome. Removal of negative lymph nodes in elective lymph node dissection certainly will have no survival benefit. Likewise, removal of positive nodes while the disease has spread beyond the nodal basin has no therapeutic impact. It still remains to be determined if survival is affected by the removal of microscopic disease in the sentinel lymph node or regional lymph node if microscopic disease is the only disease remaining after resection of the primary cancer.

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