DNA-Mutation Inventory to Refine and Enhance Cancer Treatment (DIRECT): A Catalog of Clinically Relevant Cancer Mutations to Enable Genome-Directed Anticancer Therapy

Paul Yeh[1], **Heidi Chen**[1], **Jenny Andrews**[2], **Riyad Naser**[1], **William Pao**[1], and **Leora Horn**[1]

[1]Vanderbilt-Ingram Cancer Center, Nashville, Tennessee

[2]Mercer University School of Medicine, Macon, Georgia

Abstract

Purpose—Tumor gene mutation status is becoming increasingly important in the treatment of patients with cancer. A comprehensive catalog of tumor gene–response outcomes from individual patients is needed, especially for actionable mutations and rare variants. We created a proof-of-principle database [DNA-mutation Inventory to Refine and Enhance Cancer Treatment (DIRECT)], starting with lung cancer-associated EGF receptor (EGFR) mutations, to provide a resource for clinicians to prioritize treatment decisions based on a patient's tumor mutations at the point of care.

Methods—A systematic search of literature published between June 2005 and May 2011 was conducted through PubMed to identify patient-level, mutation–drug response in patients with non–small cell lung cancer (NSCLC) with EGFR mutant tumors. Minimum inclusion criteria included patient's EGFR mutation, corresponding treatment, and an associated radiographic outcome.

Results—A total of 1,021 patients with 1,070 separate EGFR tyrosine kinase inhibitor therapy responses from 116 different publications were included. About 188 unique EGFR mutations occurring in 207 different combinations were identified: 149 different mutation combinations were

associated with disease control and 42 were associated with disease progression. Four secondary mutations, in 16 different combinations, were associated with acquired resistance.

Conclusions—As tumor sequencing becomes more common in oncology, this comprehensive electronic catalog can enable genome-directed anticancer therapy. DIRECT will eventually encompass all tumor mutations associated with clinical outcomes on targeted therapies. Users can make specific queries at http:// www.mycancergenome.org/about/direct to obtain clinically relevant data associated with various mutations.

Introduction

The treatment of patients with cancer in the 21st century has evolved into a complicated algorithm, requiring knowledge of an individual patient's tumor mutation status before initiating therapy. Making mastery of knowledge even more difficult, mutational profiling studies have revealed that within a single type of cancer, even a single gene can harbor multiple different mutations in different individuals. EGF receptor (EGFR) mutations in non–small cell lung cancer (NSCLC) represent a prime example of the complexity of disease at the molecular level. In North America, about 17% of patients with non–small cell lung cancer (NSCLC) harbor EGFR mutations (1), of which approximately 80% to 90% are composed of exon 19 deletions and L858R point mutations (2), which inherently confer sensitivity to therapy with the EGFR tyrosine kinase inhibitors (TKI), gefitinib or erlotinib (3). However, there are rare mutations that can be associated with primary drug sensitivity, primary drug resistance, or secondary resistance, while other rarer EGFR mutations are of less clear clinical significance (4). This rapid evolution of information promises to overwhelm practicing clinicians who will need a mechanism whereby they can quickly and conveniently reference the most accurate therapeutic options for patients with known tumor mutations.

Here, we report on the development of a catalog of clinically relevant somatic mutations, named the DNA-mutation Inventory to Refine and Enhance Cancer Treatment (DIRECT). This clinical database is designed to enable a genetically informed approach to cancer medicine by providing clinicians access to tumor gene therapy–response information based on individual patient data published in the literature. As proof-of-principle, we started the catalog on EGFR mutations in lung cancer, aiming to provide a comprehensive database on rare and common mutations associated with disease control and disease progression to EGFR TKI therapy.

Materials and Methods

Searches of the PubMed database from June 2005 to May 2011 were completed to identify relevant studies that reported on EGFR mutations in patients with NSCLCs. The search strategy used the terms Receptor, Epidermal Growth Factor [Mesh] OR (EGFR protein, human [Substance Name]) AND Lung Neoplasms [Mesh]. Additional articles were identified from searching the bibliographies of retrieved articles. An English language restriction was applied. Details of the search strategies are available on request from the authors. All articles were manually reviewed to determine whether the publication met minimum criteria for inclusion by 2 independent reviewers (P. Yeh and J. Andrews).

