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The introduction of systematic genomic testing for patients with non-small cell lung cancer

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Abstract

Background—Genomic testing to identify driver mutations that enable targeted therapy is emerging for patients with non-small cell lung cancer (NSCLC). We report the implementation of systematic prospective genotyping for somatic alterations in *BRAF, PIK3CA, HER2*, and *ALK*, in addition to *EGFR* and *KRAS*, in NSCLC patients at the Dana-Farber Cancer Institute.

Methods—Patients with NSCLC were prospectively referred by their providers for clinical genotyping. Formalin-fixed, paraffin embedded tumor samples were analyzed by Sanger sequencing for mutations in selected exons of *EGFR, KRAS, BRAF, PIK3CA*, and *HER2. ALK* rearrangements were detected by FISH or immunohistochemistry.

Results—Between 7/1/2009 and 8/1/2010, 427 specimens from 419 patients were referred for genomic characterization; 344 (81%) specimens were successfully genotyped with a median turnaround time of 31 days (range, 9-155). Of the 344 specimens, 185 (54%) had at least one identifiable somatic alteration (*KRAS:* 24%, *EGFR*: 17%, *ALK*: 5%, *BRAF*: 5%, *HER2*: 4%, *PIK3CA*: 2%). As of 8/1/2011, 63/288 (22%) advanced NSCLC patients had received molecularly targeted therapy based on their genotypic results, including 34/42 (81%) patients with *EGFR*

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mutations, 12/15 (80%) with *ALK* rearrangements, and 17/95 (18%) with *KRAS, BRAF* or *HER2* mutations.

Conclusions—Large scale testing for somatic alterations in *EGFR, KRAS, BRAF, PIK3CA, HER2* and *ALK* is feasible and impacts therapeutic decisions. As the repertoire for personalized therapies expands in lung cancer and other malignancies, there is a need to develop new genomics technologies that can generate a comprehensive genetic profile of tumor specimens in a time and cost effective manner.

Keywords

Lung cancer; cancer genomics; molecular targeted therapy

Introduction

Targeted cancer therapy is transforming the care of patients with non-small cell lung cancer (NSCLC) and driving efforts to incorporate tumor genotyping into clinical decision-making. For example, it is now standard care to examine tumor specimens from patients with NSCLC for somatic alterations in *EGFR* to identify those with sensitizing mutations for initial therapy with gefitinib or erlotinib.[1] Similarly, the ALK tyrosine kinase inhibitor, crizotinib, has shown response rates in excess of 60%, progression-free survival greater than 10 months, and median survival in excess of two years from the start of crizotinib therapy in patients with advanced NSCLC bearing *ALK* rearrangements.[2, 3] Crizotinib is recommended as initial therapy for *ALK*-rearranged NSCLC in the National Comprehensive Cancer Network guidelines.

Thus far, approximately 15 to 20% of NSCLC patients from Europe and North America have tumors bearing *EGFR* mutations or *ALK* rearrangements, with drugs available to treat these genomic changes. Other potential therapeutic targets emerging in 2009 in patients with NSCLC included activating mutations in *KRAS, BRAF, HER2,* and *PIK3CA. KRAS* is the *RAS* family member most frequently mutated in lung adenocarcinomas, with mutations in codons 12, 13, and 61 detected in approximately 20% of cases. *KRAS* mutations are a negative predictive marker for response to EGFR tyrosine kinase inhibitors, as well as a potential therapeutic target.[4] Activating mutations in *BRAF, HER2*, and *PIK3CA* have each been reported at lower frequencies in NSCLC, ranging from 1% to 3%.[5-8] Ongoing research at our institution and others is attempting to determine whether therapeutic inhibition of KRAS, BRAF, HER2, and PIK3CA will be an effective strategy in NSCLC, and to identify additional driver mutations that can be successfully targeted with existing or novel compounds. Therefore, consistent multiplex genotyping is needed for patients with NSCLC to inform therapeutic choices and to expand the possible candidates for personalized lung cancer therapies.

