Pervenio™ Lung NGS

Next-Generation Sequencing

Find the optimal therapy for your NSCLC patient with the Pervenio Lung NGS assay

Recent studies have identified an increasing number of genomic alterations associated with non-small cell lung cancer (NSCLC), with many present at low frequency. As the number of alterations increases, the current paradigm of sequentially testing single gene targets is unsustainable given the limited availability of tissue. Advances in next-generation sequencing technology allow for the rapid assessment of multiple targets simultaneously from limited tissue samples.

Pervenio Lung NGS was developed and validated specifically for NSCLC, using state-of-the-art next-generation sequencing-based technology.

Assesses mutations and translocations associated with 25 genes

- Mutation analysis in 22 genes, including *EGFR*, *BRAF*, and *KRAS*
- Translocation analysis for 3 genes:[1] *EML4-ALK*, *RET*, and *ROS1*

Uses tissue-sparing techniques specifically designed for small specimens

- Ten 7 µm unstained slides[2]

Generates a current, clinically relevant, and well-referenced report

- Updated therapeutic and clinical trial information

Delivers accurate and reproducible results

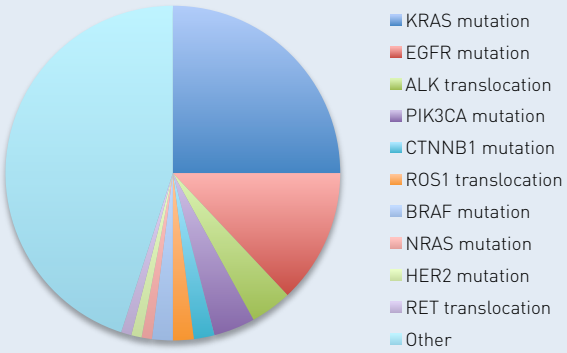
- ≥99% accuracy, reproducibility, analytical sensitivity and specificity

Provides rapid turnaround time

- Generally within 10[3] days of sample receipt[2]

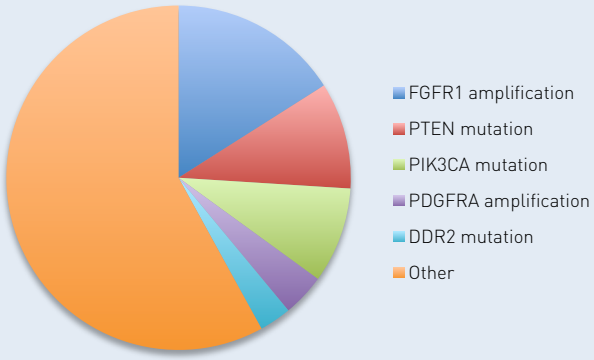
[1] Currently use qPCR for these three genes, with transition to NGS planned
[2] Request to perform confirmatory testing by FISH, if an *ALK* translocation is detected, adds 3-4 days in turnaround time and three 4 µm slides.
[3] Samples requiring repeat testing may result in an increased turnaround time

Mutation Spectrum in Adenocarcinoma



Mutations in *TP53* and *STK11*/*LKB1* are common occurrences, not included in pie chart due to high overlap with other mutations

Mutation Spectrum in Squamous Cell Carcinoma



Mutations in *TP53* are a common occurrence, not included in pie chart due to high overlap with other mutations