Additional articles were identified from searching the bibliographies of retrieved articles. The review of articles was conducted manually as most of the individual patient-level data, if presented, was within a table or image form, undetectable by automated, computerized methods. After the cutoff date for the PubMed search query, a retrospective search on the database was conducted to identify duplicate patient data published in multiple studies. The database was further verified by having 2 independent investigators (L. Horn and W. Pao) recheck at least every tenth patient data entry with the primary source reference. Any discrepancies were resolved through collective agreement.

All data was entered into an open source, electronic data capture program, the Research Electronic Data Capture (REDCap) project, from Vanderbilt University (Nashville, TN; refs. 5, 6). Only articles with individual patient data that provided the minimum following criterion were included: EGFR mutation, type of systemic treatment, and associated outcome including response rate (RR), progression-free survival (PFS), and/or overall survival (OS). At this time, only patients treated with the EGFR TKIs, gefitinib and erlotinib, were included. Data from studies with second-generation EGFR TKIs, such as afatinib (BIBW2992), neratinib (HKI-272), dacomitinib (PF-00299804), and XL647, will be included in future studies.

Response rate was classified into two categories, disease control and disease progression. Disease control was used to describe patients who achieved a complete response (CR), partial response (PR), or stable disease (SD), as defined by Response Evaluation Criteria in Solid Tumors (RECIST; ref. 7), following treatment with gefitinib or erlotinib. Disease progression was categorized as patients who had progressive disease (PD) as defined by the RECIST (7) following treatment with gefitinib or erlotinib. If a particular mutation had patients in both groups, that particular mutation was classified in the disease control or disease progression group based on whatever group had the higher percentage of a particular response. Additional data included if available were patient age, gender, ethnicity, smoking status, tumor histology, cancer stage at diagnosis, Eastern Cooperative Oncology Group (ECOG) performance status, and number of prior therapies. The PubMed ID, primary author name, journal name, and year of publication were recorded for each individual patient. Patients with acquired resistance to EGFR TKI therapy were catalogued separately ($n = 61$ from 9 different studies) from those with *de novo* mutations (i.e., disease previously untreated with EGFR TKIs).

Descriptive statistics, including medians, and ranges of continuous variables, as well as percentages and frequencies for categorical variables, were tabulated. The demographic analysis of the final DIRECT patient pool for this article was conducted using R Statistical Computing Program Version 14·1 (8).

Results

The initial search identified 2085 publications including prospective studies, retrospective studies, and case reports (Fig. 1). Of the 2085 publications, 1,939 articles were excluded for not meeting minimum inclusion criteria. The most common reason for exclusion was data presented in an aggregate population form such as a clinical trial. Of the 2085 publications,

146 papers met minimum criterion for inclusion into the DIRECT database. The number of therapy–response entries exceeds the number of individual patient entries, as some patient reports included more than one line of treatment.

After filtering out patients treated with only with other therapies (e.g., chemotherapy regimens), 1,149 patients with a total of 1,207 unique therapy–response entries still remained. Forty-two patients (with 47 therapy–response entries) were excluded because the exact EGFR mutation was unreported, and 79 patients (with 83 therapy–response entries) were excluded because the responses to EGFR TKI therapies were not reported. An additional 7 patients (with 7 therapy–response entries) were deemed duplicate data and deleted. About 1,070 separate therapy–response entries to gefitinib or erlotinib therapy from 1,021 patients and 116 different studies were used in our analysis. The number of unique patients does not match the number of therapy– response entries as one patient may have been treated with EGFR TKIs in different lines of treatment and thus have multiple associated therapy–response entries.

The median age of patients was 62 years (Table 1). The majority of the DIRECT patients with reported clinical features was female (42.8% vs. 28.0% male), Asian (66.6% vs. 18.5% Caucasian), never smokers (42.4% vs. 26.5% current/former smokers) with adenocarcinoma histology (65.8% vs. 24.8% other histology). About 63.6% had stage IIIB/IV or IV lung cancer (32.5% had unrecorded cancer staging). A significant portion of the patient pool had unknown demographic features (Table 1).

The mutations from each of the 1,021 patients were then classified into mutations associated with disease control, disease progression, or acquired resistance (Supplementary Table S1, Tables 2 and 3). Several mutations had patients that fit more than one category. For example, 2 of 3 patients with L861R mutation had disease control with EGFR TKI treatment; L861R was classified as a mutation with EGFR TKI disease control. Five mutation combinations that had a perfectly even split between 2 groups were classified as mutations associated with disease control because half of the patient sample achieved a SD, PR, or CR response, indicating the mutation seems at least somewhat sensitive to EGFR TKI therapy.