The Lowe Center for Thoracic Oncology at the Dana-Farber Cancer Institute, in conjunction with the Center for Advanced Molecular Diagnostics in the Pathology Department at the Brigham and Women's Hospital and the Laboratory for Molecular Medicine at the Partners Healthcare Center for Personalized Genetic Medicine, introduced prospective genotyping of advanced NSCLC for somatic alterations in *BRAF, HER2, PIK3CA*, and *ALK* in July 2009, in addition to routine mutational analysis of *EGFR* and *KRAS*, which had been ongoing since 2004. Three years have passed since we initiated this expanded genomic testing, allowing adequate time for determining the success of genomic characterization and assigning patients to therapies based on the molecular findings. The purpose of this report is to provide data on the ability of our center to generate this information and to guide other

institutions as they develop and modify their procedures for multiplex genomic characterization of lung cancers and other solid tumors.

Materials and Methods

Study population

Patients eligible for this analysis included those with histologically or cytologically confirmed NSCLC who underwent genomic characterization of *BRAF, PIK3CA* and *HER2* when added to the ongoing standard characterization of *EGFR* and *KRAS*, from July 1, 2009 until August 1, 2010. Specific genotyping studies were ordered at the discretion of the treating provider; in a majority of cases, *ALK* testing was also performed. Patients could be genotyped anytime during the course of their therapy. Patients were identified through a query of patient information for subjects prospectively enrolled in the Clinical Research Information System (CRIS) within the Lowe Center for Thoracic Oncology at the Dana-Farber Cancer Institute that tracks all of the patients referred for genomic testing from our center. This patient information has been used for previous reports.[9-11] Patients studied during a period of 13 months were evaluated to include a full year of data, including those during the first month start-up phase. Patients whose tumors were tested after August 1, 2010 were excluded from this analysis to assure at least one year of clinical follow-up after testing. During the study period, *EGFR* and *KRAS* tumor genotyping were considered routine clinical tests without the need for patient consent. Patients provided written informed consent for *BRAF, PIK3CA, HER2* and *ALK* testing, as well as for the collection of baseline information, details on their treatments, clinical outcomes information, and ability to contact them for potential trials. The collection of clinical information on patients referred for genotyping was approved by the local institutional review board at the Dana-Farber Cancer Institute.

Genomic characterization

Tumor specimens submitted for genomic characterization consisted of formalin-fixed paraffin-embedded (FFPE) material and were pre-screened by a board-certified pathologist (NIL) to confirm adequate tumor material for testing. Specimens were analyzed for the presence of somatic mutations of *EGFR* (exons 18 to 21), *KRAS* (exons 2 and 3), *BRAF* (exons 11 and 15), *PIK3CA* (exons 8, 10 and 21), and *HER2* (exon 20) by bidirectional Sanger dideoxyterminator sequencing according to described methods.[12] This method allows detection of expected key driver mutations in the genes tested as well as other genetic changes that may have clinical significance. Mutation analysis was performed at the Laboratory for Molecular Medicine at the Partners Healthcare Center for Personalized Genetic Medicine under conditions certified according to the Clinical Laboratory Improvement Amendments. Only mutations detected on both forward and reverse strands and confirmed by testing a second aliquot of DNA were reported as positive. Sequences were independently interpreted by two technologists, a molecular geneticist (VAJ), and a pathologist (NIL).

Fluorescence *in situ* hybridization (FISH) was performed on 4-micron sections of FFPE tumor samples cut onto glass slides using an ALK break-apart probe (Abbott Vysis, Abbott Park, IL), according to previously described methods.[2, 3] FISH-positive specimens were defined as separated orange and green signals, with a split distance of at least 2 probe diameters, in greater than 15% of tumor cells. FISH slides were independently interpreted by two technologists, a cytogeneticist (VAJ), and a pathologist (NIL). In some cases, immunohistochemistry (IHC) was initially performed, and samples scored as positive or equivocal for ALK expression were confirmed by FISH analysis as previously reported.[13] Before the laboratory became more efficient at handling high throughput, a number of

samples were referred to a commercial laboratory for *ALK* FISH testing. Other samples were prospectively assessed for *ALK* rearrangements at a central laboratory as part of eligibility screening for clinical trials.

