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Roadblocks to translational advances on metastasis research

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Abstract

Promising advances in cancer therapy stemming from an increasing understanding of the molecular and genetic underpinnings of the tumorigenic process have been fueled by a strong, determined scientific community, influential patient advocacy groups and committed funding bodies. Despite these efforts, the development of effective drugs to prevent systemic dissemination of cancer cells or to eliminate overt metastasis in secondary organs remains a challenge to both researchers and physicians. In an attempt to tackle the most relevant and timely translational issues, a meeting held in 2012 as a result of a successful partnership between the Volkswagen Foundation and *Nature Medicine* brought together a group of metastasis research experts to identify the most important hurdles and help create a framework for potential clinical and translational strategies.

Metastasis-driving concepts: plasticity or aberrant genetics

Metastasis is responsible for more than 90% of cancer-associated mortality; thus, the clinical need to prevent or target metastasis is high. Without a doubt, the stepwise accumulation of alterations in oncogenes and tumor suppressor genes is a major driver of malignant progression toward the colonization of distant tissues and formation of macrometastasis[1], as initially shown for colorectal cancer[2]. However, this alone cannot explain important clinical observations.

Many solid cancers, such as the very common adenocarcinomas, show a plastic phenotype with a differentiated primary tumor mass and undifferentiated areas particularly at the invasive front, but, strikingly, re-differentiated metastases[3]. Therefore, an additional view has arisen within the last decade in which an exceptional phenotypic and functional plasticity of cancer cells can trigger metastasis[4,5]. Thereby, it is not the fixation in an

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aggressive phenotype that favors metastatic progression but rather the aberrant ability of at least subgroups of cancer cells to switch from one state to the other, allowing permanent adaptations to the demanding conditions. This supports the role of a strong regulatory input from the changing tumor environment in addition to endogenous and irreversible genetic alterations.

Two alternative concepts were in line with aberrant cellular plasticity in metastasis. First, cancers can be hierarchically organized with undifferentiated cancer stem cells (CSCs) as tumor and metastasis-initiating cells, which self-renew, proliferate and differentiate, thereby building up the differentiated main mass of both the primary tumor and metastases[6]. And second, cancer cells can activate the embryonic epithelial-to-mesenchymal transition (EMT) program, inducing de-differentiation and dissemination from the primary tumor. Later a re-differentiation, or mesenchymal-to-epithelial transition (MET), is necessary for colonization and growth of metastases. Thus, in the second concept, rounds of EMT-MET processes drive metastasis[3,5,7]. Both notions were linked in a model in which migrating cancer stem cells form the basis of metastasis[8], and by showing that EMT inducers can also confer 'stemness'[9]. A combined EMT and stemness phenotype both triggers motility and dissemination and allows export of the tumor initiating capacity to distant sites. Thus, the function and characteristics of invading and particularly circulating tumor cells (CTCs) and disseminating tumor cells (DTCs) are central for metastasis[10].

In this context, key questions on metastasis biology must be answered: Do metastasis-initiating CSCs and CTCs exist in patients? Do CTCs (or a subfraction of CTCs) exert an EMT phenotype or stemness? If so, is it linked to quiescence or dormancy and therefore one reason for long-term latency[11]? It is also unclear as to what niche keeps CSCs—and potentially CTCs—in an EMT and stem cell–like state and whether this phenotype in the case of CTCs is associated with therapy resistance (as already shown for CSCs) and therefore may be a source for tumor recurrence and metastasis. Finally, although the CSC and EMT concepts probably explain the cellular plasticity of metastastic cells, other possibly related mechanisms may also account for this flexible behavior, such as the existence of semistable epigenetic states[12].

Although clearly detectable in human cancer[3] and validated in experimental models[13-15], it is not fully clear why metastases often revert to a differentiated, or MET, phenotype. One reason could be that the quiescence associated with EMT and stemness requires a reversion to a differentiated, growth-associated phenotype to colonize and form metastases[3,13]. Again key questions remain to be answered. First, is re-differentiation (MET) the rate-limiting step in metastasis? What environmental signals trigger it at the distant site? It is still unknown whether such signals are key determinants of a metastatic niche, whether they are different depending on the tumor type (and therefore involved in the organ specificity of metastases) and whether they may be potential therapeutic targets to prevent colonization and metastasis.

