

## A Systematic Study of Cancer Metastasis Tumor Evolution and Progression Working Group

### What is the recommendation (1-3 sentences)?

Launch a multi-disciplinary effort to elucidate the metastatic lesion and its vulnerabilities from the earliest to most advanced stages of cancer in both pediatric and adult populations, with a focus on exemplary tumor types and anatomic sites.

### Where are we now (2-3 paragraphs)?

Metastatic cancer accounts for most cancer-associated morbidity and mortality. Evidence in patients and experimental models has demonstrated that metastatic dissemination of cancer cells from primary tumors may occur early (in some tumor types even before progression to invasive stages), yet most disseminated cancer cells will not develop into “macro-metastatic” lesions. Rather, they remain dormant (sometimes for many years) as individual cells or small clusters. Thus, the outgrowth of metastatic lesions likely requires additional factors such as non-cell-autonomous effects provided by the microenvironment (e.g., tissue injury-related inflammatory signals or signals from the cells of the metastatic niche) or by cooperating cancer cells (i.e., due to clusters of cancer cells disseminating together or subsequent self-seeding). At the same time, evasion from the immune system undoubtedly contributes, but this too may occur at earlier stages—possibly even at the time that invasive disease first develops in situ. However, our understanding of the genesis and maintenance of metastatic states remains fragmentary.

Until recently, we lacked the ability to model and perturb the metastatic process using patient-derived tumor cells. Although mouse models yielded valuable insights into mechanisms governing metastasis, high-order genetic manipulation remained time- and labor-intensive. Similarly, our ability to query the salient heterogeneity of malignant and microenvironmental cells and how these might promote the metastatic niche was under-developed. However, recent technological advances such as genome editing and high-resolution analysis offer the promise of overcoming these barriers, thereby bringing a new understanding of metastatic states and how they are maintained in patients.

### Where do we need to be (in 1-5 years)?

#### **Priority 1. Gain a comprehensive understanding of the dependencies operant in metastatic states.**

New experimental methodologies such as genome editing make it possible to identify genes and pathways that are essential to tumor cells in various contexts. Such approaches could be leveraged to characterize genes or pathways that are required for survival in various metastatic states. This may involve querying the malignant cells directly or probing effectors from the microenvironment that may provide key inputs into metastatic programs.

Approaches of interest may include (but are not limited to) functional interrogation of patient-derived models; systematic or mechanistic studies of genetically engineered mouse models that reflect critical aspects of metastasis (and dependencies therein); new technologies that may

assess cancer dependencies in primary tumor material directly; and tractable systems that model specific metastatic challenges (e.g., brain or bone metastases).

**Priority 2. Construct “3-dimensional” and “4-dimensional” cancer cell atlases of paired primary and metastatic cancers, or metastases to different anatomic sites.**

Emerging technologies are making it possible to produce high-resolution and even single-cell characterization of all major cell types (malignant, microenvironment, and immune) in tumor tissues. Leveraging such technologies, it would be of interest to perform ultra-high (e.g., single-cell) resolution analyses of biopsies or resections obtained from individual cancer patients throughout the course of disease and treatment, including the advent of drug resistance. In addition, new *in situ* technologies that read out cell/tissue topology could be used to ascertain the cellular adjacencies that may influence particular functional states. Atlases generated by this approach should ideally be linked to model systems that allow experimental testing of the hypotheses generated (see Priority 3, below).

Such atlases could provide, for the first time, a view into the heterogeneity of salient programs and states operant in metastatic foci, how they are influenced by different microenvironmental and immune factors, and how they evolve over time or during treatment.

**Priority 3. Develop and characterize new cancer model systems designed to fill key gaps in existing studies of metastasis.**

For many cancer types, we still lack appropriate experimental model systems that would allow us to study the salient tumorigenic programs governing metastasis, and to discover new therapeutic targets. Recent years have witnessed advances that could enable a dramatic expansion in various types of models, including patient-derived xenografts, and the possibility of generating tumor-bearing mice with “humanized” immune systems. These advances could be leveraged to generate new models of key metastatic sites (e.g., brain metastases) that are less well represented at present, and use these models to characterize states and dependencies operant therein.