Statistical methods

Baseline clinical characteristics were determined by prospective collection from a patient-administered questionnaire. Smoking status was categorized as never (<100 lifetime cigarettes), light (≤10 pack-years) or heavy smoker (>10 pack-years). Tumor histology was classified using the 2004 WHO criteria.[14]

Patients who had a somatic alteration in at least one of the six genes were categorized as being treated with a molecularly targeted therapy if they received an agent targeted against that genomic change or closely related downstream pathway. Patients were also considered to have been assigned to a molecularly targeted therapy if they participated in a trial where prospective identification of a mutation was required for enrollment. For instance, the patients with *KRAS* mutations who were entered on the trial where they were assigned either to docetaxel or docetaxel plus selumetinib were categorized as being treated with targeted therapy (NCT00890825).

Summary statistics are provided regarding the demographic and disease characteristics of the 419 patients tested and are summarized by mutation status. Wilcoxon rank sum and Fisher's exact tests were used to compare the characteristics between patients with genomic changes and wild-type tumors. Turnaround time was calculated as the interval from the time of test requisition until genotyping report finalization and included the time required to obtain the FFPE tumor material, which had to be requested from outside hospitals in some cases; to section and review the submitted material for adequacy; to extract, amplify and sequence DNA, and to re-extract DNA in the case of inadequate malignant tissue amount or integrity in the original tested sample; to transport the sample to and from the laboratory with genomic characterization capability; and to interpret and sign out the results. The turnaround time was calculated for *BRAF, HER2* and *PIK3CA* mutational analysis; specimens were most frequently also tested for mutations in *EGFR* and *KRAS*, although in some cases, mutational analysis of *EGFR* and *KRAS* had previously been performed. The turnaround time could not be reliably assessed for specimens referred for *ALK* testing as different laboratories were involved. All reported P values are based on two-sided hypothesis tests. The statistical analysis was performed using SAS 9.2.

Results

Patient characteristics

Between July 1, 2009 and August 1, 2010, 427 specimens from 419 patients with NSCLC were prospectively referred for clinical genotyping for mutations in *BRAF, PIK3CA*, and *HER2*. Table 1 shows the demographic and clinical characteristics of these 419 patients. The median age of the study cohort was 61 years (range, 24-95 years). There were 173 (41%) men and 246 (59%) women, and 36% of patients were never or light smokers (≤10 pack-years). Most patients were White, non-Hispanic, reflecting the predominant ethnic background of our clinic population. The majority of patients had stage IV or relapsed NSCLC at the time of genetic test requisition (80%). Histology was predominantly adenocarcinoma (87%), reflecting the patient population primarily targeted by clinical genotyping at our institution. Eight patients had two different samples tested; 4 of these patients had a second specimen submitted because the original sample contained insufficient tumor material for genetic analysis, two patients had samples from two different body sites

tested, and two patients underwent surgical resection of synchronous primary tumors and each tumor was successfully genotyped.

Genomic characterization

Of the 427 specimens referred for genomic characterization of *BRAF, HER2* and *PIK3CA*, 344 (81%) specimens from 341 patients were successfully tested. Table 2 outlines the reasons for the 83 samples that did not complete genetic analysis. Genotyping failures occurred more commonly in specimens obtained from bone (5 of 9 samples did not complete genetic analysis). Correspondingly, recent reports have shown that fixatives with a low pH, such as bone decalcifying solutions, can affect the quality and quantity of DNA in the samples, thereby interfering with molecular testing.[15] Therefore, subsequent bone samples were excluded from this study and genomic testing on bone specimens is no longer routinely performed at our center. Of the 344 samples successfully tested for *BRAF, HER2* and *PIK3CA* mutations, 336 (98%) samples were also successfully tested for *EGFR* mutations, 328 (95%) were successfully tested for mutations in *KRAS*, and 310 (90%) underwent successful *ALK* testing.

Among the 344 genotyped specimens, the median turnaround time was 31 days (range, 9-155 days). The turnaround time was significantly longer when pathology specimens were obtained from outside institutions (n=138) compared with specimens available in the Department of Pathology at the Brigham and Women's Hospital (n=206) (median 36 *v* 28 days, respectively; p<0.001). Sixty-four specimens had a turnaround time greater than 50 days, including 3 specimens with a turnaround time greater than 100 days. Of those 3 specimens, two required DNA re-extraction to meet quality standards, which significantly prolonged the turnaround time, and were subsequently successfully tested. The remaining specimen was obtained from an outside hospital; 127 days elapsed before the tumor block was received, after which genomic testing was successfully completed and reported in 28 days.

