A long-standing problem in oncology drug development is the frequent failure of preclinical mouse tumor therapy models to accurately predict clinical activity.1,2 Highly positive results obtained in mice are often followed by eventual negative phase III clinical trial outcomes. Historically, most such encouraging preclinical studies have involved treatment of mice with established primary tumors, usually obtained after transplantation of cultured mouse or human tumor cells obtained from long-established cell lines. The discrepancy in preclinical versus clinical outcomes was a major factor in stimulating the development and use of 2 (hopefully superior) alternative tumor therapy models in mice, namely, genetically engineered mouse models (GEMMs) of human-like spontaneous mouse tumors4 or patient-derived xenografts (PDXs).8 Both such models almost always involve treatment of established primary tumors5 rather than advanced metastatic disease, an extremely challenging circumstance to achieve highly encouraging therapeutic results in the clinic, at least in terms of prolonging survival or cure. Thus, beginning a decade ago, a research program was initiated in my laboratory to develop models of advanced, late-stage spontaneous metastasis in mice to use for experimental therapeutic studies.6 The rationale was that the results obtained from using such models would have a greater probability of clinical relevance, and hence clinical translation, at least with respect to predicting subsequent results in metastatic cancer patients enrolled in clinical trials, compared with the results obtained using conventional approach of treating mice with primary tumors only.1 The strategy we adopted involved use of established human tumor cell lines, including those of breast, colorectal, and renal cell carcinoma as well as melanoma; they were subjected to an in vivo serial (2-step) selection process involving orthotopic primary tumor growth, surgical resection of primary tumors, and isolation of subsequently arising overt spontaneous lung or liver metastases, which were established as cell lines.6 The more aggressive metastatic variants were then “tagged” with markers to allow detection of visceral metastasis by imaging or to assess overall tumor burden over time in response to therapy. Here, we describe various results showing correlation between preclinical and some prior phase III clinical trial outcomes involving antiangiogenic therapies, as well as results of “metronomic” chemotherapy, which helped contribute to undertaking phases II and III clinical trials of this treatment concept. Additional results from studies using these models that have led to insights into understanding the basis of resistance to antiangiogenic drugs and the biology of brain metastasis are also summarized. Disadvantages and limitations of the models for investigational therapeutics are also discussed, as are strategies for improvements.

The Methodology Used
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breast cancer patient. Although long-established cell lines have their drawbacks due to such factors as selection on plastic and “genetic drift,” they have several advantages for the goal we have of developing models of postsurgical metastatic disease that can be used for experimental therapeutics in a practical way. Thus, established cell lines can be easily genetically manipulated by gene transduction techniques (in contrast, e.g., to using intact PDX tumor tissue). Such techniques can be used to insert genes encoding biomarkers to detect visceral metastatic disease and its response to therapeutic interventions. Two such markers have been used for our studies. The first is luciferase for whole-body optical bioluminescent imaging for detection of macroscopic established metastases, and the second is the secreted circulating $\beta$-subunit of human chorionic gonadotropin hormone $\beta$-hCG detected in mouse urine that serves as a surrogate marker of disease burden. While these biomarkers have proven highly useful for our metastatic therapy studies, they are not without technical drawbacks and limitations. To cite an example, we have observed in some cases that the procedures involving luciferase tagging and selection can result in a partial loss of metastatic aggressiveness or a loss of the bioluminescent signal (unpublished observations). Such changes may not be apparent in short-term (e.g., 2 weeks to 1 month) therapy experiments. However, in our case, therapy experiments can sometimes be quite prolonged, for example, 5 to 6 months of continuous daily therapy with oral antiangiogenic drugs by gavage and/or oral chemotherapy drugs, the latter also administered by gavage at low doses, that is, “metronomic” chemotherapy. Such protracted investigations increase the chances that alterations in the phenotypes of luciferase-mediated bioluminescent signaling or metastatic aggressiveness may be lost or reduced. The alterations in phenotype could be due to factors such as epigenetically silencing of the luciferase gene inserted caused by tumor environmental changes such as hypoxia.

