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ARTICLE

Biomarker Testing for Breast, Lung, and Gastroesophageal Cancers at NCI Designated Cancer Centers

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Background Molecular biomarkers, a cornerstone of precision oncology, are critical in breast, gastroesophageal, and non–small cell lung cancer management (BC, GEC, NSCLC). Testing practices are intensely debated, impacting diagnostic quality and affecting pathologists, oncologists and patients. However, little is known about testing approaches used in practice. Our study described biomarker practices in BC, GEC, and NSCLC at the leading US cancer centers.

Methods We conducted a survey of the National Cancer Institute (NCI) designated centers on BC, GEC, and NSCLC biomarker testing. We used simple frequencies to describe practices, two-sided Fisher's exact test and two-sided McNemar's test for cross-cancer comparison. All statistical tests were two-sided.

Results For BC human epidermal growth factor receptor 2 (HER2), 39% of centers combine guidelines by using in situ hybridization (ISH) and immunohistochemistry (IHC) concurrently, and 21% reflex-test beyond guideline-recommended IHC2+. For GEC HER2, 44% use ISH and IHC concurrently, and 28% reflex-test beyond IHC2+. In NSCLC, the use of IHC is limited to 4% for epidermal growth factor receptor (EGFR) and 7% for anaplastic lymphoma kinase (ALK). 43.5% test NSCLC biomarkers on oncologist order; 34.5% run all biomarkers upfront, and 22% use a sequential protocol. NSCLC external testing is statistically significantly higher than BC ($P < .0001$) and GEC ($P < .0001$). NSCLC internally developed tests are statistically significantly more common than BC ($P < .0001$) and GEC ($P < .0001$).

Conclusions At the NCI cancer centers, biomarker testing practices vary, but exceeding guidelines is a common practice for established biomarkers and emerging practice for newer biomarkers. Use of internally developed tests declines as biomarkers mature. Implementation of multibiomarker protocols is lagging. Our study represents a step toward developing a biomarker testing practice landscape.

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Molecular biomarkers play a transformative role in cancer management and facilitate emergence of precision oncology (1–6). Over 600 molecular tests are included in oncology guidelines (7), and a host of new biomarkers will soon enter clinical practice (8,9). For many biomarkers, multiple testing methods and algorithms are available, each with limitations, causing quality variation and fueling ongoing debate on which testing practice is best (10–13). Guidelines include a range of alternative testing methods and algorithms, leaving room for institutional choice of practices (14–20). The knowledge of real-world practices, among other factors, may inform institutional decisions and the overall debate on biomarker testing. However, this real-world knowledge is presently limited. We undertook this study to identify the practices National Cancer Institute (NCI)–designated cancer centers employ for biomarker testing in three common cancers. Our goal is to take a step toward developing a practice landscape for cancer biomarker testing, starting with NCI cancer centers—oncology thought leaders in the United States.

Biomarker testing practices affect accuracy and timeliness of results that guide decisions on potentially life-saving therapies (10,13,17,20,21). Thus, these practices are of keen interest to not only pathologists, but also oncologists, surgeons, radiologists, and other disciplines involved in oncology care. Notably, experts call for multidisciplinary involvement in institutional decisions on biomarker testing (17,20,22–24). Payers, patients, and the general public are also drawn into the discussion, as illustrated by testing for human epidermal growth factor receptor 2 (HER2) in breast cancer (25–31).

Breast cancer (BC), gastric/esophageal cancers (GEC) and non–small cell lung carcinomas (NSCLC) are among cancers where biomarkers and targeted therapies have brought dramatic benefit: they transformed management of BC (32–34), extended survival in

advanced GEC (35), and revolutionized management of NSCLC (Table 1) (36–39). Biomarker testing practices in these cancers are the subject of keen interest and extensive debate (13,17–19,22,55–60,78–81), with a spectrum of available testing methods and approaches, as summarized in Table 1. However, knowledge of practices employed at US cancer centers is limited to reports of institutional experiences (76,96,102,104–108) and expert estimates (45). Proficiency surveys, although highly important, rightfully focus on technical aspects rather than algorithm and protocol practices (109,110).