A somatic alteration in at least one of the 6 genes was identified in 185 of the 344 (54%) genotyped specimens; seven of the 185 specimens harbored two mutations. Table 3 lists the somatic mutations identified. Overall, we identified 60 *EGFR* mutations in 56/336 (17%) specimens, 79 *KRAS* mutations in 79/328 (24%) specimens, 16 *BRAF* mutations in 16/344 (5%) specimens, 15 *HER2* mutations in 15/344 (4%) specimens, and 6 *PIK3CA* mutations in 6/344 (2%) specimens. A rearrangement of the *ALK* gene was detected in 16 of the 310 (5%) successfully tested samples (FISH-positive: 12; IHC-only positive: 4). Three specimens harbored concurrent mutations in *PIK3CA* and either *KRAS* (n=2) or *EGFR* (n=1). Two specimens with *EGFR* T790M had concurrent sensitizing *EGFR* mutations in exon 19. Two additional specimens harbored two *EGFR* mutations involving G719. Two novel *BRAF* mutations not previously reported in NSCLC patients were identified. *BRAF* p.1794_1796dupTAC (c.T599_V600insT), a somatic change previously described in a female patient with pancreatic cancer, was identified in one sample.[16] Another specimen harbored *BRAF* p.1405_1407delGGA (c.G469del), a somatic change not previously reported in the literature; other missense mutations in *BRAF* codon 469 have been detected in solid tumors. One hundred and twenty eight of 313 (41%) specimens were wild-type at all predefined exons and negative for the *ALK* rearrangement.

The demographic and clinical characteristics of the patients with tumors bearing an identifiable oncogenic driver mutation or pan-negative (wild-type) tumors are shown in Table 4. Consistent with previous reports, patients with *ALK*-rearranged tumors were significantly younger than patients with wild-type tumors (p=0.005).[17] Further comparative analyses showed that never or light smoking history (≤10 pack-years) was significantly associated with mutations in *EGFR* (p<0.001), *BRAF* (p=0.073), *PIK3CA* (p=0.048), and

HER2 (p<0.001), as well as A*LK* rearrangements (p<0.001), while heavier smoking was significantly correlated with *KRAS* mutations (p=0.007). The two patients with tumors bearing concurrent mutations in *PIK3CA* and *KRAS* were a 30-year-old white female never smoker with adenocarcinoma and a 58-year-old white male former heavy smoker with adenosquamous carcinoma. An 81-year-old white female, never smoker, had adenocarcinoma that harbored an *EGFR* exon 19 deletion and a *PIK3CA* mutation before exposure to any systemic therapy.

Clinical and therapeutic implications

As of August 1, 2011, 288 of the 341 patients (84%) were diagnosed with stage IV or relapsed metastatic NSCLC. Of the 288 patients, 152 (53%) had at least one identifiable somatic alteration (*EGFR*: 41, *EGFR* + *PIK3CA*: 1, *KRAS*: 62, *KRAS* + *PIK3CA*: 2, *BRAF*: 15, *HER2*: 13, *PIK3CA*: 3, *ALK*: 15). Thirty-five of the 152 (23%) patients enrolled in a study of molecularly targeted therapy, including 9 of 42 patients with *EGFR* mutations. An additional 25 *EGFR* mutant advanced NSCLC patients were treated with erlotinib off protocol. Eight patients with *EGFR* mutations were not treated with an EGFR tyrosine kinase inhibitor. Of those 8 patients, two were treated at outside institutions with no therapeutic information available; three had exon 20 insertion mutations, which are not sensitive to treatment with erlotinib or gefitinib; two were asymptomatic requiring no systemic therapy for their advanced NSCLC; and one died shortly after the identification of a sensitizing mutation of *EGFR*. Twelve of 15 patients with *ALK*-rearranged advanced NSCLC enrolled in a clinical trial, including 2 patients who were treated at outside institutions. Three patients with *ALK* rearrangements were not treated with crizotinib. Of the 3 patients, one remained on maintenance pemetrexed with prolonged stable disease at the time of this analysis; the remaining two patients were not eligible for trial enrollment due to poor performance status and died shortly after the identification of an *ALK* rearrangement. Finally, three patients with *HER2* mutations received a trastuzumab-containing regimen off protocol. Thus, a total of 63 of 152 (41%) patients received molecularly tailored therapy based on their genetic alterations (Figure 1).