The basic methodology to select variant sublines having a much more aggressive ability to metastasize “spontaneously” to multiple distant organ sites from a (resected) primary tumor consists of several steps, which have been described and reviewed previously. The approach is a variation pioneered by I. J. Fidler, who selected successive melanoma cell lines for increasingly more aggressive metastasis by intravenous injection of tumor cells and recovering so-called “artificial” metastases. Instead, we decided to use a similar approach for selecting variants that have a more aggressive spontaneous metastatic phenotype and disseminate from a primary tumor before resection. The first is orthotopic transplantation of the biomarker “tagged” (or untagged) tumor cells; for example, breast cancer, cells are injected into the mammary fat pad, melanoma cells into the skin, colorectal cancer cells into the cecum, and renal cell carcinoma cells into the kidney capsule. This procedure is known to promote and accelerate metastasis, in comparison to “ectopic” transplantation, which generally has involved subcutaneous transplantation. However, overt spontaneous metastases can often take months to become established in our experience, at least when using human tumor cell lines. This means that the established primary tumors must be resected to prolong the survival of the mice to allow seeded microscopic metastases sufficient time to expand into macroscopic lesions. In the case of most of our studies, such metastases usually first (and sometimes only) appear in the lungs, and in the case of colorectal cancer, in the liver. An individual metastasis is removed, or several metastases are pooled from the same organ, and a cultured cell line established from the disaggregated metastases. Using this cell population, a second (serial) selection in vivo is repeated consisting of the same sequence of steps. Our limited experience has been that this second selection results in accelerated development of metastases with the number (and often the size) of distant metastases both increased, which is indicative of selection of a variant subpopulation of tumor cells. An individual metastasis from the second serial selection step is isolated and used to establish a new cell line, for example, the LM2.4 (i.e., lung metastasis, 2nd selection, 4th mouse) isolated from the MDA-MB-231 human breast cancer cell line. This line is used untagged, or subsequently tagged, for example, with luciferase, and later used for therapy experiments. Typically, the cells are injected orthotopically, and resultant primary tumors, once established, are resected. Therapy is initiated within 4 to 6 weeks once overt distant metastases are formed based on imaging or known from prior experience using the untagged tumor cells. Survival is then used as a primary endpoint to assess therapeutic efficacy. In a number of cases, the results are compared with treatment of control mice with unJECTED primary tumors, where the impact of the therapy is assessed only on the growth of such primary tumors, that is, undertaking a historically conventional therapy approach and then determining if the outcomes are similar or different in the 2 different treatment settings. A variation of this is to initiate therapy in the postsurgical metastasis models immediately or very soon after primary tumor resection when overt metastases are not present, but the mice are at future risk of developing such metastases. This would mimic the circumstances of potentially curable adjuvant therapy of early-stage microscopic metastatic disease. One technical problem we have encountered that should be highlighted with this methodology is regrowth of primary tumors at the site of surgical resection. Another is the heterogeneity of metastatic disease in terms of extent, timing, and location from one mouse to another. This can sometimes be overcome with procedures such as increasing the number of mice at the start of the experiment and avoiding the use of mice with primary tumor regrowth or no metastases that are visible. There are also metastatic models we have used that do not involve surgical resection of primary tumors, for example, advanced intra-peritoneal metastasis of human ovarian cancer after intraperitoneal injection of luciferase tagged clones selected for aggressive spread within the peritoneal cavity. We have also developed models of locally advanced orthotopic primary tumors, for example, of HCC (human hepatocellular carcinoma).

Results Using Preclinical Models of Metastatic Disease: Correlation With Clinical (Trial) Outcomes

An obvious approach to assess and validate, at least in part, whether models of metastatic disease for therapy testing might be superior in predicting future clinical activity is to first undertake studies of a therapy that failed to show efficacy in a phase III trial involving treatment of metastatic patients but was preceded by highly encouraging preclinical results using standard in vivo drug testing techniques involving treatment of primary tumors. To this end, we undertook studies of several different antiangiogenic drugs, either alone or with chemotherapy, to treat late-stage overt metastatic breast cancer of human tumor xenografts, using the LM2.4 metastatic variant selected from the MDA-MB-231 human breast cancer cell line, as described above and previously. For example, previous studies, mostly involving treatment of established primary tumors, in a variety of different models, including GEMMs or chemically induced breast cancers in rats or human tumors grown in mice, showed that treatment with sunitinib could cause reasonable antitumor efficacy in many of the primary models, the extent of which could be increased by such procedures as either increasing the drug dose or combining