We identified more than 188 unique EGFR mutations, occurring in 207 different combinations. For example, some patients had either L858R alone ($n = 225$ from 62 studies), T790M alone ($n = 6$ from 3 studies), or both L858R and T790M in combination ($n = 27$ from 11 studies), resulting in an analysis of just 2 unique mutations (L858R and T790M) but 3 different noted mutation combinations. Of the 207 EGFR mutation combinations, 149 (72.0%) met criteria for disease control, 42 (20.3%) were associated with disease progression, and 16 (7.7%) were linked to acquired resistance (Table 1).

L858R

About 210 of the 235 therapy–response instances (89%) from 225 patients with a single L858R mutation achieved disease control with either gefitinib or erlotinib. L858R mutations were also frequently found with a second de novo mutation ($n = 31$). Including all combinations of L858R mutation with concurrent *de novo* mutations, 237 of the 266

therapy–response instances (89%) from 256 unique patients achieved disease control. L858R mutations occurring in combination with either A871E, L747S, *de novo* G719S, *de novo* T790M, L861P, or R776G were associated with disease progression.

Del19

A total of 191 of the 215 therapy–response instances (89%) from 204 separate patients with a single del19 E746-A750 mutation achieved disease control. Including patients with del19 E746-A750 mutation and a concurrent *de novo* mutation, 201 of the >225 therapy–response instances (89%) from 213 separate patients achieved disease control. Factoring in all patients from the 53 mutation combinations involving a del19 mutation, including those with an unspecified amino acid deletion in exon 19 (del19 unspecified) and those with concurrent *de novo* mutation(s), 424 of the 466 therapy–response instances (91%) from 439 patients were classified as having disease control. Del19 unspecified in combination with either ins20 unspecified or V769M were noted to be associated with disease progression. Only one patient with each combination was in DIRECT, making any determination of their therapeutic significance difficult.

G719X

Fourteen of 18 therapy–responses and 18 patients (78%) with a single G719X mutation— G719A, G719C, G719D, and G719S—achieved disease control. Expanding the pool to all patients with a G719X and concurrent *de novo* mutations, 20 of 24 therapy–response instances and 24 patients (83%) fit disease control criteria.

L861Q

Twelve of 14 therapy–response instances (86%) from 13 patients with a single L861Q mutation achieved disease control. Including all patients with L861Q and concurrent de novo mutations, 17 of 19 therapy–response instances (89%) from 18 patients had disease control. However, only 7 of these 17 (41%) disease control therapy–responses were partial responses; the other 10 met stable disease criteria. In contrast, 177 of the 237 disease control therapy–responses (75%) for L858R, 332 of the 424 disease control therapy– responses (78%) for del19, and 16 of the 20 disease control therapy–responses (80%) for G719X were partial responses, suggesting that L858R, exon 19 deletion, and G719X mutations respond more favorably to gefitinib or erlotinib than L861Q.

T790M

Both therapy–responses from 2 patients in DIRECT with a *de novo* T790M mutation had progressive disease. Five of 6 therapy–response instances (83%) and 6 patients with *de novo* L858R + T790M had disease progression.

Acquired resistance

Fifty-seven of 60 patients (95%) with acquired resistance to EGFR TKI therapy had T790M. The other 3 patients developed a D761Y, L747S, or T854A resistance mutation, in line with known acquired resistance mutations to EGFR TKI therapy (9). Other resistance mechanisms (e.g., MET amplification, PIK3CA mutation, etc.) were outside the focus of

this current report but will be included in future studies. Del19 E746-A750 was found with T790M acquired resistance mutation in 14 patients from 5 studies, whereas the L858R mutation was found with acquired resistance mutation T790M in 21 patients from 6 studies.

Discussion

The DIRECT database represents the most comprehensive catalog of EGFR mutations and response to gefitinib or erlotinib therapy to date. The majority of the mutations in the DIRECT database are rare mutations with limited clinical data that may not be readily accessible to the busy clinician. As expected for more common mutations, L858R and exon 19 deletions were associated with disease control, whereas the T790M mutation was associated with disease progression on an EGFR TKI. We further confirm that G719X and L861Q mutations confer sensitivity to EGFR TKIs, although L861Q mutations were associated with a lower response rate. As an increasing number of patients have their tumors genotyped, there will be (and is already) a dire need for an interactive easily accessible educational tool that provides up-to-date information to physicians on the clinical relevance of mutations in cancers. The database is currently available for query at http://www.mycancergenome.org/about/direct, where users can complete a brief questionnaire on their mutation of interest, and a detailed report on the specific mutation is emailed back (10). Since its inception, we have averaged 5 to 6 queries per month from around the world, the majority (>80%) for rare mutations. The number of queries continues to increase monthly. With time and with proper data sharing agreements for data from large clinical trials, we intend to change this into an interactive platform that will provide immediate data at the point of care to aid in therapeutic decisions.