Table 1. Biomarkers included in this study

Marker/protocol	Clinical relevance	In practice since	Testing methods* and algorithms†	Relevant issues	Why important example of practices
Breast cancer					
HER2	Predicts response to anti-HER2 therapies: trastuzumab (32–34), lapatinib (40,41), pertuzumab (42), ado-trastuzumab emtazine (43)	1998	Multiple methods exist: IHC, ISH (FISH, CISH, SISH, DISH) (44–49); other methods are studied (eg, RT-PCR50) Guidelines recommend 2 algorithms: "IHC, reflex‡ IHC2+ to ISH" and "ISH, confirmed by counting additional cells, repeat ISH or reflex to IHC" (14,20) Literature also recommends expanded reflex testing to increase accuracy: reflex IHC0-IHC1+ ("believe the positive") algorithm (51); reflex IHC3+ (52–54)	Accuracy and reproducibility of methods, especially FISH vs IHC, are hotly debated (11,55-60) FISH more accurate but more costly, more complex than IHC and other ISH; IHC widespread, cheaper but more prone to quality problems (44,45) >46 studies compare FISH and IHC (61), but no consensus; other methods less studied (45,61) Testing quality problems found in the past (62–64); national efforts to improve quality (17,65); quality is improving but still deficient (66)	HER2 expanded to gastric cancers; is studied in other cancers (67–69) HER2 challenges are relevant to other markers where multiple testing methods and algorithms are debated (10,59,70) Future new markers may complicate HER2 algorithm and require multibiomarker protocols (11)
ER, PgR	Prognosis; stratification; ER guides use of endocrine therapy (71–73)	1970s	IHC is the only method recommended in guidelines (21)	Testing quality problems found (74) National efforts to improve quality (21)	IHC is broadly used for other markers; studied as a screening method for molecular testing, eg, for ALK, EGFR (75,76)
Multibiomarker protocols	Confirm triple-positive or -negative diagnoses	N/A	Retest HER2 for triple-positive (HER2+, ER+, PgR+) or triple-negative (HER2-, ER-, PgR-) results to ensure quality	Emerging practice to ensure accurate biomarker assessment (77)	Example of a potential emerging practice to improve testing quality for most difficult patient subgroups
Gastric and esophageal cancers (GEC)					
HER2	Predicts response to trastuzumab in advanced cancers (35)	2010	Multiple methods exist: IHC, ISH (FISH, BISH, CISH, dc-SISH, dc-CISH) (19,78,79) Guidelines recommend "IHC, reflex IHC2+ to ISH" (16) Literature also recommends: ISH (68,78,80) concurrent ISH and IHC (80,81); expanding reflex testing: IHC1+ (82), IHC0-IHC1+ (for biopsy samples) (83)	Lack of detailed guidelines similar to ASCO CAP guidelines in breast HER2 testing (81,83) Testing algorithms differ from BC HER2 (35,84) Relatively small number of studies to inform practices (81,83) Emerging debate on FISH vs IHC and ISH methods (79,85)	Opportunity to understand how testing practices for one marker expand to additional tumor sites The number of biomarkers in GEC is expected to increase (86,87),requiring future multimarker protocols

(*Table continues*)

Table 1 (Continued).

Marker/protocol	Clinical relevance	In practice since	Testing methods* and algorithms†	Relevant issues	Why important example of practices
Non–small cell lung cancer, nonsquamous carcinomas (NSCLC)					
EGFR	Predicts response to EGFR inhibitors (gefitinib, eroltinib) (36–38)	2009	EGFR mutation testing is guideline recommended (15,18,88); multiple methods exist (sequencing, PCR, other) IHC is emerging but controversial (12): IHC proposed as screening, followed by molecular analysis (18,75) EGFR amplification is not guideline recommended (18)	Guidelines leave room for multiple methods: any validated EGFR mutation test can be used (18) Various methods have pros and cons (89,90)Emerging debate on methods, including IHC algorithms (70,75)	Example of how HER2 debate on testing methods permeates new biomarker areas (70,75)
ALK	Predicts response to ALK inhibitor crizotinib (39)	2011	FISH is FDA approved and guideline recommended (15,18); other methods are emerging: IHC (in an algorithm with FISH) (18,91–93), RT-PCR (94,95), CISH (95)	Remarkably rapid time from discovery to approval (23,96) FISH gold standard, but more costly, complex; IHC widespread, easier, cheaper (76,91-93) Debate emerges on which method and algorithm are best (76,92,93)	Example of a rapidly developed biomarker, requiring expedient implementation (23,96) Example of how HER2 debate on testing methods permeates new biomarker areas (76,91–93)
KRAS	Prognosis (15); role as predictive marker for therapy controversial (12,18,97)	2000s	May be used as screening test to "rule-out" EGFR and ALK mutations (18) Methods used: RT-PCR, pyrosequencing, Sanger sequencing (98)	Guidelines vary (15,18)	Novel use of a test as a rule-out may be applicable to other cancers where markers are mutually exclusive
Multimarker protocols§	Facilitate testing of multiple biomarkers	N/A	EGFR, ALK, and KRAS mutations are mutually exclusive, allowing a sequential "rule-out" protocol (18,91,99) Literature recommends various sequential protocols (18,91,99) or concurrent testing (96,100)	Protocols are debated and practices vary (13,18,76,100–102); guidelines recommend 5–10 day result turnaround time (18) Concurrent testing provides rapid turnaround and better sample management (13,96,101) Sequential testing is more economical, but longer total turnaround time (18)	NSCLC is a paradigm for multimarker testing in cancer (103)