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the drug with standard-of-care chemotherapy regimens. There are many similar studies published in the preclinical literature showing efficacy of single-agent antiangiogenic, including oral tyrosine kinase inhibitors (TKIs) in common solid tumor types, especially when such drugs are combined with chemotherapy. In contrast, treatment of common solid tumors in the clinic with antiangiogenic TKIs whether used as a monotherapy or when combined with chemotherapy has resulted in extremely high rates of failure in randomized phase III trials, in part because of excessive toxicity, thus necessitating drug dose reductions or drug holidays. In the case of sunitinib, a total of 4 such trials were undertaken, 2 in the first-line and 2 in the second-line treatment settings, and 3 of them involving concurrent chemotherapy, using various different drugs including paclitaxel, docetaxel, and capecitabine. These findings clearly represent a major failure of preclinical models in mice predicting efficacy in the clinic—at least at the randomized phase III clinical trial level.

We therefore undertook an analysis of the impact of 3 different antiangiogenic drugs, sunitinib, pazopanib, and DC101, a vascular endothelial growth factor receptor 2 (VEGFR-2) antibody. Although all 3 of these drugs showed efficacy when treating primary tumors (manifested mainly growth delays), none of them showed an ability to prolong median overall survival (OS) in mice with advanced metastatic breast cancer, after the primary tumor had been resected. Moreover, in the case of sunitinib, combination with paclitaxel did not result in a benefit compared with paclitaxel alone. In contrast, the DC101 antibody, when combined with paclitaxel, was found to cause a statistically significant OS advantage compared with the untreated control mice and a trend toward an increase in survival when compared with mice treated with paclitaxel. The antibody targeting the VEGF pathway plus paclitaxel result reflected to some extent the benefit observed in a phase III trial in metastatic breast cancer patients evaluating bevacizumab plus paclitaxel against paclitaxel alone, where a significant benefit in progression-free survival (PFS) of almost 6 months was observed. In our case, as with almost all therapy studies, we did not assess PFS. Had we done so, based on the survival results, it is likely that a benefit in PFS would have been detected using the 2-drug combination treatment versus the paclitaxel monotherapy treatment group.

The conclusion that an antibody targeting the VEGF pathway when combined with chemotherapy is superior to an antiangiogenic TKI plus chemotherapy in metastatic breast cancer (and that this is reflected in the aforementioned preclinical metastatic model therapy results) may be challenged in view of the randomized phase III ROSE/TRIO-12 trial results. This trial evaluated first-line docetaxel once every 3 weeks plus ramucirumab, a VEGFR-2 monoclonal antibody in metastatic breast cancer patients, and the PFS and OS results were negative or insignificant. However, this result stands as an exception to the general finding that bevacizumab and the VEGF antibody plus chemotherapy showed varying, albeit modest, benefits in metastatic disease, where the results were found to be highly encouraging and which subsequently helped contribute to the decision to undertake randomized clinical trials, especially at the phase III, where the results were positive. We have undertaken this kind of “prospective” analyses. It has mainly involved evaluation of low-dose continuous “metronomic” chemotherapy regimens administered either alone or in combination with a targeted therapy, usually an antiangiogenic drug, either an antibody targeting the VEGF pathway or an oral antiangiogenic TKI. The term “metronomic chemotherapy” refers to the administration of conventional chemotherapy drugs in an unconventional/investigational fashion, namely, on a frequent regular schedule (e.g., daily) using relatively low and less toxic doses of drug, with no drug-free break periods. This is fundamentally different than the common method of using maximum tolerated doses (MTDs) separated by long break periods, for example, 2 to 3 weeks, which are
necessary to allow recovery from the toxic adverse effects of such chemotherapy regimens, such as myelosuppression. The rationale, at least in part, is that the long break periods can also facilitate and accelerate recovery of the drug-treated tumors. In contrast, putting the tumors under a constant and continuous chemotherapy pressure would avoid this, but would require using doses that are below the MTD each time the drug is administered. However, the cumulative dose over time may be similar, less, or even more than the respective MTD regimen of the drug.