Currently, DIRECT catalogs only patients with NSCLCs and published individual patient data on EGFR mutations. We are working with collaborators to expand the database to include individual patient data from large randomized phase II and III trials on patients with EGFR mutations and response to EGFR TKIs and chemotherapy. We also plan to incorporate other known mutations in solid tumors (e.g. melanoma, gastrointestinal stromal tumors, etc.) for which mutation–response data exist. To truly realize the promise of personalized medicine, all publications involving genotype-driven trials should at a minimum publish Supplementary Tables listing individual patients by tumor mutation status and treatment outcome.

This study has several limitations. First, not all demographic data including gender, smoking status, tumor histology, and stage were available for inclusion. These data do not affect the reported mutation–response data but do not allow classification of specific mutations within demographic categories. Second, of our 207 mutation combinations, 67% or 138 mutation combinations (97 disease control, 33 disease progressing, and 8 acquired resistance) are rare mutations for which there are only data from one patient, making any determination of their therapeutic significance difficult. Finally, we did not record the specific method of EGFR mutation testing from each study and thus this article does not take into account the variance in EGFR mutation detection between differing studies. Nevertheless, these data may serve as an initial guide in selecting therapy. As more and more patients have their tumors

genotyped, the number of patients with such "rare mutations" will grow and their clinical significance will emerge.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Translational Relevance

Tumor gene mutation status is becoming increasingly important in the treatment of patients with cancer. A comprehensive catalog of tumor gene-response outcomes from individual patients is needed, especially for actionable mutations and rare variants. Currently, no such catalog exists. Therefore, we created a proof-of principle database [DNA-mutation Inventory to Refine and Enhance Cancer Treatment (DIRECT)], starting with lung cancer-associated EGF receptor mutations, to provide a resource for clinicians to prioritize treatment decisions based on a patient's tumor mutations. As tumor sequencing becomes more common in oncology, this comprehensive electronic catalog can enable genome-directed anticancer therapy. DIRECT will eventually encompass all tumor mutations associated with clinical outcomes on targeted therapies.

PubMed Query: 2,085 papers identified

1,939 papers excluded for not having individual-level patient data

146 papers containing 1,373 unique individual patient-level data with 1,779 individual therapy–response entries[a] verified

224 patient entries with 572 therapy–response entries removed after filtering for patients treated with only EGFR TKI (Gefitinib or Erlotinib) therapies

1,149 unique patient entries with 1,207 separate therapy–response entries[a] treated with EGFR TKI therapy remained for statistical analysis

121 patient entries and 130 therapy–response entries removed after filtering out patients with undocumented mutations and /or undocumented responses to EGFR TKI therapy

1,028 unique patients with 1,077 separate therapy–response entries[a] remained for statistical analysis

7 patient and 7 therapy–response entries deemed duplicates and removed from analysis