Is there a way to bring both postulated driving forces of metastatic progression—genetic alterations and cellular plasticity—together? Evidence suggests that basic genetic alterations precede aberrant stemness maintenance and enhanced response to EMT-inducing signals, thereby paving the way for extensive cellular plasticity. Therefore, both driving forces may act in parallel and in an overlapping way on the route to metastasis. Cellular plasticity, triggered by the changing tumor environment, may be predominant in common well to moderately differentiated carcinomas (plasticity-driven metastasis). In contrast, accumulating genetic alterations with less signs of cellular plasticity may prevail in undifferentiated, highly metastatic tumors (genetic-driven metastasis)[4], such as triple-negative breast cancers and various anaplastic cancers, which induce an early and fast metastatic progression.

These concepts offer new treatment options to prevent or fight metastasis, but they also uncover potential translational hurdles[4], as the postulated different routes of metastatic progression may require different interference strategies. Which phase of the plasticity route toward re-differentiated metastasis is the best to target—the EMT and stemness state or the re-differentiated and MET phenotype? Cells with an EMT and stem-like phenotype in all stages of tumor progression (primary tumor cells, CTCs, DTCs, migrating cancer stem cells, metastases), which are probably the most therapy-resistant subtypes, might be the major obstacle in successful cancer treatment and, therefore, the key target to successfully fight metastasis. New drugs selectively targeting such cells are currently being developed. As an alternative strategy, differentiation-inducing agents, such as certain already available epigenetic drugs, may be used. Differentiation therapy might open a therapeutic window by shifting the EMT- and stemness-associated drug-resistance phenotype to a sensitivity phenotype and thus may resensitize the tumors to standard therapy. However, these drugs may risk awakening low-cycling, potentially dormant CTCs and DTCs to produce proliferating progeny with high colonizing capacity, raising the question of whether we should rather keep cells in or induce an EMT and stemness state to prevent colonization. Finally, does long-term chemotherapy shift a plasticity type to a genetic type of metastatic progression by selecting for genetic alterations generating a stable, highly aggressive, highly metastatic and drug resistant EMT and stem cell–like phenotype[16]? Different types of metastasis will require different treatment strategies, and a single target strategy will never be successful. Do we need to target all types of cancer cell subpopulations (cancer stem cells, CTCs, DTCs and differentiated cancer cells) at the same time? Such concerns underscore the importance of developing new combination therapies targeting different steps in the metastatic cascade and identifying new prognostic and predictive biomarkers for metastatic CSC and CTC subfractions to assess the risk for metastasis and to monitor treatment response. —*TB*

Deciphering the earliest phases of metastatic evolution

Clinical studies and treatment designs are based on the traditional view that tumor cells determine their own fate. This notion supports a linear progression model of tumorigenesis and metastasis in which established primary tumor growth, local invasion, intravasation, survival in the circulation, extravasation and micrometastatic and macroscopic metastasis at a distant organ site occur sequentially. Because clinicians consider metastasis a late-occurring event, cancer treatment strategies are skewed accordingly, and although radiation and chemotherapy yield little or no meaningful survival benefits for patients with metastatic cancer, they remain the standard treatment plan in this setting. Deciphering and understanding the complex set of events in the metastasis cascade will promote the development of truly effective therapies that block metastasis—the primary culprit in patient mortality. It is therefore crucial that the cancer research community focuses on identifying common pathways in metastasis, especially those in the early phases of metastatic progression.

Recent studies have challenged the concept of metastasis as a late step in cancer progression and have called into question the linear progression model for cancer metastasis, suggesting that the primary tumor and its metastases may evolve distinctly—that is, in parallel. In mice with spontaneously arising mammary tumors, Christoph Klein and his colleagues showed that mammary tumor cells disseminate to the bone marrow and undergo metastatic growth at distant sites, even before the earliest signs of hyperplasia within the mammary gland[17]. In addition, Harold Varmus and his colleagues have demonstrated that untransformed cells can bypass transformation at the primary tumor site after oncogenic induction, highlighting that metastasis can occur seemingly before the establishment of the primary tumor[18]. In patients with ductal carcinoma *in situ*, Simon A. Joose and Klaus Pantel have shown that

cytokeratin-positive tumor cells can be detected in the bone marrow[19]. Cancer cell migration to distant sites and the formation of favorable tumor microenvironments for future metastatic disease can therefore occur far earlier than previously thought. Ongoing research aimed at characterizing these early disseminated tumor cells will offer new insights into treatments that prevent their transit to and survival at distant metastatic sites.