- **Rationale for investing (Why is this priority ripe for accelerating?)**
  - **Opportunity brought about by recent development in science, technology, practice:** New technologies for high-resolution tumor characterization (e.g., single-cell analysis, multiplexed molecular imaging, and other approaches) and perturbation (e.g., genome editing) together with advanced and emerging model systems offer considerable new opportunities for studies of metastasis.
  - **Does it address an unmet need or important gap in knowledge or practice? Yes:** knowledge of metastatic states and vulnerabilities therein remains a crucial unmet need in many cancers.
- **What would be needed for success?**
  - **New or expanded resources:** support for sampling of tumor tissue and blood over the course of disease/metastasis and from specific anatomic sites (including autopsy specimens, if appropriate); deployment of technologies and analytical capabilities for high-resolution characterization of these tumors; implementation

of experimental approaches to perturb appropriate metastasis models *in vivo*; tools for sharing and analysis of omic and experimental data that emerge.

- **Barriers/roadblocks eliminated or reduced:** support scaling of existing experimental efforts, augment existing infrastructures for biopsies and blood collection to ensure collections of all sizes are supported; support for quality data generation efforts; establishment of new computational teams focused on deconvolving the biology linked to metastatic states
- **New or enhanced technologies:** scalable functional/editing studies; studies of heterogeneity; single-cell analysis, high-content tissue topographic analysis, model system dissemination

### **Strategy: What will it take to get there?**

We recommend that the NCI develop a coordinated research effort to study all aspects of the metastatic program from the earliest stages of dissemination through overt metastasis at the molecular and biological levels. This effort may involve a particular focus on selected exemplary tumor types. This effort will require access to historical patient specimens (presumably early lesions) and paired metastatic and primary tumor specimens from more advanced cases. Another component would involve pairing the tissue collection efforts with the development of relevant functional models that both inform dissemination and metastasis and allow for functional analyses of metastatic programs operant in various anatomic sites (liver, bone, brain, etc.). Rapid autopsy programs may also be useful for assessing the molecular evolution of multiple metastatic lesions from the same individual.

### **These priorities will also require:**

**1. Scalable research biopsy and data generation programs.** These initiatives will require fresh and/or serial biopsies of metastatic and drug-resistant specimens for deep tumor/microenvironmental characterizations and generation of *ex novo* models. Thus, the cancer moonshot should support collaborative efforts to procure these biopsies at scale and link them to state-of-the-art technologies for data generation and analysis. Liquid biopsy protocols should be paired with tissue biopsy efforts to provide complementary cancer-derived materials (circulating tumor cells/DNA, exosomes, etc.). Collecting liquid (blood) biopsies between collections of tissue samples would help to build a model for better characterization. Furthermore, bridging data garnered from liquid biopsies and imaging studies would facilitate translation activities; deep characterization of the tissue and liquid biopsies together with imaging data on a low number of patients might yield information generalizable to a larger number of patients. Materials obtained from these research biopsies should be seamlessly integrated with workflows capable of generating a wide range of data types.

**2. Computational analysis capabilities.** A critical need exists to develop algorithms that integrate and extract therapeutic meaning from data generated from metastatic biopsies using the latest technologies. New algorithms will help to identify relevant variations in heterogeneous tumors. Thus, we envision the establishment of collaborative efforts whose mission to design and implement such tools.

**3. *Ex vivo* cultivation, perturbation, or target validation activities.** Expansion of cancer models in vitro and in vivo would be aided by increased capacity for handling, distributing, and propagating cancer cell line and patient-derived xenograft models. Focused efforts to optimize approaches for generating and maintaining these models, building robust collections, and perhaps hosting research on these models done by individual investigators or moonshot teams should be considered.

**What does success look like?**

Successful completion of this project would yield new insights into the specific cell-autonomous, non-cell autonomous, soluble, and microenvironmental programs and effectors that drive the metastatic process, and how these intersect with related challenges such as drug resistance. Such knowledge may yield new insights into therapies that could be applied either at the time of metastatic cancer or earlier in disease (e.g., during treatment of primary tumors) to interrupt this lethal process.

## **New Therapeutic Targets to Overcome Cancer Drug Resistance (Joint Recommendation from Pediatric Cancers and Tumor Evolution)**

### **What is the recommendation (1-3 sentences)?**

Launch multi-disciplinary studies to identify new drug targets elaborated by cancer drug resistant states. Such studies will include approaches to overcome drug resistance in exemplary pediatric and adult tumor types and therapeutic contexts.

### **Where are we are now (2-3 paragraphs)?**

- **Summary of the current state of the science/practice**

Most cancer patients die because their tumors exhibit intrinsic resistance or develop acquired resistance to available therapies. However, our knowledge of the spectrum and mechanistic underpinnings of drug-resistant cell states remains incomplete. It has become well-recognized that resistance can be highly multifactorial and heterogeneous, with multiple independent resistance mechanisms operant in the same patient, tumor focus, or even the same tumor cell. Furthermore, some drug resistance programs may be non-cell autonomous and may overlap significantly with programs that drive metastasis and overall tumor survival/maintenance.