* Method, type of technology for testing a biomarker. ALK = anaplastic lymphoma kinase; ASCO = American Society of Clinical oncology; BISH = bright-field situ hybridization; CAP = College of American Pathologists; CISH = chromogenic in situ hybridization; dc-CISH = dual color chromogenic in situ hybridization; dc-SISH = dual color silver in situ hybridization; DISH = dual in situ hybridization; EGFR = epidermal growth factor receptor; FDA = Food and Drug Administration; FISH = fluorescence in situ hybridization; HER2 = human epidermal growth factor receptor 2; IHC = immunohistochemistry; ISH = in situ hybridization; KRAS = v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; PgR = progesterone receptor; RT-PCR = reverse transcription polymerase chain reaction; SISH = silver in situ hybridization.

† Algorithm, a sequence of methods for testing one biomarker, when multiple methods are used.

‡ Reflex testing, confirming equivocal or borderline results by another method.

§ Multibiomarker protocol, a sequence of testing multiple biomarkers.

Our study included clinically relevant biomarkers in BC, GEC, and NSCLC (Table 1): HER2, estrogen receptor (ER), progesterone receptor (PgR) in BC, HER2 in GEC, and epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), and v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) in NSCLC.

Taken together, BC, GEC, and NSCLC represent an epitome of molecular profiling practices. The HER2 debate, notably immunohistochemistry vs fluorescence in situ hybridization (FISH), is relevant to other cancers where HER2 amplification becomes a therapeutic target (67–69) and is permeating other areas (ALK, EGFR) where immunohistochemistry is proposed to augment existing methods (70,75,76,91,92). GEC exemplifies how testing practices transcend across cancers (81), and NSCLC has become a paradigm for multibiomarker profiling (103).

Introduction of new biomarkers is expected to accelerate (6,8,9,96,111) with potential application of targets across cancers (112) and adoption of next generation sequencing of tumor tissue (113–118). Understanding the current testing landscape may contribute lessons learned across cancers and inform integration of new biomarkers and technologies into practice. Our study is a step toward developing such a practice landscape.

Methods

Study Design and Survey Development

The study was approved by the institutional review board of Northwestern University. We developed a cross-sectional internet-based survey, with expert input from three pathologists and three oncologists in BC, GEC, and/or NSCLC. We piloted the survey individually with six pathologists and six oncologists in BC, GEC, and/or NSCLC and adjusted the instrument based on their feedback. The final survey instrument contained 12 BC, three GEC, and eight NSCLC questions about methods for testing individual biomarkers, multimethods algorithms for assessing one marker, multibiomarker testing protocols, whether testing was conducted internally or externally, and whether commercial tests or internally developed tests were used (Supplementary Methods, available online). The instrument contained multiple-choice questions, including "not used" and "I don't know" options as relevant. A comment area followed each question. An internet survey provider (www.surveymonkey.com, SurveyMonkey, Palo Alto, CA) was used to configure and administer the survey.

Survey Sample and Recruitment

The unit of analysis was cancer centers. We followed the American Association for Public Opinion Research (AAPOR) guidelines for establishment surveys to identify target respondents (119). Of the 66 NCI-designated cancer centers listed on the NCI website (120), we identified 58 providing adult oncology care. We contacted directors of the 58 centers asking to identify target respondents—pathologists and oncologists in BC, GEC, and NSCLC. The majority of directors (75%) provided contact information. We identified target respondents for remaining centers from their websites. We included additional contacts when suggested by our target respondents.

Data were collected between July and November 2012. Individual emails were sent from the principal investigator describing the study and providing a link to the survey. A study investigator tracked responses to study identification numbers to determine which centers and/or targets had responded, and to link individual responses to the center level in an anonymous secure fashion. No other investigator had access to the identifiable information. Up to three follow-up emails were sent to nonrespondents, following the Dillman tailored design method for internet surveys (121).

Statistical Analysis

Information collected was coded and rolled up to de-identified institutions following the AAPOR establishment survey methodology (119). If only one participant responded from an institution, that response was used. If multiple responses were received per institution, two investigators independently coded the consented results to the institution identification number according to a predetermined hierarchy. The investigators compared their coding and had no conflicts at the institutional level. Coding was reviewed by the principal investigator. Coded results by de-identified institution were entered into statistical software (STATA 12, StataCorp, College Station, TX). Simple frequencies were used to describe practices, two-sided McNemar's test of matched proportions to compare BC and GEC HER2 testing practices, and two-sided Fisher's exact test to compare in-house testing and development practices between cancers. All statistical tests were two-sided.