Historically, cancer research has focused on the tumor cell itself with the assumption that intrinsic cellular and molecular events dictate metastatic pathways and the development of overt metastatic disease. But metastasis seems to be an 'inefficient' process when based solely on the direct effects of tumor cells; only a very small percentage of syngeneic tumor cells (0.01%) intravenously injected in animals will form metastatic lesions[20]. Thus, we must also consider an all-encompassing approach to find other common factors in early cancer metastasis. Long before tumor cells disseminate to future metastatic sites, tumor-secreted factors enter the circulation and prepare local and distant microenvironments to create a hospitable and suitable milieu for arriving metastatic tumor cells[21]. Notably, tumor-conditioned medium alone can enhance the metastatic potential of metastatic cells, as shown in mice treated with melanoma-conditioned medium before tail vein injection of melanoma cells[22]. Preconditioned medium promotes premetastatic niche formation, including bone marrow–derived progenitor cell recruitment, in multiple organs and substantially increases the metastatic burden compared to control animals treated with nonconditioned medium. The contribution of bone marrow progenitor cells at pre-metastatic sites is paramount for dictating metastatic progression. This observation highlights the role of tumor-derived factors in stromal cell recruitment and thus in creating a favorable metastatic microenvironment for metastatic initiation. But tumor-conditioned medium can also dictate metastatic organotropism. To this effect, injection of Lewis lung carcinoma cells (which normally metastasize to the lung) along with melanoma-conditioned medium endowed these cells with the capacity to colonize multiple organ sites commonly observed in melanoma metastasis. The circulating tumor cell may be merely an 'innocent bystander' that establishes a metastatic niche after adhering to a favorable microenvironment 'prepared' by tumor-secreted factors. Interestingly, these tumor-secreted factors also facilitate coagulation, vascular leakiness, hypoxia and the creation of an inflammatory milieu. Therefore, tumor-secreted factors have the power to influence the formation of premetastatic niches, which in turn determine not only the sites of metastasis but also the overall metastatic burden.

Of the tumor-secreted factors, growth factors and chemokines have been best studied as therapeutic targets in metastatic disease. Hal Dvorak, Judah Folkman and Napoleone Ferrara recognized early on that growth factors 'common' to nearly all cancers may exist. For instance, both metastatic and nonmetastatic tumors secrete the potently angiogenic vascular endothelial growth factor (VEGF); however, clinically targeting VEGF alone has proven challenging in human trials[23]. Combination therapy, where anti-VEGF drugs would be used in conjunction with 'common pathway' inhibitors that block other tumor-derived growth or secreted factors crucial to the metastatic process, may prove effective. In this vein, Albert Zlotnik and his colleagues showed that chemokine receptors expressed on breast tumor cells, such as CXCR4 and CCR7, and chemokine ligands in target organs, such as CXCL12 and CCL21 in lymph nodes and the lungs, promoted organ-specific metastasis[24]. Therefore, the specific chemokine expression pattern in future metastatic organs may help predict organotropic disease involvement.

In pre-metastatic niche studies, bone marrow–derived progenitor cells establish pre-metastatic sites before the arrival of tumor cells[22]. Tumor-derived particles, later identified as tumor-derived exosomes, were also detected at pre-metastastic sites in parallel with the arrival of bone marrow–derived cells[25]. Exosomes are 100-nm endoplasmic reticulum–derived microvesicles produced by myeloid cells as well as megakaryocytes and platelets,

and they are thought to maintain normal vascular homeostasis. Tumor-derived exosomes have been recently shown to mediate the cross-talk between tumor cells and tumor-associated stromal cells, explaining the existence of stromal-derived oncogenes. In fact, tumor-derived exosomes carry tumor protein and oncogenic cargo (c-MET oncoprotein) and can circulate and fuse with local stromal cells, such as with fibroblasts at pre-metastatic sites, as well as distal bone marrow–derived progenitor cells, 'educating' these cells to a provasculogenic and pro-metastatic phenotype. Tumor-derived exosomes are capable of initiating clot formation, vascular leakiness, extracellular matrix production, enzymatic activation, inflammation and bone marrow progenitor cell recruitment at distant organ sites, which together establish the pre-metastatic niche, contributing to metastasis initiation and formation of early metastatic lesions. Notably, as all tumor cells seem to secrete exosomes, there may be a common tumor-secreted factor worth exploring further as a possible effector of the metastatic cascade.