1,021 unique patients with 1,070 separate therapy–response entries[a] remained for statistical analysis and inclusion in this paper
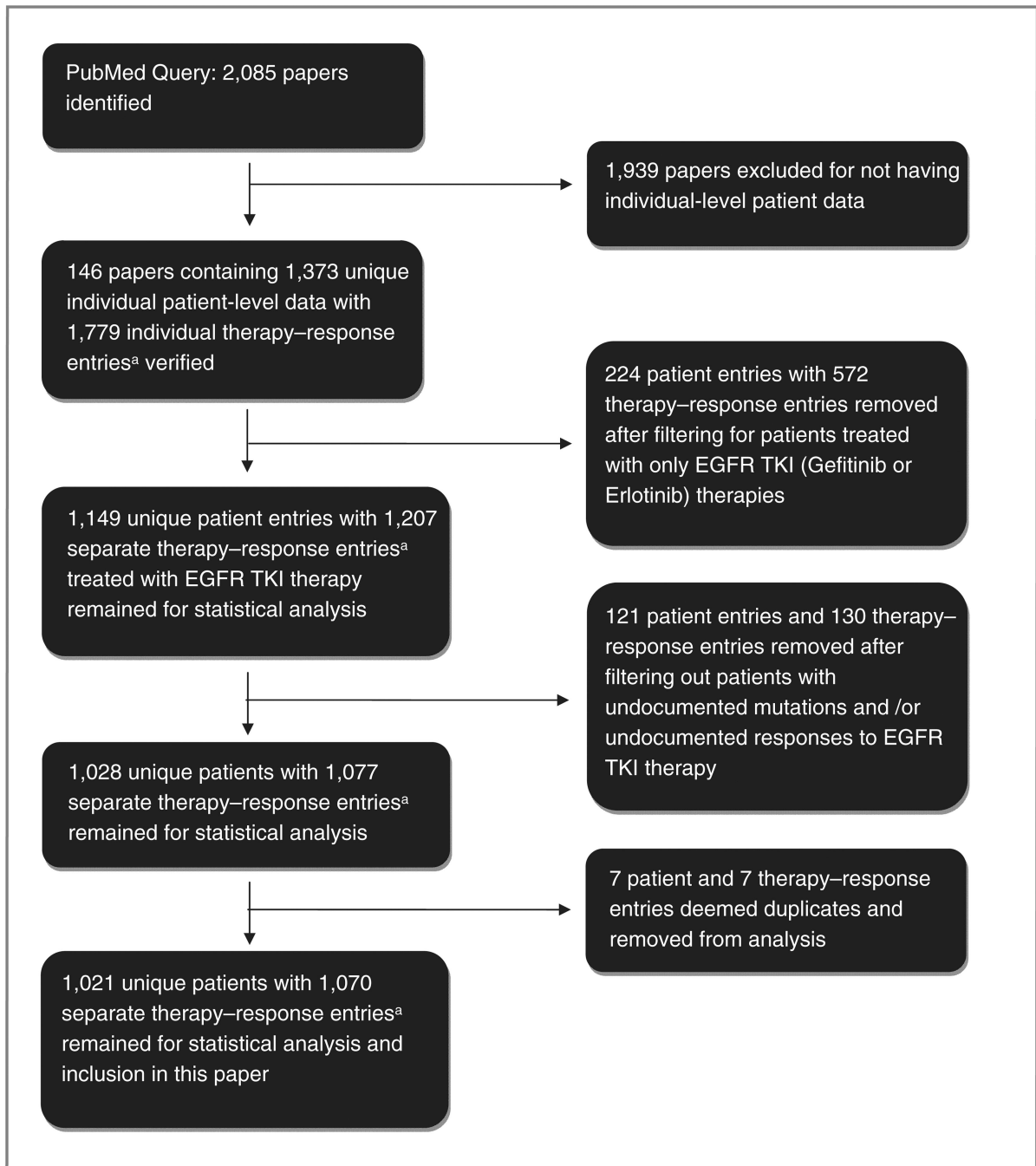
Figure 1. Schematic flowchart of DIRECT database and patient cohort formation. Number of unique patients does not match the number of therapy-response entries as one patient may have been treated with EGFR TKIs in different lines of treatment and thus have multiple associated therapy-response entries

Table 1

Demographic data for DIRECT patients treated with EGFR TKIs

Category	n (% of total cohort)
Median age (range), y	62 (27–92)
Gender	
Female	437 (42.8)
Male	286 (28)
Unknown	298 (29.2)
Ethnicity	
Caucasian	189 (18.5)
Asian	680 (66.6)
African-American	2 (0.2)
Unknown	150 (14.7)
Histology[a]	
Adenocarcinoma	672 (65.8)
Adenosquamous	5 (0.5)
Bronchioloalveolar	23 (2.3)
Large cell	15 (1.5)
Squamous	34 (3.3)
Spindle	1 (0.1)
Unknown	96 (9.4)
Smoking status	
Current	55 (5.4)
Former smokers	215 (21.1)
Never smokers	433 (42.4)
NSCLC nos[b]	175 (17.5)
Unknown	318 (31.2)
Stage	
I	4 (0.4)
IB	1 (0.1)
IIA	3 (0.3)
IIB	5 (0.5)
III	2 (0.2)
IIIA	25 (0.25)
IIIB	39 (3.8)
IIIB/IV	401 (39.3)
IV	209 (20.5)
Unknown	332 (32.5)
ECOG performance status	
0	11 (1.1)
1	35 (3.4)
2	21 (2.1)

Category	n (% of total cohort)
3	8 (0.8)
4	5 (0.5)
Unknown	941 (92.1)
Number of prior therapies before starting EGFR TKI therapy	
0	179 (17.5)
1	49 (4.8)
2	86 (8.4)
3	18 (1.8)
4	9 (0.9)
6	2 (0.2)
Unknown	678 (66.4)
Total number of mutation combinations by response category	
De novo disease control	149 (72.0)
De novo disease progression	42 (20.3)
Acquired resistance	16 (7.7)
TOTAL unique mutation combinations	207 (N/A)

Abbreviation: N/A, not applicable.