An abundance of mostly preclinical studies have shown that the antitumor effects of metronomic chemotherapy may be fundamentally different than MTD chemotherapy antitumor mechanisms, which only or mainly involve direct tumor cell cytotoxicity. Instead, metronomic chemotherapy may work by fundamentally different mechanisms as shown in Figure 1. These include antiangiogenic/vascular targeting, antivasculogenesis, stimulation of the immune system, especially using certain drugs such as cyclophosphamide, and inhibition of hypoxia-inducible factor 1α (HIF-1α) using topoisomerase 1 inhibitors such as topotecan or camptothecin, as well as possible direct tumor cell–targeting effects, which may include the cancer stem cell subpopulation. The antiangiogenic/vascular–targeting effects stem from the presumed presence of dividing endothelial cells in newly forming blood vessels, which should render such cells potentially vulnerable to cytotoxic chemotherapy drugs that preferentially target any cycling cell population. The antivasculogenic effects may be related to the impact of low-dose continuous chemotherapy inhibiting the mobilization and tumor homing/invasion of circulating bone marrow–derived cell populations including endothelial progenitor cells, whereas in contrast, MTD chemotherapy regimens may be associated with increasing the number of mobilized endothelial progenitor cells, that is, causing a “spike”. These multiple mechanisms may help explain why, counterintuitively, resistance may actually develop more slowly to low-dose metronomic chemotherapy regimens, compared with the respective MTD regimen of the drug. In contrast, putting the tumors under a constant and continuous chemotherapy pressure would avoid this, but would require using doses that are below the MTD each time the drug is administered. However, the cumulative dose over time may be similar, less, or even more than the respective MTD regimen of the drug.

We have observed a number of preclinical situations where a metronomic chemotherapy regimen can have surprisingly potent effects on prolonging OS in mice with advanced metastatic disease. An example of this is shown in Figure 2. The regimen used was a “doublet” metronomic combination therapy involving daily oral cyclophosphamide administered through the drinking water and oral UFT (a 5-fluorouracil prodrug) administered daily by gavage to treat established metastatic breast cancer after the primary tumor in the mammary fat pad had been resected. Neither drug used alone had a discernible benefit on survival, but the 2-drug combination clearly did have such an effect. Interestingly, when the same 2-drug combination was used to treat established orthotopic primary tumors of the same tumor cell line in control experiments, the effects were far less impressive; only a modest growth delay was noted. This pattern of result stands in complete contrast to the previously described results utilizing sunitinib, pazopanib, or DC101, an example of which is also shown in Figure 2. Specifically, an antitumor effect of sunitinib is seen in the primary tumor treatment setting, but no significant effect is observed in the metastatic setting. Aside from bolstering the rationale for metronomic chemotherapy regimens as a possible effective treatment for metastatic disease in the clinic, these results also suggest that metronomic chemotherapy does not cause antitumor effects solely or predominantly by inhibiting tumor angiogenesis. If it did, the pattern of tumor response would presumably be similar to that seen with the previously mentioned antiangiogenic drugs.

The previously mentioned metronomic chemotherapy findings along with the results of a number of other groups have helped contribute to the decision to undertake phase II and even phase III randomized trials evaluating metronomic chemotherapy in the metastatic as well as adjuvant setting. One randomized phase III trial has now been completed, and the results reported. This is the CAIRO3 trial evaluating a maintenance regimen consisting of daily lower-dose capcitabine combined with bevacizumab in metastatic colorectal cancer patients. Patients in this trial were first treated for approximately 4.5 months with

![FIGURE 1](image-url)

**FIGURE 1.** Multiple mechanisms proposed to account for the antitumor effects of metronomic chemotherapy. BMDCs indicates bone marrow–derived cells; EPCs, endothelial progenitor cells.
6 cycles of conventional chemotherapy consisting of capecitabine and oxaliplatin combined with bevacizumab ("CAPOX-B"). Capecitabine was administered at a dose of 1000 mg/m² twice a day for 2 weeks followed by a 1-week break when it was given up-front as an induction therapy. Provided that patients showed a clinical benefit to this regimen, they were then randomized into 2 different arms. Patients in the first received no further treatment until disease progression. Patients in the second arm received capecitabine once again, but “metronomically” at a lower daily dose of 625 mg/m² twice a day with no breaks, plus bevacizumab, until progression as a maintenance therapy. Once patients progressed and assessed for PFS or "PFS-1", they were then retreated with the original CAPOX-B induction regimen, and PFS was again measured (called PFS-2), the primary endpoint of the trial. Patients in the maintenance treatment arm had a significant benefit in PFS-1 and PFS-2. Moreover, the treatment was found to be very tolerable. This positive trial result provides a foundation for further investigations of metronomic chemotherapy treatment in the metastatic setting would not have been predicted from the primary tumor therapy results.