- **Identify barriers to progress and/or emerging opportunities**

Barriers to progress in understanding cancer cell resistance exist on genetic, molecular, cellular, and physiological levels. Understanding why, when, and how resistance develops is complicated by gaps in understanding regarding, but not limited to, tumor cellular heterogeneity; cell plasticity among potential cancer stem cell/tumor initiating cell populations; rewired and/or reprogrammed signaling pathways; compensatory signaling mechanisms; positive/negative signaling feedback loops; contributions of genetic polymorphisms (SNPs, CNVs); and the contribution of non-cancer cell components within the tumor microenvironment. Moreover, this multifactorial and heterogeneous nature of resistance means that multiple mechanisms can be operant in the same patient and even the same cell. That said, a growing body of evidence suggests that many individual resistance mechanisms may converge onto certain drug-resistant cell states, the understanding of which may provide new opportunities for combination therapies capable of circumventing this challenge.

### **Where do we need to be (in 1-5 years)?**

1. **Apply systematic experimental studies in appropriate model systems to define spectra of resistance mechanisms and dependencies linked to drug-resistant states.**

New genome editing (e.g. CRISPR) and unbiased small molecule screening to systematically discover their vulnerabilities and make it possible to identify genes and pathways that are essential to tumor cells that harbor specific genetic or molecular alterations. Specifically, it is paramount that there is a focus on pediatric cancers with a low probability of cure (metastatic solid tumors, select CNS tumors, AML, certain high risk subsets of ALL, and all refractory and recurrent cancers). These approaches may be leveraged to discover individual resistance

mechanisms, common resistant cell states onto which they may converge, and genes/pathways that become essential after evolution to drug resistance. The interrogation of translocation-based tumors and identification of ways to expand the view of signal transduction pathways, particularly those involved in metastatic disease, is important. This effort should yield many new insights into tumor pathways and molecular contexts underpinning drug resistance that could be exploited using existing or future therapeutic regimens.

Priority should be given to efforts that approximate the clinical environment linked to drug resistance as closely as possible. Examples include diverse models (e.g., organoids, patient-derived xenografts, co-cultures in physiologic/"hypoxic" conditions, genetically engineered mouse (GEM) models, etc.), and assessment of drug-resistant states in addition to "steady-state" 2-D cell culture. Patient-derived models will be of particular interest but mouse models capable of interrogating aspects of tumor evolution as they relate to drug resistance are also important, particularly as they allow investigators to address these processes in the context of an intact immune system. New technologies that assess drug resistance mechanisms in primary tumor material directly will be a plus. In addition, model systems that assess non-cell autonomous effectors of cancer drug resistance (e.g., derived from the microenvironment or immune cells) would also be of interest.

## **2. Comprehensive characterization of drug-resistant clinical specimens, including 3-dimensional and 4-dimensional cancer cell atlases linked to drug-resistant states.**

Emerging single-cell technologies are making it possible to produce high-resolution characterization of all major cell types (malignant, microenvironment, and immune) in tumor tissues. Both this recommendation and the accompanying recommendation on metastasis could include single-cell and/or multiplexed *in situ* cellular analysis of biopsies obtained from individual cancer patients throughout the course of disease and treatment, including the advent of drug resistance. Single-cell analysis will ideally be combined with new *in situ* technologies that read out cell/tissue topology to ascertain the cellular adjacencies that may influence particular functional states. Moreover, the atlases generated by this approach should be linked to model systems that allow experimental testing of the hypotheses generated. Such information could bring forth major new insights into tumor biology and heterogeneity, as well as cell states that identify new therapeutic targets and predict treatment response in metastasis and drug resistance.

## **3. Develop a collection of drug-resistant cancer models designed to fill key gaps and emphasize areas of unmet medical need.**

For many cancer types, we still lack appropriate experimental model systems that would allow us to study the salient tumorigenic programs linked to drug resistance and to discover new therapeutic targets. Recent years have witnessed advances that could enable a dramatic expansion in various types of models, including cell culture systems (e.g., organoids and tissue slice cultures where cells are in their unperturbed environment), patient-derived xenografts, genetically engineered mouse models, and the possibility of generating tumor-bearing mice with "humanized" immune systems. Thus, the above recommendations may include new cancer model generation that is most representative of clinical areas of unmet medical need.

