

Power in Numbers: Meta-analysis to Identify Inhibitor-Sensitive Tumor Genotypes

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Widespread tumor genotyping has increased the complexity of lung cancer care, often identifying mutations of uncertain clinical significance. In the accompanying article, the authors carried out a meta-analysis of the published literature on EGF receptor (*EGFR*) genotype and erlotinib/gefitinib sensitivity to develop a publicly accessible database to inform patient care. *Clin Cancer Res*; 19(7); 1634–6. ©2013 AACR.

In this issue of *Clinical Cancer Research*, Yeh and colleagues present a new strategy to address the complexity of genotype-directed cancer therapy: using meta-analysis of the published literature to develop a database of actionable somatic EGF receptor (*EGFR*) mutations (1). Genotyping of *EGFR* is now a standard component of non-small cell lung cancer (NSCLC) care, as with *KRAS* and *BRAF* for colorectal cancer and melanoma, respectively. But when these biomarkers were first developed, early studies intentionally simplified the complexity of tumor genotype by dichotomizing cancers into "mutant" or "wild-type". In this fashion, drug-sensitive phenotypes were identified: *KRAS*-wild-type colon cancers gain a survival benefit from cetuximab, *EGFR*-mutant lung cancers are best treated with *EGFR* tyrosine kinase inhibitors (TKI), and *BRAF*-mutant melanomas gain unique benefit from vemurafenib.

However, closer scrutiny of the data reveals a greater degree of complexity. The pivotal studies of first-line gefitinib and erlotinib were limited to cancers carrying *EGFR* exon 19 deletions or L858R mutations. The landmark study of cetuximab efficacy in colorectal cancer only included sequencing of *KRAS* exon 2 and the BRIM3 trial of vemurafenib versus dacarbazine only randomized melanomas harboring V600E mutations. Is the drug-sensitive phenotype limited to the genotypes studied in these landmark randomized trials? This question is particularly important because genotyping does not only detect these genotypes but less common somatic mutations are also detected, though many are not well established as clinical biomarkers. To achieve

precision genotype-directed therapy, we must determine how to manage patients harboring these uncommon genotypes.

In the case of NSCLC, less common mutations constitute 10% to 15% of all *EGFR*-mutant lung cancer. Yet, their exclusion from many pivotal trials leaves clinicians uncertain as to whether they are best treated with targeted therapy or conventional chemotherapy. Because the low prevalence of these genotypes makes them difficult to study, several approaches (Fig. 1) have been used to gain an understanding of their behavior:

- Single-center experiences: Several cancer centers in Asia and the United States have been routinely conducting genotyping since *EGFR* mutations were first discovered nearly a decade ago. On the basis of these experiences, it has been identified that G718X and L861Q mutations are associated with a 53% to 60% response rate to erlotinib or gefitinib (2), that exon 20 insertions are unlikely to respond and are associated with a poorer survival (2, 3), and that a subset of exon 19 deletions is less likely to respond to erlotinib or gefitinib (4). The primary limitation of single-center experiences is the possibility that results are specific to certain assays and may not generalize.
- Multicenter experiences: Collaborative efforts between multiple cancer centers can create an opportunity to study genotypes too rare to study as a single center. For example, combining the experiences of 2 large referral centers, a recent study of 12 patients with exon 19 insertions found these mutations to be associated with TKI sensitivity (5). The most comprehensive multicentered effort may be the French HERMETIC IFCT network, through which 1048 *EGFR*-mutant cancers were identified over a 6-year period (6); results have not yet been published. Multicentered trials have occasionally included patients with uncommon *EGFR* mutations, though generally a small number; the LUX-Lung 3 trial of afatinib versus chemotherapy included 37 patients with uncommon mutations, with objective responses seen in association with several uncommon genotypes (7).

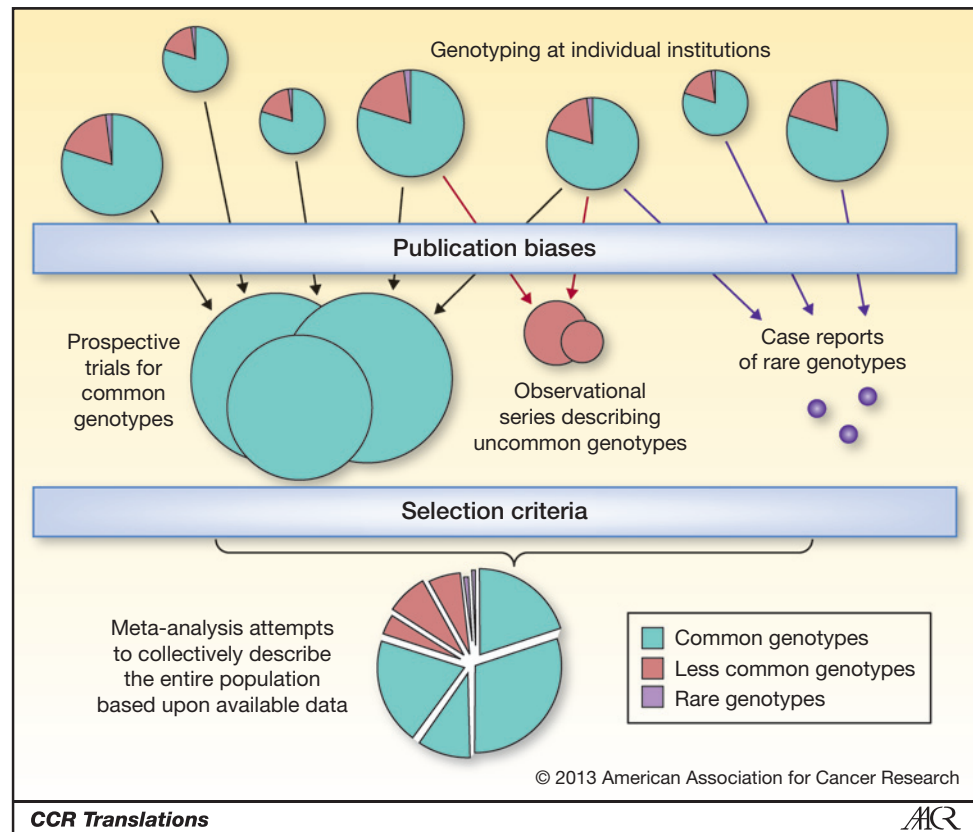
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Figure 1. Approaches to studying and interpreting tumor genotypes. Genotyping carried out as part of routine clinical care occasionally identifies uncommon genotypes, which may lend sensitivity to targeted therapy (top). However, not all data on specific genotypes and their association with drug sensitivity reaches the published literature (middle). Common genotypes are often included in prospective trials (aqua), less common genotypes may be described in observational series (red), and the rarest genotypes may only be presented as case reports (purple). Meta-analysis can allow collective study of these individual published experiences (bottom), though the completeness of the data inherently depends upon selection criteria as well as publication biases.