Studies of Adjuvant Therapy of Early-Stage Micrometastatic Disease

While most of our studies involving models of metastasis for investigational therapeutics have involved advanced/established metastatic disease, we have also undertaken studies of treatment involving micrometastatic earlier stage disease, that is, adjuvant therapy studies, after surgical resection of primary tumors. These studies have involved adjuvant therapy of breast cancer, colorectal cancer, and renal cell carcinoma. The first such study
involved treatment of micrometastatic breast cancer using the LM2.4 variant of MDA-MB-231 and assessing the impact of an antiangiogenic therapy, namely, sunitinib. The protocol that was adopted involved a high dose of sunitinib (120 mg/kg) given by gavage for a short period, that is, 1 week. The short treatment duration was necessary because many TKIs, including sunitinib, can be toxic to SCID mice, the recipient mouse strain for the tumor xenografts used in these studies. We found that the sunitinib treatment actually accelerated metastatic progression and shortened survival times compared with vehicle control. As a result of this single study, we questioned the wisdom of undertaking hugely expensive adjuvant trials of antiangiogenic drugs without further preclinical information that would provide support for undertaking such clinical trials. The paradigm in oncology is that if a drug or therapy shows efficacy in the metastatic setting, then it is likely to show an equal or even better benefit in the adjuvant treatment setting. However, there are known exceptions to this rule, and this has also seemed to be the case based on our limited preclinical studies. Since publication of our studies, there have been multiple randomized phase III trials of adjuvant antiangiogenic drugs alone or with chemotherapy in breast cancer, colorectal cancer, and hepatocellular and renal cell carcinomas. All of these trials have failed to show a benefit in disease-free survival and are considered failures. Only in 1 case has a worse outcome been noted, that is, in the so-called “AVANT” adjuvant colorectal cancer trial, and, although there is no evidence of a general trend of worsened outcomes, our cautionary note about undertaking adjuvant antiangiogenic trials in the absence of more preclinical information was correct.

This last point deserves more comment. This is because of the failures to find clinical evidence that tumor progression and metastasis are accelerated or enhanced by an antiangiogenic drug treatment, even though many other reports by other groups indicated similar findings to ours and Paez-Ribes et al., indicating exacerbation of metastatic disease as a result of an antiangiogenic therapeutic intervention. In addition, our study has been criticized on the basis of using a very high dose of sunitinib. There are several points to consider in this regard. The high dose that we used for our adjuvant experiments and its short duration were also assessed in a conventional primary tumor treatment setting, as shown in Figure 3, where an antitumor effect was observed, in contrast to the worsened outcome in the postsurgical setting.
treatment setting. Second, with respect to observations in the clinic, it should be noted, at least with bevacizumab, that all adjuvant trials using this drug involved upfront combination with chemotherapy followed by maintenance bevacizumab. Such combinations have not been duplicated in preclinical studies, with 1 exception. In this study, the prometastatic effects of sunitinib observed in a preclinical ovarian cancer model were counteracted by concurrent chemotherapy. These reservations are important, not only because they may help resolve the discrepancy between the preclinical versus clinical outcomes, but also because they suggest that it may not only be the case that an antiangiogenic drug can improve the efficacy of chemotherapy, but the opposite may be true as well—the chemotherapy can improve the overall effects of the antiangiogenic drug by preventing or suppressing the possibility of any proinvasive/metastatic effect the antiangiogenic treatment might have. Such promalignancy effects in some cases may be secondary to the increases in tumor hypoxia caused by the antiangiogenic drug treatment, which could elevate expression of HIF-1α, which in turn may up-regulate various genes involved in tumor cell invasion/migration and metastasis. Thus, drugs that have a secondary effect of suppressing elevated HIF-1α caused by an antiangiogenic drug may be extremely useful for combination with antiangiogenic agents. An example of this is the use of low-dose topotecan and a camptothecin nanoparticle drug conjugate called CRLX101.