**Rationale for investing (Why is this priority ripe for accelerating?)**—see above

**Opportunity brought about by recent development in science, technology, practice:** The advent of new tools to perturb cancer cells (e.g., through systematic gain- and loss-of-function studies), to culture such cells ex vivo or in PDX settings, and to conduct serial sampling of tumor cells throughout the course of treatment offer unprecedented opportunities

**Does it address an unmet need or important gap in knowledge or practice?**

The development of drug resistance underlies cancer recurrence and accounts for significant cancer-associated mortality. Notably, despite significant progress made in the treatment of children with cancer, in the U.S. cancer remains the leading cause of death from disease in children, with intrinsic and acquired resistance being central to mortality. With no current means to predict who will develop resistance, or when resistance will arise, there is a substantive gap in knowledge and a clinically unmet need.

**What would be needed for success? For example:**

- **New or expanded resources:** support for serial collection of tumor tissue and blood during treatment and upon frank drug resistance; deployment of technologies and analytical capabilities for high-resolution characterization of these tumor cells prior to treatment, during treatment, and upon resistance; implementation of experimental approaches to perturb appropriate models ex vivo, in vitro, or in vivo;
- **Barriers/roadblocks eliminated or reduced:** support scaling of existing experimental efforts, augment existing infrastructures for biopsies and blood collection; support for data generation efforts; establishment of new computational teams focused on deconvolving the biology linked to resistance
- **New or enhanced technologies:** scalable functional studies (gain-of-function studies, loss of function studies, genome editing efforts); single-cell analysis, high-content tissue topographic analysis, etc.

**Strategy: What will it take to get there?**

- **Concrete actions to take in the next 1-5 years**

We recommend that the cancer moonshot effort pursue a multi-disciplinary effort that consists of both systematic experimental studies and comprehensive characterization of clinical specimens obtained prior to treatment and upon relapse to exemplary cancer therapeutics in selected tumor contexts (targeted therapy, immunotherapy, and/or chemoradiotherapy). Collaborative efforts dedicated to the study of childhood cancers, which could include establishment of centers of excellence, in addition to separate studies of adult cancers should include: 1) adult and pediatric dependency screening; 2) pediatric and adult cancer model generation; 3) preclinical therapeutic testing. In addition, there should be a dedicated effort to develop and test circulating free DNA (cfDNA) methods in pediatric and adult cancers. This effort will incorporate technologies such as single-cell sequencing as well as tissue-based characterization, which may allow specific investigations into the roles of microenvironmental cells and specific patterns of heterogeneity in the overall tumor drug-resistant state. In parallel, both systematic and in-depth functional studies of drug resistance will be conducted using appropriate tumor model systems so that correlative features

observed in clinical specimens could be characterized mechanistically (and conversely, resistance mechanisms identified in vitro could be queried using the clinical data).

**Similar to the “Metastasis” recommendation, these priorities may also require:**

**1. Scalable research biopsy and data generation programs.** These initiatives will require fresh and/or serial biopsies of metastatic and drug-resistant specimens for deep tumor/microenvironmental characterizations and generation of ex vivo models. Thus, the cancer moonshot should support collaborative efforts, such as the establishment and maintenance of centers of excellence, to procure these biopsies at scale and link them to state-of-the-art technologies for data generation and analysis (below Liquid biopsy protocols should be paired with tissue biopsy efforts to provide complementary cancer-derived materials (circulating tumor cells/DNA, exosomes, etc.). Materials obtained from these research biopsies should be seamlessly integrated with workflows capable of generating a wide range of data types.

**2. Computational analysis capabilities.** A critical need exists to develop algorithms that integrate and extract therapeutic meaning from data generated from metastatic biopsies using the latest technologies. Thus, we envision the establishment of collaborative efforts whose mission to design and implement such tools.

**3. Ex vivo cultivation, perturbation, or target validation activities.** Expansion of cancer models in vitro and in vivo would be aided by increased capacity for handling, distributing, and propagating cancer cell line and patient-derived xenograft models. Focused efforts to optimize approaches for generating and maintaining these models, building robust collections, and perhaps hosting research on these models done by individual investigators or moonshot teams should be considered.

**What does success look like?**

A cancer drug resistance landscape project, applied to representative tumor and therapeutic contexts (e.g., specific targeted therapy, immunotherapy, and chemo-radiotherapy regimens) in adult and pediatric cancers, should produce new information about the biology of drug-resistant states that directly informs the development and clinical testing of novel therapeutic combinations. The initiatives should make it possible to non-invasively detect and molecularly characterize recurrences at the earliest possible time point so that salvage therapy can be initiated at a point of minimal tumor burden, with minimal molecular diversity. By the end of five years, several of these might emerge that could be administered up-front in cancer patients and circumvent prevalent drug-resistant states (or even “push” cells into drug-sensitive states).