Results

We achieved an overall survey response rate of 98% (57/58 institutions), with response rates of 98% to BC, 93% to GEC, and 95% to NSCLC questions. We found no conflict in responses from participants within sites. Table 2 summarizes respondents' characteristics.

Breast Cancer Biomarker Testing

All BC respondents conduct HER2, ER, and PgR testing. For HER2 testing, 81% use IHC, all in an algorithm with ISH. All sites use ISH, alone or in an algorithm with IHC: 91% use fluorescence

Table 2. Respondent institution characteristics*

Characteristic	No. (%)
Designation	
NCI-designated Comprehensive Cancer Center	57 (100)
NCCN member institution (23 total) (122)	23 (40)
Location	
Located in large metropolitan area	30 (53)
Located in nonmetropolitan area	27 (47)
Geography	
Geography - Midwest United States	13 (23)
Geography - Northeast United States	13 (23)
Geography - Southern United States	17 (30)
Geography - Western United States	14 (25)
Institution size (based on the number of medical oncologists	
Larger number of medical oncologists (>40)	27 (47)
Smaller number of medical oncologists (≤40)	30 (53)

* NCCN = National Comprehensive Cancer Network; NCI = National Cancer Institute.

in situ hybridization (FISH), 5% use silver in situ hybridization (SISH), 4% use chromogenic in situ hybridization (CISH), and 2% (one site) use FISH and CISH (Table 3). Among the seven distinct algorithms reported for HER2 testing, "IHC, reflex to FISH" is most common (42%), followed by concurrent FISH and IHC testing (30%), and by "FISH alone" (18%). Concurrent IHC and SISH or CISH testing is conducted at 9% of respondents, and one site uses "CISH only." Overall, 39% of centers use IHC and a type of ISH (FISH, SISH or CISH) testing concurrently. While all 24 institutions utilizing HER2 "IHC, reflex to FISH" algorithm reflex-test IHC2+ results, 50% (12/24) of them, or 21% (21/57) of all responding centers, also reflex other IHC results. Of the 12 institutions reflex-testing beyond IHC2+, 58% (7/12) do this as a standard protocol and 42% (5/12) on oncologist order. All respondents conduct ER and PgR testing by IHC, with 14% using a second test for ER and 11% for PgR. Retesting HER2 for triple-negative and/or triple-positive cancers occurs at 11% of sites.

Table 3. Breast cancer biomarker testing methods, algorithms, and protocols (HER2, ER, PgR)

Characteristic, n = 57	No. (%)
Methods* and multimethod algorithms†	
HER2	
HER2 IHC, reflex test‡ to FISH	24 (42)
Reflex test when result = IHC2+	*12 (50)*
Reflex test when result = IHC0, 1+, 2+, and 3+	*5 (21)*
Reflex test when result = IHC2+ and 3+	*3 (13)*
Reflex test when result = IHC1+ and 2+	*2 (8)*
Reflex test when result = IHC0, 1+ and 2+	*1 (4)*
Reflex test when result = IHC1+, 2+, and 3+	*1 (4)*
Concurrent HER2 FISH and IHC	17 (30)
HER2 FISH	10 (18)
Concurrent HER2 IHC and SISH	3 (5)
Concurrent HER2 CISH and IHC	1 (2)
Concurrent HER2 CISH, FISH, and IHC	1 (2)
HER2 CISH	1 (2)
ER	
ER- IHC	49 (86)
ER IHC and RT-PCR	5 (9)
ER IHC and LBA	3 (5)
PgR	
PgR- IHC	51 (89.5)
PgR IHC and RT-PCR	4 (7)
PgR IHC and LBA	2 (3.5)
Multibiomarker protocols§	
Retest HER2 for triple-negative cancers‖	3 (5)
Retest HER2 for triple-positive¶ and triple-negative cancers	2 (4)
Retest HER2 for triple-positive cancers	1 (2)

* Method, type of technology for testing a biomarker. Italicized choices represent various algorithms for reflexing to FISH test based on IHC results (0, 1+, 2+ and/or 3+). CISH = chromogenic in situ hybridization; FISH = fluorescence in situ hybridization; HER2 = human epidermal growth factor receptor 2; IHC = immunohistochemistry; LBA = ligand-binding assay; PgR = progesterone receptor; RT-PCR = reverse transcription polymerase chain reaction; SISH = silver in situ hybridization.

