Brain metastases are one of the most deadly complications of malignancy, and their treatment remains difficult. Two recent articles in Cancer Discovery report deep molecular profiling of matched brain metastases and primary tumors. The first study by Paik and colleagues (1) of 79 stage IV squamous cell lung cancers (SqCLC) used exon capture/next-generation sequencing (NGS) and identified two major subtypes: tumors with FGFR1 amplification and tumors with alterations in the PI3K pathway, comprising PIK3CA mutations and loss of the tumor suppressor PTEN. In total, 61% of patients' tumors harbored a somatic alteration in either FGFR1 or the PI3K pathway, aberrations both potentially targetable with drugs in clinical development. Primary cancers that gave rise to brain metastases exhibited truncal loss of PTEN. There were no clonal differences in PTEN loss or PIK3CA mutations between primary tumors and brain metastases. Interestingly, patients with SqCLC with alterations in the PI3K pathway exhibited a higher metastatic burden and incidence of brain metastases and, importantly, a strikingly shorter overall survival compared with patients without these aberrations. In the second study, in this issue of Cancer Discovery, Brastianos and colleagues (2) interrogated DNA from 86 trios, also with WES. Mutations for the primary and metastases were identified separately by comparison with the matched normal DNA; then the mutations present in the metastasis and the primary tumor were compared with each other. In most cases, the primary tumor and the metastasis shared a “trunk” of mutations, but sites harbored their own subset of unique mutations. Thus, although the primary tumor and metastasis had some shared history, the metastasis diverged from the primary tumor early in their natural history and then both tumors evolved independently. Brastianos and colleagues leveraged copy number from whole-exome data plus mutation allele frequency to estimate the fraction of tumor cells containing each mutation, dubbed the cancer cell fraction (CCF). A CCF of 1.0 means that all tumor cells contain the mutation. The shared truncal mutations had a CCF of 1.0 in both the primary tumor and the brain metastasis. However, both tumor sites harbored mutations with a CCF of 1.0 that were unique to each site, suggesting that all cancer cells in each respective tumor have these mutations. These mutations must have arisen after the brain metastasis and the primary tumor diverged and then grew to dominate each respective site. Importantly, mutations unique to the brain metastases with a CCF of 1.0 were not detected in the primary tumor, not even as a subclonal mutation or a rare population. This is an important result, as it suggests that the primary tumor and the metastasis diverged early and, from then on, followed separate evolutionary histories.
This analysis critically depends on adequate and deep sampling of the primary tumor, because it is possible that a rare subclone is already present in it but is not detected. Brastianos and colleagues (2) calculated the minimum CCF of mutations in each primary sample for which they had a detection power ≥ 95%. In most cases, this number was significantly <10%. For example, in a mouse model of lung cancer, a rare metastasis-founding subclone was detected when a large percentage of the tumor was available for sampling (3). A limitation of these studies is the inability to sample many different regions of the tumor. Indeed, several studies where multiregion sampling and sequencing were performed have demonstrated that unique mutations can be detected in different regions of the primary tumor (4, 5). In these studies, however, CCFs were not estimated. Consistent with the results from these two recent studies, in the few samples tested, the metastasis shared truncal mutations with the primary, but also had a unique set of private mutations as well.

The large cohort of Brastianos and colleagues (2) allowed comparison of brain with extracranial metastases in the same patient as well as comparison of multiple metastases resected at different times from different sites in the brain. Both intrasional heterogeneity (by sampling multiple areas of the same brain metastasis) and interlesional heterogeneity were evaluated in a small subset of patients. All brain metastases from the same patient shared mutations that were not present in the primary tumor, suggesting a role for the brain microenvironment in regulating metastatic tropism and/or brain-specific evolutionary branching. Of potential importance for the treatment of these patients, almost all clinically relevant mutations were shared among brain metastases. These included alterations known to be sensitive to ERBB receptor, PI3K/AKT/mTOR, FGFR, MAPK, and CDK inhibitors. In eight cases that also had an extracranial metastasis, this metastasis had a variable relationship to the brain metastasis. In most cases, although the extracranial and brain metastasis and primary tumor were clearly related, each had private mutations, suggesting that profiling primary tumor and extracranial metastases would be insufficient to predict the profile of brain metastases.

Selection pressures such as chemotherapy or targeted therapies can enrich for subclones within heterogeneous tumors with intrinsic or acquired drug resistance (6, 7). In turn, these subclones can dominate a tumor mass and drive tumor progression. The two studies discussed herein are small and do not provide systematic information on prior treatment. It is interesting, however, that several brain metastases in patients with HER2-amplified breast cancer treated with trastuzumab exhibited additional alterations in HER2 (mutation), EGFR, and PI3KCA that were not detected in the primary tumor. All these lesions are known to confer resistance to trastuzumab (8). These findings challenge the notion that brain metastases are drug resistant for purely “pharmacologic” reasons, i.e., poor drug penetration of the blood–brain barrier. They suggest, first, drug-specific mechanisms of acquired resistance and, second, the need to always molecularly profile these lesions. Actionable mutations identified in brain metastases would assist in the selection of targeted drugs that can be used after resection of these metastases and/or stereotactic radiotherapy in order to control residual microscopic disease in the central nervous system (CNS). In Paik and colleagues (1), all brain metastases exhibited heterozygous loss of PTEN and had a pattern of gene expression consistent with PTEN loss. Almost all of these patients had been previously treated with chemotherapy. These observations coupled with the markedly poor outcome of SqCLC with PI3K pathway alterations suggest that aberrant PI3K signaling is involved in resistance to chemotherapy and cancer progression in the brain and other metastatic sites. Randomized adjuvant trials with PI3K inhibitors in patients with PI3K-mutant SqCLC using time to recurrence in the brain as one endpoint may determine if altered PI3K signaling is causal to brain metastases.

The shared truncal (clonal) mutations provide additional molecular evidence to what has been established clinically, that subclinical micrometastases are present in the CNS at the time of diagnosis of an early surgically resected cancer. If these were pathogenic to the cancer, postsurgical (adjuvant) systemic therapies that penetrate the brain with drugs targeting those truncal mutations could eliminate these micrometastases. This is important because current drug development and diagnostic approaches using NGS for genotype-specific trials only consider the presence or absence of a driver mutation but do not take into account their clonal or subclonal frequency. Although this makes intuitive sense, we recognize that therapeutically targeting a clonally dominant, truncal driver has not yet been shown to be a more effective drug development strategy (9). If important for treatment decisions, quantification of this heterogeneity and clonality could have a major impact in the development of companion diagnostics for genotype-specific drugs (10). Of note, the Deciphering Anti-tumor Response With Intratumor Heterogeneity (DARWIN) trial will test whether targeting a clonally dominant driver event results in a better outcome compared with targeting the same driver mutation when present subclonally. The two studies discussed herein provide additional rationale for studies like the DARWIN trial.

Both of these studies were conducted in tumors from patients with stage IV solid tumors for whom currently there are no curative therapies. Although targeted therapies may induce a clinical response in patients with metastatic disease selected based on a genomic alteration in their tumor, these responses are transient. The primary tumor–metastasis and intermetastatic heterogeneity reported in the studies by Paik and colleagues (1) and Brastianos and colleagues (2) has been associated with multiple mechanisms of intrinsic and acquired drug resistance and supports the transient responses (or inefficacy) seen with single-agent–targeted therapies. These studies also reinforce the concept of targeting truncal events with neoadjuvant or adjuvant systemic therapy before micrometastases become clinically apparent (the definition of stage IV incurable cancer) and before there is time for evolutionary divergence, which promotes tumor growth at metastatic sites and drug resistance.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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