Finally, one patient in our cohort had profiling of metachronous resected bilateral T2 tumors that revealed two distinct genotypes (*KRAS* G12C in one case, and wild-type for all tested sites in the other), suggesting two early stage primary tumors as opposed to metastatic disease. Another patient had a similar scenario, but the two specimens were wild type for all tested sites.

Discussion

Over the past two decades, modifications of chemotherapy combinations and the addition of the antiangiogenic agent, bevacizumab, have brought about modest gains in survival for patients with advanced NSCLC.[18-21] Treatment with erlotinib or gefitinib for NSCLC patients with *EGFR* mutations and crizotinib for patients with *ALK* rearrangements has transformed therapy for these patient subsets. Other promising agents for lung cancers that harbor uncommon genomic changes are under development and have prompted the need for extensive genomic characterization of advanced NSCLC. Research efforts published in 2010 and 2011 performed genetic profiling of resected NSCLC and defined the frequency and types of somatic mutations of *EGFR, KRAS, NRAS, HRAS, HER2, BRAF, PIK3CA, ALK* rearrangements, and *ROS1* fusions.[12, 22] Although resected lung cancers provide an abundant source of DNA for molecular profiling, the patients who more urgently need to undergo genomic characterization are those with advanced or relapsed NSCLC to guide the selection of initial systemic therapy and to identify candidates for investigational therapy.

In the present study, we report on our experience prospectively screening 427 specimens from 419 patients for mutations in *BRAF, HER2*, and *PIK3CA*, in addition to routine mutational analysis of *EGFR* and *KRAS*, during an initial 13 months following implementation. In 90% of cases, *ALK* testing was also performed. Most specimens were successfully genotyped (344/427, 81%), and 185 of 344 specimens (54%) had an identifiable driver mutation. Drs. Dias-Santagata and Sequist previously reported on their ability to perform systematic genotyping of NSCLC.[1] In their study, 552 of the 589 patients (94%) referred for clinical genotyping had their tumors successfully genotyped. Notably, their population included a greater number of patients with resected stage I/II NSCLC (197/546 or 37% *v* 45/419 or 11% in our cohort), offering a potential explanation for the higher proportion of specimens that were successfully genotyped in their study. Likewise, their group used a genotyping technique called SNaPshot that requires smaller amounts of DNA and a less pure population of tumor cells than the 50% malignant cells needed for direct DNA sequencing used in this report.[1, 23] Correspondingly, we categorized 22 specimens that were found to be wild-type at all predefined exons of *BRAF, HER2* and *PIK3CA* as “inconclusive” because they contained fewer than 50% malignant cells. As routine lung cancer care is redefined to incorporate tumor mutational profiling into clinical decision-making, the obstacles posed by limited tissue specimens stress the need to obtain adequate tumor material at the time of diagnostic sampling.

The goals of the genomic characterization of our NSCLC patients are to help guide therapy and ultimately lead to improved outcomes for those patients with specific genomic changes. Our group identified 152 of 288 (53%) patients with advanced or relapsed NSCLC with a somatic alteration of *EGFR, KRAS, BRAF, PIK3CA, HER2*, and/or *ALK*; 63 of the 152 (41%) patients began a molecularly tailored therapy in response to genotypic results. We expect that this proportion should increase as the scope of genotype-specific clinical trials is expanding at our institution and other centers. As was to be expected with proven effective therapies, the vast majority of *EGFR* mutant advanced NSCLC patients were treated with erlotinib (34/42, or 81%), either as part of a clinical trial or off protocol. Similarly, 12 of the 15 (80%) advanced NSCLC patients whose tumors harbored ALK rearrangements were treated with crizotinib. Research is ongoing to determine whether the outcomes of NSCLC patients with other genomic changes treated with targeted therapies are improved compared with those given empiric chemotherapy.