† Multimethod algorithm, a sequence of methods for testing one biomarker, when multiple methods are used.

‡ Reflex test, confirming equivocal or borderline results by another method.

§ Multibiomarker protocol, a sequence of testing multiple biomarkers.

‖ Triple negative breast cancer, HER2-, ER-, PgR-.

¶ Triple positive breast cancer, HER2+, ER+, PgR+.

Gastric/Esophageal Cancer Biomarker Testing

All GEC respondents conduct HER2 testing (Table 4). IHC is used by 83% of respondents, all in an algorithm with ISH. All respondents use an ISH method, most commonly FISH (94%). Concurrent FISH and IHC testing is most common (41%), followed by "IHC, reflex to FISH" (39%), "FISH alone" (13%), and concurrent "IHC and SISH or CISH" (4%). Overall, 44% of centers use IHC and a type of ISH (FISH, SISH or CISH) concurrently. Among the 21 sites using "IHC, reflex to FISH" algorithm, all reflex-test IHC2+ results, as recommended in guidelines, and 71% (15/21) of these sites, or 28% (15/54) of all responding sites, reflex-test at least one other IHC result. Among the 15 sites conducting expanded reflex testing, 53% (8/15) do so as a standard protocol and 47% (7/15) on oncologist order.

Non–Small Cell Lung Cancer Biomarker Testing

All NSCLC section respondents conduct EGFR and ALK testing, and 96% test KRAS (Table 5). To determine EGFR status, all respondents test for EGFR-activating mutations, most commonly with PCR (44%), followed by sequencing (16%), and by IHC (4%). Forty-five percent also assess EGFR gene amplification. For ALK testing, 64% use FISH—alone (58%) or in an algorithm with IHC or PCR (6%). IHC is used by 7% (4/55) of respondents—in an algorithm with FISH and/or PCR (6%) or alone (2%). At 43.5% of institutions, the sequence of NSCLC biomarker testing is determined by an oncologist's order: 34.5% run all biomarkers upfront for newly diagnosed patients, and 22% use a sequential protocol, with five different protocols reported. Among those testing sequentially, 50% (6/12) run KRAS upfront, alone or with EGFR, while others test EGFR first, alone or concurrently with ALK.

Practice Comparison Across Cancers

Comparing the use of BC and GEC HER2 testing practices among 54 respondents to both sections, we determined that 61%

Table 4. Gastric and esophageal cancer biomarker testing methods and algorithms (HER2)

Characteristic, n = 54	No. %
Methods* and multimethod algorithms†	
Concurrent HER2 FISH and IHC	22 (41)
HER2 IHC, reflex test‡ to FISH	21 (39)
Reflex test when result = IHC2+	*6 (29)*
Reflex test when result = IHC0, 1+ and 2+	*6 (29)*
Reflex test when result = IHC1+ and 2+	*3 (14)*
Reflex test when result = IHC2+ and 3+	*3 (14)*
Reflex test when result = IHC0, 1+, 2+, and 3+	*2 (9)*
Reflex test when result = IHC0 and 2+	*1 (5)*
HER2 FISH	7 (13)
Concurrent HER2 CISH and FISH	1 (2)
Concurrent HER2 CISH and IHC	1 (2)
Concurrent HER2 IHC and SISH	1 (2)
HER2 SISH	1 (2)

* Method, type of technology for testing a biomarker. Italicized choices represent various algorithms for reflexing to FISH test based on IHC results (0, 1+, 2+ and/or 3+). CISH = chromogenic in situ hybridization; FISH = fluorescence in situ hybridization; HER2 = human epidermal growth factor receptor 2; IHC = immunohistochemistry; SISH = silver in situ hybridization.

† Multimethod algorithm, a sequence of methods for testing one biomarker, when multiple methods are used.

‡ Reflex testing, confirming equivocal or borderline results by another method.

Table 5. Non–small cell lung cancer biomarker testing methods, algorithms, and protocols (EGFR, ALK, KRAS)