As tumor-derived exosomes are easily isolated from the blood of patients with cancer, they provide easy access to tumor-derived material carrying essential biomarkers for predicting metastatic potential and the risk of metastatic relapse. Exosome cargo will probably be unique to each type of tumor, influencing distinct cell types in the metastatic milieu and eliciting distinct responses (i.e., provasculogenic versus inflammatory responses) from bone marrow–derived cells. In addition, tumor-derived exosomes offer new opportunities for therapeutic drug design, as blocking exosome production or fusion with stromal cells may hinder pre-metastatic niche formation and metastasis development.

In mouse models, targeting angiogenic processes in macrometastatic lesions blocks the expansion of macrometastatic disease; however, it often promotes earlier events in the metastatic evolution, such as micrometastatic lesions and pre-metastatic niche formation, which are often more pathological and ultimately result in increased morbidity. New therapies targeted against tumor-secreted factors and the cells they educate, including receptors expressed on these cells that bind components enriched in metastatic niches, may lead to improved treatments that prevent pre-metastatic niche formation, as well as the homing to and engraftment of disseminated cells within these sites. Thus, to successfully treat macrometastatic disease, treatments must be tailored to target the earlier phases of metastasis[26]. The true challenge is to teach basic researchers and clinicians that vascular instability, hypoxia and inflammation contribute to not only the formation of late macrometastatic lesions but all stages of metastatic evolution, including those in earlier phases of metastatic progression. To improve his or her success in monitoring a patient for potential metastasis, the physician should recognize that the primary tumor is often well differentiated and therefore may not be predictive of metastatic potential or progression. Instead, the assessment of a patient with cancer should initially include examination of both blood and future metastatic organs, which may be predictable in certain cancers (for example, uveal melanoma has a propensity to metastasize to the liver, whereas osteogenic sarcoma invariably metastasizes to the lung). Therefore, clinicians should not wait for radiographic evidence of macrometastatic development at the predicted organ site of metastasis and only treat the macrometastatic disease; they should shift focus proactively to identify and target the earlier pre-metastatic and micrometastatic processes as well. Understanding the earliest phases of metastatic disease evolution through the evaluation of blood and future organ sites of metastasis may help decipher and perhaps conquer metastatic disease. —*DL*

The role of the microenvironment in metastasis

It is nearly 125 years since Paget enunciated the seed-and-soil hypothesis of cancer[27]. Yet, in the modern era of cancer research, the soil has been ignored, whereas oncogenes and

tumor suppressors of the seeds, namely cancer cells, have dominated the research agenda. But this dominance has changed over the past decade, as emerging data suggested that the cellular and extracellular matrix (ECM) microenvironments—both in the primary tumor and in metastatic sites—are crucial at multiple stages of metastasis[28,29]. Targeting the tumor microenvironment in metastasis might hold promise for therapy because stromal cells are not mutated and the effects may be widespread, as the ECM interacts with multiple tumor cells. Despite the potential of the tumor stroma as therapeutic target, studies on its composition and function in the metastatic process still lag behind studies on the tumor cells.

The first step in metastasis, which has been the focus of numerous studies, involves local invasion followed by entry into the circulation, a dissemination process considered unique to metastasis and an early event that may precede frank malignancy[30]. There is also interest, both clinically and from a basic science perspective, in circulating tumor cells; however, it is still unknown whether they predict metastatic potential, as even nonmalignant epithelial cells can be found in circulation in acute inflammation in patients lacking tumors[30-32]. Inflammation in the tumor microenvironment seems to have a major role in dissemination. Cytokines and growth factors from stromal immune cells and fibroblasts promote EMT of tumor epithelial cells, which boosts the invasiveness and dissemination of tumor cells. However, it is still unclear which immune cell type—macrophages, neutrophils, dendritic cells or T cells—is the crucial regulator of tumor cell dissemination, as it may depend on the nature of the tumor. Thus, a more thorough understanding of how inflammation contributes to the basic dissemination processes is worthy of investigation and could lead to new therapeutic interventions in metastasis.

