Tumor heterogeneity encompasses interpatient heterogeneity, intrapatient heterogeneity among disease sites within a patient, and intratumoral heterogeneity within a single disease site. While tumor profiling provides a glimpse of interpatient heterogeneity, oncologists lack adequate tools in our diagnostic toolkit to fully characterize intrapatient and intratumoral heterogeneity, which remain critical barriers to developing targeted anticancer therapies, particularly in gastroesophageal adenocarcinoma (GEA).\textsuperscript{1-3} Although distal gastric cancer, historically associated with \textit{Helicobacter pylori}, is becoming less common, proximal cancers arising near the gastroesophageal junction are becoming more common in Western countries owing to their associations with obesity and the Western diet.\textsuperscript{4} These cancers have extensive chromosomal instability leading to copy number alterations, including amplifications of genes encoding potentially actionable receptor tyrosine kinases, such as \textit{ERBB2} (OMIM 164870), \textit{EGFR} (OMIM 131550), \textit{MET} (OMIM 164860), and \textit{FGFR2} (OMIM 176943).

Trastuzumab is approved for patients with \textit{ERBB2}-positive GEA based on the landmark ToGA trial,\textsuperscript{5} which demonstrated a significant survival advantage for trastuzumab. However, subsequent phase 3 trials targeting \textit{ERBB2} (ie, TyTan, TRI0-013/L0GIC, GATSBY, and JACOB) have found no statistically significant differences, despite successes with the same therapies in \textit{ERBB2}-positive breast cancer. Beyond lack of drug efficacy, the failure of these trials was likely associated with the greater intratumoral heterogeneity of \textit{ERBB2} positivity that has been documented for GEA compared with breast cancer. Indeed, GEA cells can commonly harbor \textit{ERBB2} amplification in combination with secondary oncogene amplifications\textsuperscript{6} or tumors where \textit{ERBB2} is amplified in only a subset of the tumor cells. Either state would likely have diminished benefit from \textit{ERBB2}-targeted agents. Currently, we lack metrics for tumor heterogeneity in the clinical setting.

To this end, Chao et al\textsuperscript{7} performed a retrospective analysis of 41 patients who had undergone resection of their primary tumor from 1989 to 2013 without receipt of neoadjuvant therapy to identify the prognostic significance of GEA intratumoral heterogeneity on survival. Heterogeneity was characterized using 2 approaches. First, Chao et al used a single nucleotide variation (formerly \textit{single nucleotide polymorphism}) array for copy number alteration quantification, assessing only a single region of the tumor from the resected specimens with clonality inferred using a previously published bioinformatic pipeline. Kaplan-Meier survival analysis was then performed stratified by heterogeneity (\(\geq 2\) clones or not). As expected, copy number changes were more commonly seen in the proximal tumors, where chromosomal instability predominates, than in the distal stomach, where Lauren diffuse-type tumors more commonly occur. Nearly half of patients had at least 2 clones identified. However, even after accounting for stage, adjuvant therapy, and Lauren histological subtype, patients with heterogeneous tumors exhibited worse survival (multivariate hazard ratio, 4.55; 95% CI, 1.09-19.04; \(P = .04\)).

Second, in conjunction with the computational approach to demonstrate heterogeneity, Chao et al\textsuperscript{7} also performed multisite and multiprobe fluorescence in-situ hybridization (FISH) in a subset of patients with heterogenous tumors to ascertain the spatial distribution of these potentially targetable copy number alterations. By evaluating multiple sites within 5 mm of each other, Chao et al uncovered heterogeneous amplifications of \textit{MET}, \textit{EGFR}, \textit{FGFR2}, and \textit{PIK3CA} (OMIM 171834). Multisite FISH in these patients highlights how the initial computational approach underestimated the degree
of intratumoral heterogeneity found in these tumors, which further highlights the utility of more comprehensive testing. Chao et al acknowledge several limitations of their retrospective study, including use of untreated primary tumors and lack of targeted therapy data to correlate with their findings. If the study had evaluated heterogeneity between synchronous disease sites or at multiple time points, they would have likely found even more extensive heterogeneity and that therapeutic selective pressure determines clonal fitness and thus, proliferation.

These analyses have striking implications for targeted therapy trial design. Targeted therapy trial enrollment can depend on immunohistochemistry expression or next generation sequencing amplification of a biomarker on a single biopsy specimen. Aside from the fact that many studies use archival specimens that may not reflect the time of enrollment, the findings from the study by Chao et al suggest that biopsy results may depend on which region of the tumor is sampled by the endoscopist and whether the 4- to 6-μm slide from a biopsy sample (often measuring ≤10 mm) contains the biomarker in question. Chao et al demonstrated in 1 instance that a difference of 0.67 mm may have determined if a patient was enrolled in an FGFR2-selected trial. Returning to the ToGA example, this may explain how some patients' biopsy results were immunohistochemistry-negative, yet FISH-amplified (albeit recognizing the lower cutoff for FISH positivity in ToGA) and why if these patients' tumors were more heterogenous, their inferior survival should not be surprising. Similarly, coalteration of genes, such as KRAS (OMIM 190070) or PIK3CA, are also associated with inferior survival; and it could be inferred from the study by Chao et al that even when not identified, resistant clones likely preexist targeted therapy in many patients.

These results have several implications. One is that trials have probably rejected therapies for patients with GEA that might have been successful if given to a better-selected patient population. Indeed, it is likely that several of the ERBB2 agents that are successful in breast cancer would had efficacy in a homogenously ERBB2-positive subpopulation. Additionally, although not approved by the US Food and Drug Administration, therapies against targets such as EGFR or FGFR2 will likely have efficacy in tumors with more homogenously positive amplification of these targets. Improved biomarker strategies will likely require multiregion sampling or integration of tissue-based biomarkers with assessment of cell-free DNA. Furthermore, we will have to address the question of how to optimally target tumors in which marked heterogeneity occurs, as these patients will likely be either refractory to oncogene-directed therapy or will be prone to rapid emergence of resistant disease. Such therapies are anxiously awaited in this patient population.


