Everything you need to know about *ERBB2 (HER2)* testing in NSCLC in 2 minutes

Introduction

Human epidermal growth factor 2 receptor (HER2) is a transmembrane protein encoded by the *ERBB2 (HER2)* gene located on the long arm of chromosome 17 (17q21). HER2 is a member of the ERBB receptor tyrosine kinase family. Unique among the other ERBB family members, HER2 is activated in a ligand-independent manner through homodimerization or heterodimerization with other HER proteins. Dimerization of HER2 leads to activation of the downstream phosphatidylinositol-3-kinases (PI3K)–mediated protein kinase B (AKT) pathway and methyl ethyl ketone (MEK)–mediated extracellular signal regulated kinase (ERK) pathway, both of which determine cellular proliferation and migration [1].

HER2 alterations, namely *HER2* amplification and overexpression, are well established in breast and gastric cancers. The role of HER2 alterations in dysregulation is an active area of investigation in other solid tumors, including non-small cell lung cancer (NSCLC).

HER2 alterations in NSCLC

Three types of HER2 alterations in NSCLC have been described: *HER2* mutations, *HER2* amplification, and HER2 protein overexpression. *HER2* gene amplifications are found as *de novo* or acquired alterations, as one of the mechanisms responsible for acquired EGFR tyrosine kinase inhibitor resistance [1]. HER2 protein overexpression is characterized by an overabundance of HER2 proteins expressed on the cell surface that increases the formation of HER2-containing heterodimers, which lead to activation of several oncogenic signaling pathways [2]. In contrast to breast cancer in NSCLC, the correlation between *HER2* amplification and HER2 protein overexpression is poor, suggesting distinct roles in tumorigenesis.

A wide range of somatic *HER2* mutations is found across several malignancies, including NSCLC, further defining a distinct molecular subset that is typically mutually exclusive with other oncogenic drivers. *HER2* mutations encompass many single-nucleotide variants (SNVs) or insertions and deletions that can occur in the extracellular, transmembrane/juxtamembrane, and extracellular domains. The functional implications of dysregulation largely depend on where the mutation is located [3].
The majority of *HER2* mutations occur in exons 18–20 of the kinase domain as in-frame duplications or insertions, ranging

	HER2 mutations	*HER2* amplification	HER2 protein overexpression
			
Description	Mutations in the *ERBB2 (HER2)* gene	Abnormally high number of copies in *ERBB2 (HER2)* gene	Overabundance of HER2 receptors expressed on the surface of tumor cells
Testing method(s)	NGS, RT-PCR	FISH	IHC
Prevalence	2–4% [1]	3–13% [1]	2–38% [1]

from 3 to 12 base pairs. The most common of these is the YVMA insertion-duplication variant involving an in-frame exon 20 insertion at codon 776, accounting for up to 90% of all *HER2* mutations [1,2,4].

Less frequently observed mutations are SNVs or other insertions in exon 20 or other exons that affect the transmembrane and juxtamembrane domains [5]. The significant heterogeneity observed in *HER2* alterations in NSCLC requires distinct methods for identification.

HER2 testing methods

HER2 protein overexpression is commonly detected by immunohistochemistry (IHC). In contrast to breast and gastric cancers, positivity in NSCLC determined by scoring is not universally established and requires further investigation.

HER2 amplification refers to a copy number increase at a specific chromosome location relative to the whole chromosome and, is primarily detected using fluorescence *in situ* hybridization (FISH). Alternatively, *HER2* amplification can also be detected by NGS, with the advantage of simultaneously detecting variants across numerous genes.

Reverse-transcription polymerase chain reaction (RT-PCR) is the most common method used to detect *HER2* mutations. The limitation of RT-PCR is its ability to only detect known and well-characterized mutations, and its inability to detect less common variants.

NGS is becoming an invaluable method in clinical research, particularly with NSCLC for its ability to simultaneously analyze multiple genes from limited tumor material, including the identification and characterization of heterogeneous *HER2* mutations. The ideal method for *HER2* clinical research should be able to identify relevant types of variations in *HER2,* including exon 20 insertions, missense point mutations, and copy number variation and amplification, with a low requirement for input DNA high speed, and high repeatability [6].

Molecular profiling for NSCLC research, including *HER2* alterations, with Oncomine Solutions

Oncomine™ Solutions are complete end-to-end NGS workflows, including bioinformatics, for precision oncology research. Requiring as little as 10 ng of DNA or RNA, Oncomine Solutions can generate results from limited tissue and small biopsies in as little as 24 hours.

Oncomine Solutions provide an ideal method for molecular profiling in NSCLC because of the ability to identify relevant variants in *HER2*, including exon 20 insertions, SNVs, and amplifications, with low input requirements, high sensitivity, and a short turnaround time.

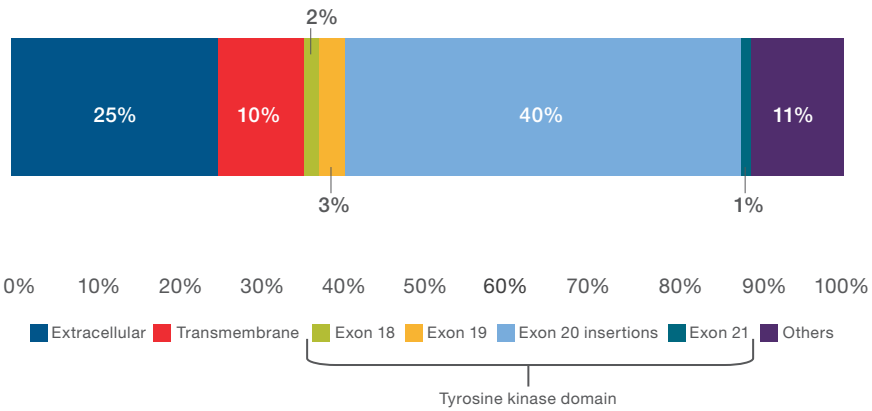

Figure 1. Distribution of *HER2* mutations in NSCLC [4].

Table 1. Oncomine Solutions for NSCLC research.

		Ion Torrent™ Oncomine™ Comprehensive Assay v3	Ion Torrent™ Oncomine™ Comprehensive Assay Plus	Ion Torrent™ Oncomine™ Precision Assay GX
Panel details	ALK, BRAF, EGFR, ERBB2, KRAS, MET, NTRK, ROS1, RET	•	•	•
	Mutational signatures	–	MSI, TMB	–
	Specimen types	FFPE tissue	FFPE tissue	FFPE tissue and plasma
	Alteration types	Mutations, insertions, deletions, CNVs, fusions		
	Specimen types	DNA and RNA	DNA and RNA	DNA and RNA, cfTNA
	Number of genes	161	>500	50
	DNA or RNA input amount	20 ng	20 ng	10 ng
Instrument and turnaround time (TAT)	Ion GeneStudio™ S5 System (4-day TAT)	•	•	–
	Ion Torrent™ Genexus™ System (1-day TAT)	•	–	•

• included

– not included

References

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