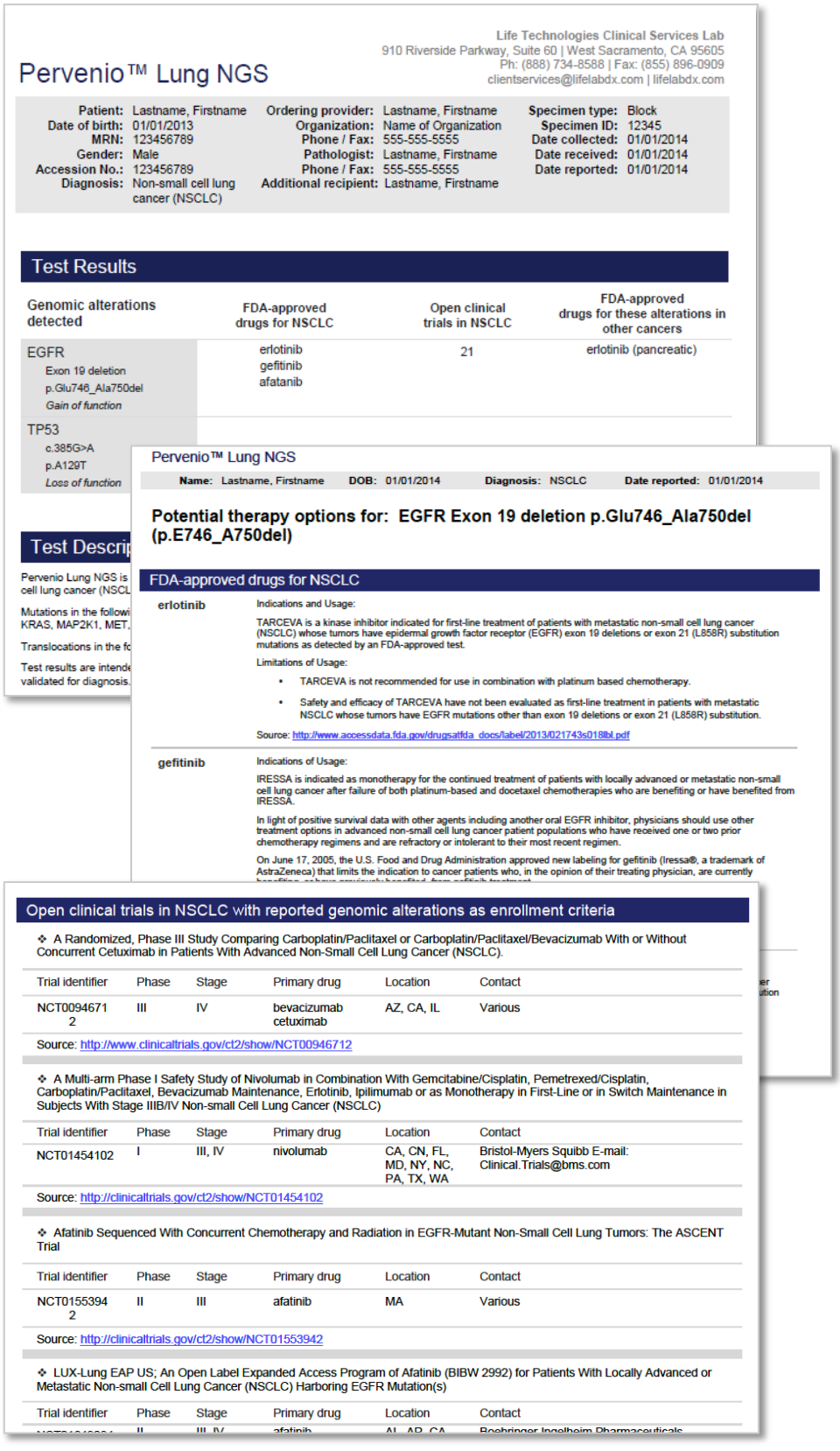
Source: Adapted from Heist RS and Engelman JA, SnapShot: Non small cell lung cancer, Cancer Cell 2012; 21:448-448e2