Characteristic, n = 55	No. (%)
Methods* and multimethod algorithms†	
EGFR, activation mutation status	55 (100)
PCR	*24 (44)*
Sequencing	*16 (29)*
IHC	*2 (4)*
Method not specified, use external lab	*13 (24)*
EGFR, gene amplification	25 (45)
FISH	*8 (32)*
Next generation sequencing	*2 (8)*
Method not specified, use external lab	*15 (60)*
ALK	55 (100)
FISH	*32 (58)*
PCR	*3 (6)*
PCR and FISH	*1 (2)*
IHC	*1 (2)*
IHC and FISH	*1 (2)*
IHC and PCR	*1 (2)*
IHC, PCR, and FISH	*1 (2)*
Method not specified, use external lab	*11 (20)*
Method not specified, use internally developed test	*4 (7)*
KRAS	53 (96)
Pyrosequencing	*14 (26)*
RT-PCR	*11 (21)*
Full sequencing	*10 (19)*
method not specified, conduct internally developed test	*10 (19)*
method not specified, use external lab	*8 (15)*
Multibiomarker protocols‡	
Sequence is based on oncologist order	24 (43.5)
Run all biomarker tests upfront	19 (34.5)
Run biomarkers sequentially	12 (22)
Run KRAS, if negative, run EGFR, if negative, run ALK	*3 (25)*
Run KRAS and EGFR upfront; if both negative, run ALK	*3 (25)*
Run EGFR, if negative, ALK (KRAS not used or not routinely used)	*3 (25)*
Run EGFR, if negative, run KRAS and ALK	*2 (17)*
Run EGFR and ALK simultaneously, if negative, run KRAS	*1 (8)*

* Method, type of technology for testing a biomarker. Italicized choices represent various technologic methods used for determining specific biomarker status. ALK = anaplastic lymphoma kinase; EGFR = epidermal growth factor receptor; FISH = fluorescence in situ hybridization; IHC = Immunohistochemistry; KRAS = v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; PCR = polymerase chain reaction; RT-PCR = reverse transcription polymerase chain reaction.

† Multimethod algorithm, a sequence of methods for testing one biomarker, when multiple methods are used.

‡ Multibiomarker protocol, a sequence of testing multiple biomarkers.

of institutions use a similar algorithm. Among sites using different GEC vs BC algorithms, 20% use "concurrent IHC and ISH" in GEC, 15% use IHC, reflex to ISH in GEC, and 4% use ISH in GEC, but not BC. The two-sided McNemar's test of matched proportions (statistical significance cutoff $P = .05$) showed lack of statistically significant association between the BC and GEC HER2 practices within these institutions: 39% use "concurrent IHC and ISH" for BC, while 46% use this algorithm for GEC ($P = .35$); 43% use "IHC, reflex to ISH" for BC, while 39% use this algorithm for GEC ($P = .64$); and 19% use "ISH alone" for BC, while 15% use this algorithm for GEC ($P = .41$).

BC and GEC HER2 and ER/PgR testing are performed predominantly in-house (Table 6), with a similar rate of internal testing between BC and GEC (93% vs 92%, $P = .8232$), while the rate of external NSCLC testing, at 36%, is statistically significantly higher than BC at 7% ($P < .0001$) and GEC at 8% ($P < .0001$). In-house testing is performed with internally developed tests more commonly in NSCLC than in BC or GEC (80% vs 10%, $P < .0001$, and 80% vs 21%, $P < .0001$, respectively), and more commonly in GEC than in BC (21% vs 10%, $P = .0175$). The choice of methods, algorithms or protocols in BC, GEC, and NSCLC had no association with institution size, metro vs nonmetro location, or whether the institution held NCCN designation. The two-sided Fisher's exact test was used here (statistical significance cutoff $P = .05$).

Discussion

We surveyed the NCI-designated cancer centers on their biomarker testing practices in three common cancers: breast, gastroesophageal, and non–small cell lung. While testing practices vary across NCI centers, findings indicate several similarities. These include testing beyond guidelines as a common practice for established biomarkers and an emerging practice for new biomarkers. Using internally developed tests is lower for mature than for emerging biomarkers. Implementation of institutional multibiomarker protocols lags guidelines.

We found variability of testing practices across the NCI centers: we identified seven distinct HER2 testing algorithms in BC and GEC, three or more testing methods for each NSCLC biomarker, and six NSCLC multibiomarker protocols. Variability is hardly surprising, given that respective guidelines allow multiple testing approaches (14–20) and published studies advocate the merit of additional approaches beyond guidelines (51–54,79,80,82,83). Establishing an experience-based minimally acceptable standard for biomarker testing may be desirable to support institutional decision-making between guideline- and literature-recommended choices. However, practice variability, even at the leading centers, is a barrier to establishing such standards. The evolving nature of the biomarker field and emergence of new testing methods and protocols could make standardization a moving target. Further research should elucidate drivers and consequences of various testing practices, including patient outcomes, cost, and cost-effectiveness, to inform institutional decision-making and pave the way to establishing a standard.