[a] 2004 World Health Organization (WHO) Classification used.

[b] NSCLC not otherwise specified.

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Table 2

EGFR mutations associated with disease progression

| Mutation | N | | Number of unique | | |
	Therapy–response instances	PD responses	Patients[a]	Studies[b]	References
A763V	1	1	1	1	11
A859T	2	2	2	2	12, 13
Del19 A767-V769	1	1	1	1	11
Del19 unspecified + ins20 unspecified	1	1	1	1	14
Del19 unspecified + V769M	1	1	1	1	14
E709G + G719C	1	1	1	1	14
E711K	1	1	1	1	15
G719A + S768I	2	2	2	2	16, 17
G721D	1	1	1	1	18
G729R	1	1	1	1	15
I744M	1	1	1	1	19
I759T	1	1	1	1	20
Ins20 769–770insGVV	1	1	1	1	18
Ins20 A767-V769dupASV	1	1	1	1	17
Ins20 D770-N771insD	1	1	1	1	17
Ins20 P772-H773insYNP + H773Y	1	1	1	1	17
Ins20 S768-D770dupSVD	4	3	4	1	17
Ins20 SVD768–770	3	2	3	1	21
Ins20 unspecified	3	3	3	1	22
K806E	1	1	1	1	17
K860E	1	1	1	1	15
L703F	1	1	1	1	15
L747P	2	2	2	1	23
L777G	1	1	1	1	18
L838P + E868G	1	1	1	1	19
L858R + A871E	1	1	1	1	14
L858R + G719S	3	2	3	1	24

Mutation	N		Number of unique		References
	Therapy–response instances	PD responses	Patients[a]	Studies[b]	
L858R + L747S (de novo)	1	1	1	1	14
L858R + L861P	1	1	1	1	15
L858R + T790M (de novo)	6	5	6	5	17, 18, 21, 25, 26
L858R + R776G	1	1	1	1	17
L861Q + G719S	1	1	1	1	11
N826Y	1	1	1	1	14
N842S	1	1	1	1	14
S768I + V769L	1	1	1	1	27
S784F	1	1	1	1	14
T847A + G863S	1	1	1	1	15
T847I	1	1	1	1	14
T790M (de novo)	2	2	2	2	25, 28
V774M	1	1	1	1	14
V802I	1	1	1	1	25
V852I	1	1	1	1	29

NOTE: Disease progression noted as a PD response as defined by the RECIST criteria (7), following treatment with EGFRTKI(gefitinib or erlotinib therapy).

[a] Number of unique patients does not match the number of therapy–response entries as one patient may have been treated with EGFR TKIs in different lines of treatment and thus may have multiple associated therapy-response entries.

[b] Number of unique studies refers to the number of different studies that encompass all of the patients with a particular mutation in DIRECT.

Table 3

EGFR mutations found in patients with acquired resistance to EGFR TKIs

| Initial mutation | Acquired resistance mutation[a] | Number of unique | | References |
		Patients[b]	Studies[c]	
Del19 E746-A750	T790M	14	5	30–34
Del19 E746-T751insA	T790M	1	1	32
Del19 E746-T751insV	T790M	1	1	35
Del19 L747-A750insP	T790M	1	1	31
Del19 L747-E749;A750P	T790M	3	2	34, 35
Del19 L747-P753insQ	T790M	1	1	369
Del19 L747-P753insS	T790M	2	2	32, 34
Del19 L747-S752	T790M	2	2	31, 32
Del19 L747-T751	T790M	1	1	35
Del19 L747-T751;K754E	T790M	3	2	34, 35
Del19 unspecified	T790M	3	1	37
L858R	D761Y	1	1	32
L858R	L747S	1	1	38
L858R	T854A	1	1	35
L858R	T790M	21	6	31, 32, 34, 35, 37, 39
Wild-type	T790M	4	1	31

[a] These resistance mutations were acquired after treatment with EGFR TKI.

[b] Number of unique patients does not match the number of therapy-response entries as one patient may have been treated with EGFR TKIs in different lines of treatment and thus may have multiple associated therapy-response entries.

[c] Number of unique studies refers to the number of different studies that encompass all of the patients with a particular mutation in DIRECT.