Assesses mutations and translocations associated with 25 genes

Gene	% Frequency in NSCLC Adeno	% Frequency in NSCLC Squamous	Background Information
Mutations			
AKT1	0.5-1.2	0.6	Mutations in AKT1, a serine-threonine kinase (STK) downstream of PI3K, have been described in patients with non-small cell lung cancer (NSCLC). Randomized clinical trials (RCTs) with AKT1 inhibitors in NSCLC are currently underway.
ALK	7.8-9.6	2.2-3.4	Mutations within the ALK tyrosine kinase domain have been described in patients with NSCLC, and are associated with acquired resistance to the ALK tyrosine kinase inhibitor (TKI), crizotinib. RCTs with agents targeting ALK alterations in NSCLC are currently underway.
BRAF	1.8-9.6	4.5	Mutations in the BRAF STK have been described in patients with NSCLC, and lead to increased kinase activity, activation of downstream signaling pathways, and enhanced tumorigenicity. In contrast to melanoma where the majority of BRAF mutations occur at V600 within exon 15 of the kinase domain, BRAF mutations in NSCLC also occur at other positions within the kinase domain. RCTs with BRAF inhibitors in NSCLC are currently underway.
CTNNB1	1.2-3.9	2.2	Mutations in the proto-oncogene ß-catenin, which is encoded by the CTNNB1 gene, have been described in patients with NSCLC, and result in increased transcriptional activation of downstream target genes and enhanced tumorigenicity.
DDR2	0.6-5.5	1.1-1.7	Mutations in the receptor tyrosine kinase (RTK) DDR2 have been described in patients with NSCLC, and are associated with increased kinase activity, activation of downstream signaling pathways, and enhanced tumorigenicity. RCTs with DDR2 inhibitors in NSCLC are currently underway.
EGFR	11.8-18.4	3.4-3.9	Mutations in exons 18 to 21 of the RTK EGFR, which have been described in patients with NSCLC, increase its kinase activity, leading to hyperactivation of downstream pro-survival signaling pathways. EGFR inhibitors have been approved by the US Food & Drug Administration (FDA) for NSCLC, and RCTs with agents targeting EGFR alterations in NSCLC are currently underway.
ERBB2	1.8-4.4	1.1-2.8	Mutations in the RTK ERBB2, such as in-frame insertions within exon 20, have been described in patients with NSCLC, and lead to increased kinase activity, activation of downstream signaling pathways, and enhanced tumorigenicity. RCTs with ERBB2 inhibitors in NSCLC are currently underway.
ERBB4	4.9-8.4	6.7	Mutations in the RTK ERBB4 have been described in patients with NSCLC, and result in increased kinase activity, activation of downstream signaling pathways, and enhanced tumorigenicity.
FBXW7	1.2-4.4	6.7	Mutations in FBXW7, an F-box protein involved in ubiquitin-dependent degradation of certain cell cycle-related proteins, have been described in patients with NSCLC, and are associated with cell cycle dysregulation and enhanced tumorigenicity.
FGFR1	0.5-1.3	0.6-1.7	Mutations in the fibroblast growth factor receptor type 1 (FGFR1) RTK have been reported in patients with NSCLC, and lead to increased kinase activity, activation of downstream signaling pathways, and enhanced tumorigenicity. RCTs with FGFR1 inhibitors in NSCLC are currently underway.
FGFR2	1.2-3.1	3.9	Mutations in the FGFR2 RTK have been reported in patients with NSCLC, and result in increased kinase activity, activation of downstream signaling pathways, and enhanced tumorigenicity. RCTs with agents targeting FGFR2 alterations in NSCLC are currently underway.
FGFR3	0.4-1.6	2.2	Activating mutations in the FGFR3 RTK have been reported in patients with NSCLC, and are associated with increased kinase activity, activation of downstream signaling pathways, and enhanced tumorigenicity. RCTs with FGFR3 inhibitors in NSCLC are currently underway.
KRAS	26.2-36.8	1.1	Mutations in KRAS, such as those resulting in amino acid substitutions at position 12, 13, or 61, have been described in patients with NSCLC, and lead to constitutive activation of downstream signaling pathways. KRAS mutations in NSCLC (versus colon cancer) have not yet been shown to be negative predictors of benefit from EGFR inhibitors, but are negative predictors of radiographic response to the EGFR TKIs erlotinib and gefitinib. RCTs with agents targeting KRAS alterations in NSCLC are currently underway.
MAP2K1	1.3-2.7	1.1	Mutations in MAP2K1, a member of the extracellular signal-related kinase (ERK) family, have been described in patients with NSCLC, and result in increased kinase activity, activation of downstream signaling pathways, and enhanced tumorigenicity. RCTs with MAP2K1 inhibitors in NSCLC are currently underway.
MET	1.8-8.3	2.2-2.8	Mutations in the juxtamembrane and semaphorin domains of the RTK MET have been described in patients with NSCLC, and are associated with increased kinase activity, activation of downstream signaling pathways, and enhanced tumorigenicity. RCTs with agents targeting MET alterations in NSCLC are currently underway.
NOTCH1	0.6-4.9	7.9-8.4	Mutations in NOTCH1, a transmembrane protein receptor involved in cell fate decisions, have been described in patients with NSCLC, and lead to activation of downstream signaling pathways and enhanced tumorigenicity.
NRAS	0.4-1.8	0.6	Mutations in NRAS, which are mostly missense mutations that introduce an amino acid substitution at position 61, have been described in patients with NSCLC, and result in constitutive activation of downstream signaling pathways and enhanced tumorigenicity. RCTs with NRAS inhibitors in NSCLC are currently underway.
PIK3CA	0.6-6.5	15.2-15.7	Mutations in PIK3CA, which encodes the catalytic subunit of phosphatidylinositol 3 kinase (PI3K), have been described in patients with NSCLC. These mutations usually occur within two "hotspot" areas within exon 9 and exon 20, and are associated with increased kinase activity, activation of downstream signaling pathways, and enhanced tumorigenicity. RCTs with agents targeting PIK3CA alterations in NSCLC are currently underway.
PTEN	1.3-3.1	7.9	Mutations in PTEN, a lipid/protein phosphatase that acts as a tumor suppressor by negatively regulating the PI3K/AKT signaling pathway, have been reported in patients with NSCLC, and lead to PTEN inactivation and therefore increased PI3K-AKT signaling and tumorigenicity. RCTs with agents targeting PTEN alterations in NSCLC are currently underway.
SMAD4	1.2-3.5	2.8	Mutations in SMAD4, a tumor suppressor gene that negatively regulates the TGFß family of proteins, have been reported in patients with NSCLC, and result in SMAD4 inactivation and therefore increased TGFß signaling and tumorigenicity.
STK11	8.7-20.9	1.7	Mutations in STK11, a tumor suppressor that inhibits signaling by AMPK-related kinases and suppresses proliferation, have been described in patients with NSCLC, and lead to STK11 inactivation and increased tumorigenicity.
TP53	39.3-52.0	79.2-80.9	Mutations in TP53, a tumor suppressor gene described as the "guardian of the genome", have been reported in patients with NSCLC, and are associated with TP53 inactivation, genomic instability, and increased tumorigenicity.
Translocations			
EML4/ALK	3-7		Rearrangements, most often with the echinoderm microtubule-associated protein-like 4 (EML4) gene, have been described in patients with NSCLC, and are associated with resistance to EGFR TKIs. RCTs with agents targeting ALK translocations in NSCLC are currently underway.
ROS1	2		Rearrangements in the ROS1 RTK have been described in patients with NSCLC, and are associated with sensitivity to TKIs that have "off-target" activity against ROS1 in preclinical models, such as crizotinib. RCTs with ROS1 inhibitors in NSCLC are currently underway.
RET	1.3		Rearrangements involving RET have been described in patients with NSCLC, and result in constitutive activation of downstream signaling pathways and enhanced tumorigenicity. RCTs with agents targeting RET translocations in NSCLC are currently underway.