Another component of the stroma in the primary tumor is the ECM, which is a rich reservoir of pro- and anti-angiogenic cues that regulate neovascularization of the tumor, a crucial process in tumor cell dissemination. Interaction of tumor cells with blood and lymphatic vessels as they move through the tissue to invade other areas and the circulation emphasizes the importance of tumor angiogenesis for supporting the metastatic process[33]. A recent study in zebrafish reports how myeloid cells, such as neutrophils, cooperate with the vasculature to enhance metastasis[34]. ECM proteins also regulate EMT[35] and are implicated in chemotherapy resistance (tumor collagen I)[36]. ECM cross-linking caused by lysyl oxidases stimulates tumor progression by enhancing integrin-signaling within tumor cells, promoting phosphatidylinositide 3-kinases kinase activity and inducing invasion[36,37], although whether this works at the level of the metastasis has not been determined. Moreover, the stromal invasion of tumor cells causes a desmoplastic response or fibrosis in the primary tumor, which is a feature of poor prognosis and correlates with metastasis in pancreatic, breast and other cancers. Antifibrotic drugs are already being developed for fibrotic diseases, and they may be useful to improve chemotherapy and increase survival.

At the cellular and molecular level, the developing metastatic lesions result from a complex cross-talk between disseminating tumor cells and the different players in the microenvironment of the metastatic lesion. Carcinoma-associated fibroblasts (CAFs) are activated in incipient neoplasia to orchestrate tumor-promoting inflammation and invasion in the primary tumor[38]. CAFs express fibroblast activation protein-α, which alters the ECM and stimulates tumor invasion, and fibroblast-specific protein-1 (FSP1, also called S100A4), which contributes to metastasis by enhancing tumor cell motility. They secrete a repertoire of proinflammatory molecules, growth factors and proteinases, including interleukins, chemokines, vascular endothelial and platelet-derived growth factors, matrix metalloproteinases and ECM components (such as tenascin C, fibronectin and collagen type I), that recruit other cell types to the primary tumor and, possibly, to future sites of metastatic colonization. Immune cells recruited to the tumor stroma can then influence metastasis; for example, $CD4^+$ regulatory T cells in the stroma of mammary tumors produce

RANKL, which stimulates RANK-expressing ERBB2-driven mammary tumors to metastasize[39]. Notably, most of these RANKL-expressing T cells are located adjacent to CAFs that express the T cell–attracting chemokine CCL5, suggesting a new role for regulatory T cells in tumors.

CAFs in the primary tumor also secrete hepatocyte growth factor and stromal-derived factor-1 (also known as CXCL12), which act in a paracrine fashion to increase tumor cell proliferation and metastasis via c-Met and CXCR4, respectively[40]. There is an increasing appreciation of the importance of the c-Met pathway and its intersection with the tumor microenvironment in tumor progression and metastatic growth. In melanoma, tumor cell–derived vesicles, called exosomes, can educate bone marrow progenitor cells toward a pro-metastatic phenotype through activation of c-Met[25]. Thus, the specific recruitment of distinct populations of leukocytes and stromal cells with overlapping functions in metastasis may open new avenues to the development of metastasis-targeted therapies.

Finally, an emerging area of clinical importance is the function of the tumor microenvironment in modulating sensitivity to chemotherapy. But which aspects of the microenvironment contribute to loss of drug efficacy is still unknown, and even less is known in the metastatic setting. There may be numerous stroma-mediated drug resistance interactions, including modulation of vascular permeability and alteration of molecular signaling[41]. In the primary tumor, activation of CXCR4 signaling results in downregulation of Let-7a, leading to enhanced expression of the antiapoptotic protein BCL-X_L in tumor cells[42], which may be responsible for the chemoresistance observed in the clinical setting. Whether this pathway is also exploited by newly activated CAFs in metastatic sites to induce drug resistance remains unknown. Recent evidence points to the microenvironment as an important, but understudied, source of anticancer drug resistance and also a therapeutic target, as drugs might not need to be completely penetrant to be effective, because altering some immune cells, ECM components or the vasculature may have profound effects as seen with regulation of microRNAs[35]. Moreover, resistance mechanisms can be uncovered through the systematic dissection of interactions between tumors and their microenvironment[43].