Clinically Relevant Biologic Findings of Metastasis and the Tumor Microenvironment Emerging From the Use of Therapy Models of Metastatic Disease

While our metastatic model studies have primarily focused on therapeutic investigations, we have uncovered a number of biological findings that have clinical relevance and which have helped contribute to and foster some important areas of investigation. The most notable is spontaneous brain metastasis. Historically, studies of brain metastasis in mice have been restricted to what is known as artificial brain metastases generated by intraarterial inoculation of tumor cells. However, we generated the first model of spontaneous brain metastasis by virtue of undertaking therapy studies in some of our metastatic models involving treatment of advanced disease. Thus, treatment of mice with advanced metastatic systemic disease in organ sites such as the lungs, liver, and lymph nodes—but not the brain—relapsed with spontaneous brain metastases if their systemic disease was significantly controlled by a particular therapy. This is likely the result of a well-known and clinical phenomenon of growing importance, namely, that effective control of systemic metastatic disease for a period of time can result in a higher rate of relapse of brain metastases. This is thought to occur primarily as a result of what were seeded microscopic metastases in the brain having more time to expand into overt macroscopic lesions as a result of the efficacy benefit
in controlling systemic metastatic disease.\textsuperscript{81,82} Thus, brain metastases is likely to be an area of extremely important future investigation. In this regard, we isolated spontaneous melanoma brain metastases from mice that had their survival times prolonged allowing the eventual detection of brain metastases. Cultured cell lines were established from such brain metastases and were found to be capable of spontaneously metastasizing from an established primary and then reseeded tumor but without any need for extending survival by a therapeutic intervention.\textsuperscript{7} In other words, these cells seem to represent a selected subpopulation having the ability to spontaneously metastasize to the brain.\textsuperscript{7} Further studies implicated endothelin receptors and endothelins 1 and 3 as being possible contributors to the spontaneous brain metastatic phenotype of these sublines.\textsuperscript{83} Of considerable interest is that endothelin receptors have been implicated by Fidler and colleagues as mediators of glioblastoma growth, survival, and drug resistance.\textsuperscript{84} As a result, there is interest in treating glioblastomas and possibly in the future brain metastases in other tumor cell types with a drug that inhibits endothelin receptor function, for example, macitentan\textsuperscript{85} (www.clinicaltrials.gov, ClinicalTrials.gov Identifier NCT02254954).

Summary and Conclusions

The results summarized in this review highlight the potential advantages and benefits of using postsurgical models of spontaneous metastatic disease—whether microscopic or macroscopic in nature—for investigational therapeutics. This does not mean that such models should be used as a routine screen to initially assess potential clinical drug efficacy. Aside from the expense and impracticality of doing so, the results, ironically, could be erroneously interpreted as “false negatives.” Thus, the failure of monotherapy using antiangiogenic drugs or trastuzumab to demonstrate efficacy in the advanced metastatic treatment setting in our studies obviously does not necessarily mean the drugs are “inactive.”\textsuperscript{7} They can and do have meaningful activity when combined with certain other therapeutic modalities preclinically, as shown in a series of our studies assessing pazopanib treatment alone in ovarian cancer\textsuperscript{11} or renal cell carcinoma.\textsuperscript{9} But when the drug was combined with metronomic topotecan chemotherapy, pazopanib significantly enhanced overall efficacy compared with the metronomic topotecan monotherapy treatment,\textsuperscript{9} as shown in Figure 4. Thus, these metastatic models may be particularly useful as a “secondary” screen to better assess the possible benefits—or lack thereof—of various combination treatments in the adjuvant and metastatic treatment settings, the results of which may aid development and design considerations of clinical trials, or simply making “go versus no-go” decisions regarding whether to undertake clinical trials. These models may be used in conjunction with other models, for example, GEMMs of cancer or PDXs to gain an overall better assessment of the potential prospects of a new drug or therapy when it is later assessed in patients. In this regard, an advantage of the GEMMs is the capability to use them for assessing immune-based therapies. In contrast, use of human tumors grown in immune suppressed mice, for example, PDXs, would preclude such studies, unless such mice are “humanized” with a human-like immune system.\textsuperscript{86} When assessing the relative merits of various models—GEMMs, PDXs—versus the approach summarized here of using postsurgical models of metastatic disease, it should be noted that undertaking postsurgical models of therapy of early- or late-stage metastatic disease is very difficult because multiple primary tumors frequently arise in GEMMs and, moreover, do so asynchronously. Furthermore, metastatic disease is not commonly observed in GEMMs, although some advances are being made in achieving this goal.\textsuperscript{87–89} Similarly, PDX studies virtually always involve treatment of established primary tumors. Metastatic PDX models are rare but are now being developed.\textsuperscript{91} Taken together, these considerations argue for considering a multimodel approach for preclinical testing to better predict the likelihood that a new investigational therapy, or drug, will have some degree of efficacy when later assessed in randomized clinical trials, especially at the phase III level. While more expensive, it is a lot cheaper than a typical (failed) phase clinical trial.

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