Source: cbioportal.org, COSMIC, mycancergenome.com

Provides current, clinically relevant, and well-referenced information for > 400 genomic alterations



Pervenio™ Lung NGS

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Patient:	Lastname, Firstname	Ordering provider:	Lastname, Firstname	Specimen type:	Block
Date of birth:	01/01/2013	Organization:	Name of Organization	Specimen ID:	12345
MRN:	123456789	Phone / Fax:	555-555-5555	Date collected:	01/01/2014
Gender:	Male	Pathologist:	Lastname, Firstname	Date received:	01/01/2014
Accession No.:	123456789	Phone / Fax:	555-555-5555	Date reported:	01/01/2014
Diagnosis:	Non-small cell lung cancer (NSCLC)	Additional recipient:	Lastname, Firstname		

Test Results

Genomic alterations detected	FDA-approved drugs for NSCLC	Open clinical trials in NSCLC	FDA-approved drugs for these alterations in other cancers
EGFR Exon 19 deletion p.Glu746_Ala750del *Gain of function*	erlotinib gefitinib afatanib	21	erlotinib (pancreatic)
TP53 c.385G>A p.A129T *Loss of function*			

Test Descrip

Pervenio Lung NGS is
cell lung cancer (NSCL

Mutations in the followi
KRAS, MAP2K1, MET,

Translocations in the fo

Test results are intende
validated for diagnosis.

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Name: Lastname, Firstname **DOB:** 01/01/2014 **Diagnosis:** NSCLC **Date reported:** 01/01/2014

Potential therapy options for: EGFR Exon 19 deletion p.Glu746_Ala750del (p.E746_A750del)

FDA-approved drugs for NSCLC

erlotinib

Indications and Usage:

TARCEVA is a kinase inhibitor indicated for first-line treatment of patients with metastatic non-small cell lung cancer (NSCLC) whose tumors have epidermal growth factor receptor (EGFR) exon 19 deletions or exon 21 (L858R) substitution mutations as detected by an FDA-approved test.

Limitations of Usage:

- TARCEVA is not recommended for use in combination with platinum based chemotherapy.
- Safety and efficacy of TARCEVA have not been evaluated as first-line treatment in patients with metastatic NSCLC whose tumors have EGFR mutations other than exon 19 deletions or exon 21 (L858R) substitution.

Source: http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/021743s018lbl.pdf

gefitinib

Indications of Usage:

IRESSA is indicated as monotherapy for the continued treatment of patients with locally advanced or metastatic non-small cell lung cancer after failure of both platinum-based and docetaxel chemotherapies who are benefiting or have benefited from IRESSA.