An important issue is the length of time needed to perform multiplex diagnostic testing, which is critical for purposes of clinical decision-making. In our study, the median turnaround time was 31 days (range, 9-155 days) for mutational analysis. This included the time required to obtain FFPE samples from outside hospitals in 40% of cases, and to transport specimens to and from the laboratory with genomic characterization capability for direct DNA sequencing. Other potential delays are technical. The Sanger sequencing assay involves multiple steps, including nested PCR, with quality control assessments at several stages of the process. Turnaround times are also extended by our laboratory’s policies of confirming positive results with a second amplification from a second aliquot of DNA, and independent sequential interpretations by a total of four reviewers. Nevertheless, there is a strong desire to shorten the time needed to generate the genotypic information in order to more rapidly implement appropriately targeted therapy. The International Association for the Study of Lung Cancer (IASLC) - European Thoracic Oncology Platform multidisciplinary workshop recommended that *EGFR* mutation testing be completed within 7 working days.[24] Similarly, draft recommendations by the College of American Pathologists in conjunction with the IASLC and the Association for Molecular Pathology recommend that *EGFR* and *ALK* testing both be completed within two weeks (10 working days) of receiving the specimen in the testing laboratory. Therefore, retrieval and delivery of samples and testing within our center and others will need to take place more promptly. In

September 2010, our molecular pathology laboratory introduced a rapid *EGFR* assay that provides genotype results for the two most common sensitizing mutations, exon 19 deletions and *EGFR* L858R, within 2 days (median) of receipt of the sample in the laboratory. This has led to a significant acceleration of the implementation of targeted EGFR therapy. If this could be achieved for all molecular testing, it would be a major advance.

Similarly, there is a need for genotyping technology to evolve in order to generate a more comprehensive genetic analysis of routine clinical specimens, capturing point mutations, insertions, and deletions, as well as rearrangements and DNA copy number alterations in a single panel. This is especially relevant as we identify more non-mutational genomic targets in NSCLC, such as *MET* amplification, and *ROS1* and *RET* rearrangements.[25-27] Real-time prospective genotyping of NSCLC is currently challenged by the fact that different types of molecular alterations require different assays and necessitate tumor tissue to be processed properly in multiple ways. Moving forward, the adaptation of new technologies, such as next-generation sequencing, may offer a unifying approach to comprehensive profiling of tumor DNA, and potentially shorten the time of such analyses. Such targeted next-generation sequencing is clinically feasible and under development by commercial entities and academic centers.[27]

The primary limitations of our study are its retrospective observational design and the potential for selection bias introduced by the patients pursuing oncologic care at our tertiary referral center and in whom providers requested clinical genotyping. Nevertheless, our genotyping results are consistent with the documented mutational prevalence of the tested oncogenes.[28] Similarly, although our findings support the clinical feasibility of broad prospective genotyping, the utility of this approach remains under investigation. Multiple agents targeting KRAS, BRAF, HER2, PIK3CA, and/or downstream pathways are in various phases of clinical development in patients with advanced NSCLC and prospectively identified mutations. These studies should help determine whether a targeted therapeutic strategy will result in improved outcomes for these patients, analogous to those observed for NSCLC patients with *EGFR* mutations or *ALK* translocations.

In summary, our experience implementing systematic prospective genotyping for somatic alterations in *EGFR, KRAS, ALK, BRAF, HER2*, and *PIK3CA* demonstrates the feasibility of this approach within the clinical workflow. The genotypic information supported diagnostic decisions, directed the administration of available targeted therapeutics, and facilitated enrollment of patients into clinical trials of molecularly tailored therapy. As the repertoire of mutations for which targeted therapy may be offered expands in lung cancer and other solid malignancies, strategies to enable rapid, accurate and comprehensive clinical genotyping will be essential to successfully integrate tumor molecular analysis into the fast pace of clinical decision-making and yield its greatest potential impact on patient management.

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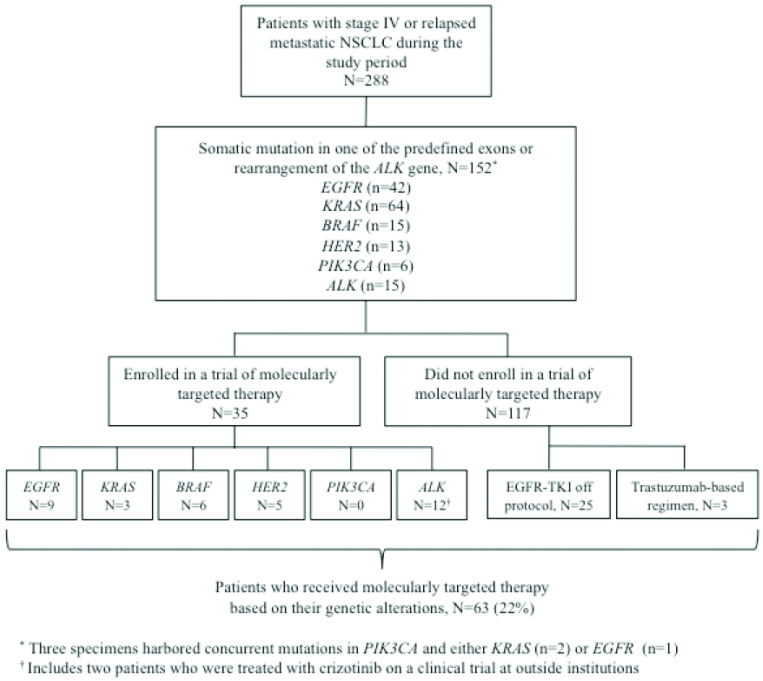