Despite variability, we found several common practices across NCI centers. First, we identified that testing beyond guidelines has become a common practice for HER2 assessment in BC and GEC. ASCO/CAP HER2 BC guidelines offer institutional choice of "IHC, reflex IHC2+ to ISH," or ISH-testing algorithms (20). Previous US estimates indicated 80% use of the former and 20% of the latter (123). However, we found that only 42% of NCI sites use "IHC, reflex IHC to ISH," and half of those expand confirmatory testing beyond IHC2+. We also found that 39% of NCI centers use concurrent testing of IHC and ISH upfront. Concurrent testing was previously documented by a utilization study (21%) (124), but we found it a more frequent practice at the NCI centers. Eleven

Table 6. Location of testing and the use of internally developed test vs commercial test

Characteristic*	Sites using test method	Internal testing with internally developed test	Internal testing with commercial test kit or reagent	Sent to external/ outside lab
	No. (%)	No. (%)	No. (%)	No. (%)
Breast cancer (n = 57)				
HER2 IHC	46 (80%)	3 (5%)	43 (75%)	0
HER2 FISH	52 (91%)	3 (5%)	43 (75%)	6 (10%)
HER2 CISH	3 (5%)	1 (2%)	2 (3%)	0
HER2 SISH	3 (5%)	0	3 (5%)	0
ER IHC	57 (100%)	6 (10%)	50 (88%)	1 (2%)
ER LBA	3 (5%)	1 (2%)	2 (3%)	0
ER RT-PCR	5 (9%)	0	0	5 (9%)
PgR IHC	57 (100%)	7 (12%)	50 (88%)	0
PgR LBA	2 (4%)	1 (2%)	0	1 (2%)
PgR RT-PCR	4 (7%)	0	0	4 (7%)
Gastric/Esophageal cancer (n = 54)				
HER2 IHC	45 (84%)	8 (15%)	36 (67%)	1 (2%)
HER2 FISH	51 (94%)	10 (18%)	34 (63%)	7 (13%)
HER2 CISH	2 (4%)	1 (2%)	1 (2%)	0
HER2 SISH	2 (4%)	0	2 (4%)	0
Non–small cell lung cancer (n = 55)				
EGFR activation mutation status - PCR	24 (44%)	13 (24%)	5 (9%)	6 (11%)
EGFR activation mutation status - Sequencing	16 (29%)	12 (22%)	1 (2%)	3 (5%)
EGFR activation mutation status - IHC	2 (4%)	2 (4%)	0	0
EGFR activation mutation status - method not specified	13 (24%)	2 (4%)	0	11 (20%)
EGFR, gene amplification - FISH	8 (15%)	6 (11%)	2 (3.5%)	0
EGFR, gene amplification - Next Gen Seq	2 (4%)	2 (4%)	0	0
EGFR, gene amplification - method not specified	15 (27%)	0	0	15 (27%)
ALK FISH	35 (64%)	18 (33%)	10 (18%)	7 (13%)
ALK PCR	6 (11%)	3 (5.5%)	0	3 (5.5%)
ALK IHC	4 (7%)	4 (7%)	0	0
ALK method not specified	15 (27%)	4 (7%)	0	11 (20%)
KRAS full sequencing	10 (18%)	9 (16%)	0	1 (2%)
KRAS pyrosequencing	14 (25.5%)	11 (20%)	3 (5.5%)	0
KRAS PCR	11 (20%)	5 (9%)	2 (4%)	4 (7%)
KRAS method not specified	18 (33%)	8 (14.5%)	2 (4%)	8 (14.5%)

* Some institutions use two or more methods. ALK = anaplastic lymphoma kinase; CISH = chromogenic in situ hybridization; EGFR = epidermal growth factor receptor; ER = estrogen receptor; FISH = fluorescence in situ hybridization; HER2 = human epidermal growth factor receptor 2; IHC = immunohistochemistry; KRAS = v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; LBA = ligand-binding assay; PCR = polymerase chain reaction; PgR = progesterone receptor; RT-PCR = reverse transcription polymerase chain reaction; SISH = silver in situ hybridization.

percent of NCI centers also retest triple-positive and/or triple-negative cases. Respondents' comments specify quality assurance and literature as reasons for the above practices.

We found similarity in overall frequencies of concurrent and expanded reflex-test algorithms between GEC and BC, as well as similarity of BC and GEC algorithms within the majority of the centers. However, the association between BC and GEC practices was not statistically significant; and a sizable minority of centers employed different approaches in these two cancers. Respondent comments indicate quality assurance, literature, and the emerging nature of GEC HER2 testing as reasons for concurrent and expanded reflex-test practices. Together, these findings suggest that both intrainstitutional and the overall field experience in BC HER2 testing inform GEC HER2 testing practices.