The microenvironment faced by cells at metastatic sites also determines whether the cells die, proliferate or become dormant. The recurrence of cancer arising from dormant cells in distant tissues is yet another microenvironmental challenge that is key to patient survival. The microenvironment of these residual disseminated cells probably differs both from the primary tumor and the metastases, but by how much? How does the microenvironment evolve from the pre-metastatic stage to established metastases and influence dormancy awakening in distant sites[11]? Although it is well established that both innate and adaptive immune cells in the primary tumor microenvironment contribute to tumor progression[28], there has been little emphasis on the stroma in metastatic sites. Determining whether regulation of dormancy and the metastatic niche arises from changes in the ECM, as may occur with senescence, decreased immune surveillance or changes in specific proinflammatory molecules, self-renewal or tumor cell motility, poses a challenge for the metastasis research community. Because of the central role of the ECM, cytokines, chemokines and the immune system in metastasis, we need a more complete understanding of the role of the metastatic microenvironment to uncover new mechanisms involved in the differentiation and recruitment of disseminating and stromal cells and how these interact with other environmental processes that promote metastasis. —*ZW*

The intractable clinical problem of metastatic disease

Why are most metastatic cancers incurable? Therapy in the metastatic setting is typically palliative, aiming for tumor shrinkage on imaging at an early time point, longer progression-free survival, and a better quality of life. Is the incurability of metastatic disease simply a problem of resistance to multiple lines of chemotherapy? This would not be the case for patients initially diagnosed with frank metastatic disease; these patients have not seen chemotherapy and yet have a poor prognosis. Another potential contributor to the incurability of metastatic disease may be what's called the 'bulky disease' problem. Most primary tumors are removed surgically; thus, drugs do not have to reach and obliterate millions of primary tumor cells via their tortuous vasculature and in the face of elevated hydrostatic pressure. In the metastatic setting, however, drugs would have to accomplish these same functions, and they apparently cannot in most cases.

Perhaps features of the metastatic process contribute to its therapeutic intractability. An emerging body of literature suggests that the same molecular pathways that make a tumor cell metastatic also make it drug resistant. So the process of metastasis may, in itself, increase malignancy and pose a challenge to treatment. Hallmark signaling players in metastasis such as activated Ras[44], chemokines[45], E-cadherin, CD44 (ref. 46), β-catenin[47], niche-derived mesenchymal stem cells[48] and certain microRNAs[49] have been recently shown to also drive chemoresistance. Even newer drivers of metastasis, such as synovial sarcoma, X breakpoint 2 interacting protein (SSX2IP), have a similar effect in promoting drug resistance[50]. These pathways could affect tumor cells—the 'seed'—or change the metastatic microenvironment—the 'soil'. Consistent with the idea that metastasis pathways promote chemotherapeutic resistance, antiangiogenic drugs worked in preclinical models of primary tumor growth but failed in the setting of advanced metastatic disease using the same cell lines[51]. It is also not understood when these dual progression and resistance pathways activate; is a micrometastasis chemoresistant or chemosensitive? The point is not arcane, as many patients with cancer are thought to harbor disseminated tumor cells at the time of initial diagnosis. Do progression and resistance pathways affect adjuvant therapy or only metastatic setting therapy?

Metastasis is traditionally defined as a movement of tumor cells to colonize a distant site, or the productive interaction of seed and soil. Another definition for metastasis—a fundamental genomic instability—may also contribute to its therapeutic intractability. Continuous genomic flux, potentially increasing with progression, would fuel the generation of variant tumor cells capable of spread and distant colonization in the face of whatever therapeutic barriers are present. Multiple aspects of genomic instability correlate with metastatic progression and poor survival in patients, but few have been mechanistically demonstrated to be participatory. Telomere dysfunction promoted genomic instability and metastasis in multiple models[52]. Other aspects, such as rates and faithfulness of DNA repair, have yet to be functionally implicated but may eventually stand as new clinical targets.