- Meta-analysis: To circumvent the logistical challenges of multicentered genotyping efforts, meta-analyses can use the published literature to systematically collect and analyze independent datasets. The best established meta-analysis of genomic data is the Catalogue Of Somatic Mutations In Cancer database run by the Sanger Institute (Hinxton, Cambridgeshire, England; ref. 8). A central aim of this effort is to identify the frequency of somatic mutations, but the database does not associate these genotypes with sensitivity to targeted therapies.

The article by Yeh and colleagues (1), accompanying this editorial, represents the first published meta-analysis, to our knowledge, describing the published literature on *EGFR* genotype and TKI sensitivity. Searching PubMed, the authors identified and reviewed 2,085 articles published between June 2005 and May 2011. They limited their analysis to 146 articles (7%), which included individual patient-level detail on *EGFR* genotype and response evaluation criteria in solid tumors response to erlotinib or gefitinib. This included 1,021 patients with 207 different *EGFR* genotypes. Importantly, the meta-analysis includes many patients from the well-characterized Taiwanese experience (2). The authors associated 72% of these individual genotypes with disease control based on a disease control rate (DCR) of at least 50%.

Are the findings from this meta-analysis consistent with other published literature? Yes, particularly as several key

articles have been incorporated into the analysis. The authors, however, categorize treatment outcomes into disease control (complete response, partial response, or stable disease) and disease progression, an approach that may overestimate the clinical effect of *EGFR* inhibitors against a particular *EGFR* genotype. High rates of objective response to targeted therapy have been seen repeatedly with oncogene-addicted cancer types. What's more, a meta-analysis of *EGFR* TKI trials found that response rate more closely correlates with survival prolongation than does DCR (9). Clarity on this issue is particularly important if the clinicians are going to use this information to decide whether to use a first-line TKI or chemotherapy for a patient with NSCLC harboring a rare *EGFR* genotype.

Can anything new be learned from this meta-analysis of rarer *EGFR* mutations? Currently, 67% of genotypes studied occurred in a single patient and only 12 occurred in more than 4 patients. One reason this series includes fewer patients than might be expected is because many larger articles, including most describing trial results, do not publish patient-level data on genotype and response. To overcome this weakness, the authors have established a database aiming to collect such data going forward: the DNA mutation Inventory to Refine and Enhance Cancer Treatment (DIRECT). It is anticipated that more information on rare genotypes will become available as the database grows. At present, the most intriguing data is that of 9 patients with the poorly described S768I mutation (8 of

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these occurring with various comutations), 6 had a response to TKI.

Perhaps the biggest limitation to the success of the DIRECT database will be the challenge of quality control. Is it appropriate to collectively study mutations identified in different laboratories using different assays? For example, one highly sensitive assay for T790M was removed from the market due to problems with false positive results (10); such "T790M-positive" cases might not exhibit resistance to TKI, whereas T790M detected using Sanger sequencing in a clinical laboratory is clearly associated with TKI resistance (and risk of an underlying germline mutation; ref. 11). Some genotyping assays only test for a limited set of mutations, potentially missing important comutations that would be identified using direct sequencing. Finally, a rare mutation identified in a research laboratory could potentially represent a sequencing artifact rather than a true oncogenic alteration. A strategy to help interpret the clinical relevance of the mutations in DIRECT would be to capture the type of assay used and whether it was carried out in a clinically certified laboratory. This is especially relevant given the poorer outcomes described when gefitinib was given to patients with lung cancer with low abundance

EGFR mutations only detected using a highly sensitive assay (12). Although for some rare genotypes, collective study using meta-analysis may be our only option for characterizing drug sensitivity, results from a single-center experience will inherently be more transparent until all centers use a single genotyping assay and share a similar degree of expertise.

Disclosure of Potential Conflicts of Interest

G.R. Oxnard is a consultant/advisory board member of Genentech and Boehringer Ingelheim. P.A. Jänne is a consultant/advisory board member of Astra Zeneca, Boehringer Ingelheim, Pfizer, Roche, Genentech, and Sanofi and receives licensing fees from Lab Corp related to intellectual property.

Authors' Contributions

Conception and design: G.R. Oxnard, P.A. Jänne

Writing, review, and/or revision of the manuscript: G.R. Oxnard, P.A. Jänne

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