Figure 1.
Flowchart of patients with stage IV or relapsed metastatic NSCLC onto molecularly targeted therapy during the study period

Table 1

Baseline patient characteristics

Total, *N*	**419**
Median age -- yrs (range)	61 (24-95)
Gender -- no. (%)	
Male	173 (41)
Female	246 (59)
Race -- no. (%)	
White, non-Hispanic	371 (89)
White, Hispanic	6 (1)
Asian	12 (3)
Black	28 (7)
Other	2 (<1)
Smoking Status -- no. (%) *	
Never-smoker	97 (23)
≤ 10 pack-years	55 (13)
> 10 pack-years	262 (63)
Histology -- no. (%)	
Adenocarcinoma	363 (87)
Adenosquamous	12 (3)
Squamous	7 (2)
Large cell carcinoma	4 (1)
NSCLC NOS	33 (8)
Stage †	
I / II / IIIA	31 (7) / 14 (2) / 30 (7)
IIIB	9 (2)
IV	217 (52)
Relapsed ‡	118 (28)

Abbreviations: NOS, not otherwise specified

* Data not available for 5 patients

† AJCC staging system, 7th edition

‡ Patients with stage I-IIIA NSCLC with relapse following definitive therapy

Table 2

Specimens referred for clinical genotyping

	No. (%)
Specimens referred for *BRAF, HER2* and *PIKC3CA* mutational analysis	427 (100)
Specimens that did not complete genetic analysis	83 (19)
Insufficient tumor material for genotyping; testing not performed *	34 (8)
Insufficient tumor material for conclusive results; testing performed †	22 (5)
Failed PCR amplification	15 (4)
Incomplete testing at all predefined exons of *BRAF*, *HER2* and/or *PIK3CA*	6 (1)
Specimen could not be located	6 (1)
Specimens successfully tested for *BRAF*, *HER2* and *PIK3CA* mutations	344 (81)

* Insufficient tumor material for genotyping identified during pathology pre-review.

† Twenty-two specimens were found to be wild-type for *BRAF*, *HER2*, and *PIK3CA* but were classified as inconclusive because there was less than 50% malignant tissue in the specimen.

Table 3

Somatic gene alterations identified

Gene	Exon	Mutations identified -- no. (%)
EGFR [a]	---	60 (100)[b]
	18	G719A: 2 (3); G719C: 1 (2); G719S: 1 (2)
	19	deletions: 13 (22); deletion-insertions: 8 (13)
		L747P (cis) or L747L/L747S (trans)[c]: 2 (3)
	20	duplications: 4 (7); insertions: 2 (3); deletion-insertions: 1 (2); deletions: 1 (2) T790M: 2 (3); S768I: 1 (2)
	21	L858R: 20 (33); L861Q: 2 (3)
KRAS [a]	---	79 (100)[d]
	2	G12C: 30 (38); G12D: 18 (23); G12V: 11 (14); G12A: 7 (9); G12S: 3 (4)
		G12F (cis) or G12C/G12V (trans)[e]: 1 (1); G12L (cis) or G12R/G12V (trans)[f]: 1 (1) G13C: 1 (1); G13D: 1 (1)
	3	Q61H: 4 (5); Q61K: 1 (1)
BRAF	---	16 (100)
	11	G466V: 1 (6); G466R: 1 (6); G469A: 1 (6); G469del: 1 (6)
	15	V600E: 9 (56); D594N: 1 (6); D594G: 1 (6) T599_V600insT: 1 (6)
HER2	---	15 (100)
	20	duplications: 9 (60); insertions: 3 (20): deletion-insertions: 2 (13) V777L: 1 (7)
PIK3CA [a]	---	6 (100)
	8	E453_P458delinsD: 1 (17)
	10	E542K: 3 (50)
	21	H1047R: 2 (33)
ALK	---	16 (100) Rearrangement: FISH-positive, 12 (75); IHC-only positive, 4 (25)

[a] Three specimens harbored concurrent mutations in *PIK3CA* and either *KRAS* (n=2) or *EGFR* (n=1).