All is not hopeless, however. The literature provides ample preclinical evidence that metastasis can be considerably prevented. In a typical preclinical metastasis experiment, tumor cells are injected into mouse tissues to form a primary tumor, or, alternatively, directly into the circulation, and eventually form metastases. A compound is administered soon after tumor cell injection and then dosed throughout the experiment, preventing metastasis formation. Such data could support clinical trials to either prevent a first metastasis in patients at risk or to prevent further metastases in patients with a limited number of lesions. Examples of metastasis-promoting pathways that have been blocked by compounds in preclinical models include the proto-oncogene tyrosine kinase SRC[53], focal adhesion kinase (FAK), transforming growth factor-β (TGF-β) and lysophosphatidic acid

receptor 1 (LPA1)[54]. Though the lack of shrinkage is usually not reported, very few compounds shrink established metastases in preclinical models[55,56].

This initial success in preclinical metastasis prevention can be improved. We need more metastatic mouse models amenable for drug testing (such as a quantifiable number of metastases that develop in a reasonable time period and mimic the pattern of the human disease) and site-specific metastasis models, as pathways that govern lung metastasis may not control liver or brain metastasis. Adding standard-of-care therapies to our experimental agents might more realistically model the patient experience; we must see a preventive effect at a clinically achievable dose with acceptable toxicity. And, finally, we need drug combinations. A 50% reduction in metastasis in mice may seem important, but translated into humans, it means that half the patients had no benefit at the experimental endpoint, and the benefit that the other half of patients observed early on will probably wither away owing to the development of resistance to any single agent. Taking a page from the book on AIDS, a cocktail of therapies—given early—is most likely to be optimally effective. The challenges to combination therapy are numerous, including finding the appropriate model systems for each drug, determining potential synergistic toxicities, selecting an effective dose and schedule, designing trials, as well as navigating the associated legal matters[57]. A funding, patent and regulatory framework needs to be set up to make conducting joint studies a win-win proposition for two drug companies.

Given the preclinical success of metastasis prevention, we need to conduct clinical trials that test these hypotheses in a meaningful, cost-effective and patient-friendly manner. Most drugs today have to shrink established metastatic tumors in early clinical trials to be promoted to metastasis prevention (adjuvant) trials, but adjuvant trials are usually large, time consuming and costly. What happens if the metastasis preventive validated in mice does not shrink an established lesion? Randomized phase 2 metastasis prevention trials have been proposed to address this problem[58]. At least two designs can be considered: prevention of a first metastasis in very high risk patients and prevention of further metastases in patients with limited, treated metastatic disease. The most important difference between the proposed new trial designs and those currently conducted would be the experimental endpoint: the time to development of a new (or original) metastasis, rather than shrinkage of a large existing lesion. A strong emphasis on low toxicity and patient quality of life would also be inherent in new trial designs as metastasis preventives will probably be taken for long periods of time. The requirements for these trials would be preclinical efficacy of the tested drug in multiple metastatic models at achievable pharmacokinetics, pharmacodynamic markers of efficacy that can be brought to the clinic, overall safety of the drugs for long term administration, phase 1 combination safety data with standard-of-care therapy, a trial design that the US Food and Drug Administration will consider for drug approval, potentially a molecularly identified patient population, and a recruitable trial. A recent example is a group of patients with metastatic HER2-overexpressing breast cancer who developed brain metastases, probably owing to HER2 promotion of metastatic competency[59] and the limited ability of trastuzumab to cross the blood-brain barrier[60]. Metastasis prevention trials are under consideration by the Southwest Oncology Group for patients with $HER2^{+}$ metastatic breast cancer and one to three brain metastases treated with localized radiation therapy. Patients will be randomized to systemic therapy and either placebo or the metastasis preventive, with an endpoint of time to the development of a new brain metastasis (G. Hortobagyi, MD Anderson Cancer Center, personal communication). Other clinicians are attempting metastasis prevention trials in urothelial cancer by selection of high-risk patients followed by randomization to treatment groups receiving potential preventive agents directed at endothelin or CCL2 aimed to disrupt the macrophage-inflammation axis (D. Theodorescu, University of Colorado, personal communication). These and other efforts

may bring our best preclinical leads forward to prevent the hopelessness associated with metastatic cancer. —*PSS*

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