In light of positive survival data with other agents including another oral EGFR inhibitor, physicians should use other treatment options in advanced non-small cell lung cancer patient populations who have received one or two prior chemotherapy regimens and are refractory or intolerant to their most recent regimen.

On June 17, 2005, the U.S. Food and Drug Administration approved new labeling for gefitinib (Iressa®, a trademark of AstraZeneca) that limits the indication to cancer patients who, in the opinion of their treating physician, are currently

Open clinical trials in NSCLC with reported genomic alterations as enrollment criteria

❖ A Randomized, Phase III Study Comparing Carboplatin/Paclitaxel or Carboplatin/Paclitaxel/Bevacizumab With or Without Concurrent Cetuximab in Patients With Advanced Non-Small Cell Lung Cancer (NSCLC).

Trial identifier	Phase	Stage	Primary drug	Location	Contact
NCT00946712	III	IV	bevacizumab cetuximab	AZ, CA, IL	Various

Source: http://www.clinicaltrials.gov/ct2/show/NCT00946712

❖ A Multi-arm Phase I Safety Study of Nivolumab in Combination With Gemcitabine/Cisplatin, Pemetrexed/Cisplatin, Carboplatin/Paclitaxel, Bevacizumab Maintenance, Erlotinib, Ipilimumab or as Monotherapy in First-Line or in Switch Maintenance in Subjects With Stage IIIB/IV Non-small Cell Lung Cancer (NSCLC)

Trial identifier	Phase	Stage	Primary drug	Location	Contact
NCT01454102	I	III, IV	nivolumab	CA, CN, FL, MD, NY, NC, PA, TX, WA	Bristol-Myers Squibb E-mail: Clinical.Trials@bms.com

Source: http://clinicaltrials.gov/ct2/show/NCT01454102

❖ Afatinib Sequenced With Concurrent Chemotherapy and Radiation in EGFR-Mutant Non-Small Cell Lung Tumors: The ASCENT Trial

Trial identifier	Phase	Stage	Primary drug	Location	Contact
NCT01553942	II	III	afatinib	MA	Various

Source: http://clinicaltrials.gov/ct2/show/NCT01553942

❖ LUX-Lung EAP US; An Open Label Expanded Access Program of Afatinib (BIBW 2992) for Patients With Locally Advanced or Metastatic Non-small Cell Lung Cancer (NSCLC) Harboring EGFR Mutation(s)

Trial identifier	Phase	Stage	Primary drug	Location	Contact

Report includes:

- All genomic alterations detected (mutations and translocations) in the tumor tissue
- Therapies that target each detected genomic alteration
  - FDA-approved drugs in NSCLC and other cancers, with links to the FDA website
- A list of open clinical trials in NSCLC with the detected genomic alteration as an enrollment criterion. Information on cancer stage, trial ID, phase, locations, contact information, and links to trial websites from clinicaltrials.gov is also provided

Given the dynamic nature of drug development, our therapeutic and clinical trial information database is updated monthly to ensure the most current information for physicians and patients.

Provides accurate and reproducible results with fast turnaround time from small tissue samples

Performance specification

Analytical Sensitivity	≥99% at ≥5% allele frequency (mutations by NGS) ≥99% at ≥1% total mRNA (translocations by qPCR)
Analytical Specificity	≥99% PPV (mutations by NGS and translocations by qPCR)
Accuracy	≥99%
Reproducibility	≥99% at ≥5% allele frequency (mutations by NGS and translocations by qPCR)
NGS Depth of Coverage	> 4,000x (average)
Turnaround Time	Generally 7 days from receipt of sample that meets acceptance criteria[1]

How to order

Visit www.lifelabdx.com to download a Test Request Form and get started today, or call the Life Technologies Clinical Services Lab at (888) 734-8588 for more information.

Billing and reimbursement support

The Life Technologies Clinical Services Lab bills insurance for its testing services. We work with patients to manage appeals and physician offices to simplify pre-certifications. Patients are kept informed throughout the process. We offer reimbursement assistance for under-insured and uninsured patients based on financial need. Please call (888) 734-8588 for more information.

Sample requirements[1]

Blocks	Single paraffin-embedded tissue block containing the highest percentage of tumor
	OR
Slides	Ten 7 µm unstained sections mounted on slides for each specimen to be tested. Each slide must be clearly labeled with the institutional block number

[1] Request to perform confirmatory testing by FISH, if an *ALK* translocation is detected, adds 3-4 days in turnaround time and three 4 µm slides.

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