[b] Four specimens had two concurrent *EGFR* mutations.

[c] Two specimens had two DNA sequence variants in *EGFR:* 2239T>C and 2240T>C. If these two variants occur on the same allele (cis), the expected amino acid change is L747P. If these two variants occur on separate alleles (trans), the expected amino acid changes are L747L and L747S.

[d] One specimen was tested at an outside facility and reported as positive for a KRAS mutation but information on the specific amino acid change was not available.

[e]Two DNA sequence variants were detected in *KRAS*: 34G>T and 35G>T. If these variants occur on the same allele, the expected amino acid change is G12F. If these variants occur on separate alleles, the expected amino acid changes are G12C and G12V.

[f]Two DNA sequence variants were detected in *KRAS:* 34G>C and 35G>T. If these variants occur on the same allele, the expected amino acid change is G12L. If these variants occur on separate alleles, the expected amino acid changes are G12R and G12V.

Table 4

Demographics of the patients successfully genotyped by mutation status*

	EGFR †	*KRAS* †	*ALK*	*BRAF*	*HER2*	*pik3ca* †	Wild-type
Proportion mutated (%)	55‡/334 (16)	79/325 (24)	16/309 (5)	16/341 (5)	15/341 (4)	6/341 (2)	128/313 (41)
Median age (range)	58 (34-81)	63 (30-82)	54 (24-79)	61 (41-77)	61 (44-75)	66 (30-81)	63 (26-87)
Gender -- no. (%)							
Male	21 (38)	28 (35)	4 (25)	7 (44)	6 (40)	3 (50)	68 (53)
Female	34 (62)	51 (65)	12 (75)	9 (56)	9 (60)	3 (50)	60 (47)
Race -- no. (%)							
White, non-Hispanic	51 (93)	74 (94)	15 (94)	15 (94)	14 (93)	5 (83)	107 (84)
White, Hispanic	0	1 (1)	1 (6)	0	0	0	4 (3)
Asian	1 (2)	1 (1)	0	0	1 (7)	0	6 (5)
Black	3 (5)	2 (3)	0	1 (6)	0	1 (17)	10 (8)
Other	0	1 (1)	0	0	0	0	1 (1)
Smoking Status -- no. (%)§							
Never-smoker	29 (53)	5 (6)	6 (38)	5 (31)	10 (67)	4 (67)	24 (19)
≤ 10 pack-years	12 (22)	3 (4)	6 (38)	3 (19)	4 (27)	0	8 (6)
≥ 10 pack-years	14 (25)	71 (90)	4 (25)	8 (50)	1 (7)	2 (33)	93 (73)
Histology -- no. (%)							
Adenocarcinoma	53 (96)	73 (92)	14 (88)	12 (75)	13 (87)	4 (67)	107 (84)
Adenosquamous	2 (4)	3 (4)	0	1 (6)	1 (7)	1 (17)	5 (4)
Squamous	0	0	0	0	0	0	3 (2)
Large cell carcinoma	0	0	1 (6)	0	0	0	1 (1)
NSCLC NOS	0	3 (4)	1 (6)	3 (19)	1 (7)	1 (17)	12 (9)

*Three patients with two separate tumor specimens successfully genotyped were accounted for only once in the respective genotype cohort.

†Three patients had tumors bearing concurrent mutations in *PIK3CA* and either *KRAS* (n=2) or *EGFR* (n=1); the demographics of the patient corresponding with each of the 3 tumor specimens were included and accounted for in the respective genotype cohort.

‡One patient had samples from two different body sites tested, both of which showed an exon 20 insertion mutation of *EGFR*. The demographics of the patient were accounted for only once in the *EGFR* cohort.

§Data not available for 3 patients in the wild-type cohort.