Respondents' comments indicate that exceeding guidelines is their strategy to assure testing quality in the HER2 field where intense debate over testing approaches persists, even within the ASCO/CAP guideline panel (125). Each guideline-recommended approach still has shortcomings, potentially leading to misdiagnosis of some patients (10,11,44,55,79,85,123). Studies show that practices of combining approaches and exceeding guidelines mitigate some shortcomings and allow incremental improvement of testing accuracy (51–54,77,80–83). However, such improvement comes at a cost. While the literature shows that some HER2 practices beyond guidelines are cost-effective (eg, reflex-testing IHC0 and IHC1+ results) (51), other practices, such as concurrent IHC/ISH or retesting triple-negative or triple-positive cases, have not been assessed for cost-effectiveness. We didn't examine whether the NCI centers analyzed or considered cost-effectiveness of their testing practices. These and other academic centers are typically well resourced, with access to donor funding, affording extra efforts to improve quality. They also conduct research, which may contribute to feasibility of their practices: trials may require certain HER2 protocols (eg, retesting all IHC cases with FISH), potentially impacting overall institutional testing practices. Community-based centers without such protocols and donor funding may not have

resources or experience for extra efforts. The costs of incremental quality improvement may be prohibitive to these centers, even if the improvements prove cost-effective. To determine applicability of NCI center practices to other settings, cost-effectiveness and net institutional budget impact of testing practices, along with patient outcomes, should be assessed.

The second common dynamic we identified is the emergence of going beyond guidelines in NSCLC. A debate on best methods, analogous to HER2 testing, is now emerging in NSCLC (70). For example, IHC is proposed as a less costly "screening" tool for EGFR (75) and ALK (76,91–93) testing, followed by molecular analysis in positive cases, and guidelines, in the case of ALK, allow this approach, when carefully validated (18). Our study showed that only a small minority of NCI centers use IHC for EGFR and/or ALK testing, indicating that they exercise the recommended caution (18,92) toward this approach, despite the cost advantage. The trajectory of the HER2 debate toward exceeding guidelines to avoid any misdiagnosing (51,123) may be emerging in NSCLC. Similarly, patient outcome, cost-effectiveness, and budget-impact implications of these practices should be assessed in NSCLC.

We found that NCI centers have universally adopted new biomarkers (GEC HER2; NSCLC ALK, EGFR mutation), but are still developing NSCLC multimarker testing protocols: nearly half of respondents run these tests based on oncologist order. Guidelines recommend either a concurrent or sequential protocol, with result turnaround time within 10 business days (18). However, we found that among NCI centers with an established NSCLC protocol, the majority assess all biomarkers upfront—a practice associated with shorter result turnaround time and better sample management, but with higher costs. Although still evolving, these practices may also signify a trend, similar to that in HER2 testing, toward higher quality despite higher costs and should be assessed for patient outcome, cost-effectiveness, and budget-impact implications.

The third common dynamic identified across NCI centers is the lower use of internally developed tests for mature vs novel biomarkers. This is yet another controversial area (10,126,127), especially given the US Food and Drug Administration's (FDA's) recent intention to tighten regulation of cancer biomarker tests (128). Our findings indicate a trend from higher external and/or internally developed testing for new biomarkers (NSCLC) to predominant use of commercial kits for mature markers (BC). This indicates that NCI centers adopt commercially available kits "voluntarily" over time, even in the absence of regulation. For example, testing for ALK, the newest biomarker in our study, with a commercial test is higher than for other NSCLC tests, perhaps because an FDA-approved test is available.

Our study had a number of limitations. Our findings were self-reported by participants, a common limitation of surveys, but mitigated by the fact that we did not receive conflicting input within responding sites. While we collected data on reasons for several specific testing practices, future studies should more comprehensively elucidate factors and motivations influencing testing practices. Practices at NCI centers may be representative of other large academic centers, but may not be indicative of practices in the community setting where donor funding and research experiences are more limited. Further studies should examine biomarker testing practices at community cancer centers, to fully understand the landscape of practices in the US. To guide applicability of our findings, and future studies, to other settings in the environment of rising healthcare costs and unaffordability concerns, the testing practice landscape should be coupled with assessment of cost-effectiveness, budget impact, and patient outcomes.

In conclusion, our survey of NCI-designated cancer centers—the leading US oncology institutions—showed that while testing approaches vary, exceeding guidelines to ensure testing quality is a common practice for established biomarkers, and an emerging practice for new biomarkers. Implementation of multimarker testing protocols is lagging and should be addressed at the NCI centers and other institutions. Our study is a step toward developing a landscape of cancer biomarker testing practices in the US. Future inclusion of community practices, expansion to other biomarkers and cancers, and studies of outcomes and cost-effectiveness of testing practices will be necessary to evolve the landscape.

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