Journal of *Molecular Pathology*

MDPI

Brief Report

ERBB2 Mutation Testing in NSCLC: A Pan-European Real-World Evaluation of the Oncomine Precision Assay

Ilaria Alborelli [1], Melanie Demes [2], Peter Wild [2], Susana Hernandez [3], Fernando Lopez-Rios [4], Olivier Bordone [5], Christophe Bontoux [5], Paul Hofman [5], Caterina De Luca [6], Giancarlo Troncone [6], Luisella Righi [7], Umberto Malapelle [6], Ricella Souza da Silva [8], Luis Cirnes [8], Fernando Schmitt [8], Eveline Keller [1], Philip M. Jermann [9], John Longshore [10] and Lukas Bubendorf [1,*]

1 Institute of Medical Genetics and Pathology, University Hospital Basel, 4031 Basel, Switzerland; ilaria.alborelli@usb.ch (I.A.); evelinebarbara.keller@usb.ch (E.K.)
2 Dr. Senckenberg Institute of Pathology (SIP), University Hospital Frankfurt, Goethe University, 60590 Frankfurt am Main, Germany; melanie.demes@ukffm.de (M.D.); peter.wild@ukffm.de (P.W.)
3 Department of Pathology, Hospital Universitario 12 de Octubre, Research Institute 12 de Octubre University Hospital (i+12), 28041 Madrid, Spain; hernandezprietosusana@gmail.com
4 Department of Pathology, 12 de Octubre University Hospital-CIBERONC, Research Institute 12 de Octubre University Hospital (i+12), Universidad Complutense, 28041 Madrid, Spain; fernandolopezriosmoreno@gmail.com
5 Laboratory of Clinical and Experimental Pathology, IHU RespirERA, FHU OncoAge, BB-0033-00025, Centre Hospitalier Universitaire de Nice, Université Côte d'Azur, 06200 Nice, France; bordone.o@chu-nice.fr (O.B.); institut_claudius_regaud@mib.claudiusregaud.fr (C.B.); hofman.p@chu-nice.fr (P.H.)
6 Department of Public Health, University of Naples Federico II, 80131 Naples, Italy; caterina.deluca@unina.it (C.D.L.); giancarlo.troncone@unina.it (G.T.); umberto.malapelle@unina.it (U.M.)
7 Department of Pathology, University of Turin, 10124 Turin, Italy; luisella.righi@unito.it
8 IPATIMUP Diagnostics, IPATIMUP–Institute of Molecular Pathology and Immunology of Porto University, 4200-135 Porto, Portugal; ricellas@i3s.up.pt (R.S.d.S.); lcirnes@ipatimup.pt (L.C.); fschmitt@ipatimup.pt (F.S.)
9 Thermo Fisher Scientific, Waltham, MA 17111, USA; philip.jermann@thermofisher.com
10 Astra Zeneca, 1800 Concord Pike, Wilmington, DE 19803, USA; john.longshore@astrazeneca.com
* Correspondence: lukas.bubendorf@usb.ch

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Academic Editor: Izidor Kern

Received: 14 February 2025
Revised: 18 April 2025
Accepted: 1 August 2025
Published: 14 August 2025

Citation: Alborelli, I.; Demes, M.; Wild, P.; Hernandez, S.; Lopez-Rios, F.; Bordone, O.; Bontoux, C.; Hofman, P.; De Luca, C.; Troncone, G.; et al. *ERBB2* Mutation Testing in NSCLC: A Pan-European Real-World Evaluation of the Oncomine Precision Assay. *J. Mol. Pathol.* **2025**, *6*, 19. https://doi.org/10.3390/jmp6030019

Abstract

Background/Objectives: The non-small-cell lung cancer (NSCLC) therapeutic landscape has undergone a profound transformation with the introduction of multiple personalized treatment options. Mutations in *ERBB2* (*HER2*) have recently emerged as promising novel targets for the treatment of non-squamous NSCLC (nsNSCLC). Accurate, rapid, and efficient molecular profiling is crucial for identifying patients who may benefit from targeted therapies, including *HER2*-directed agents. **Materials and Methods:** Here, we aimed to retrospectively assess the performance of the Oncomine™ Precision Assay* (OPA) in combination with the Ion Torrent Genexus™ Integrated Sequencer* (Thermo Fisher Scientific. Waltham, MA, USA) for detecting *ERBB2* mutations in nsNSCLC. A total of 108 archived nsNSCLC samples, consisting of biopsies, resections, and cytological specimens, were used to assess concordance with in-house-validated orthogonal tests. **Results:** The OPA showed high sensitivity and specificity with an overall accuracy of 100% for single-nucleotide variants (SNVs) and insertions and deletions (Indels). SNVs and Indels with allele frequencies as low as 5% were correctly identified across samples with a tumor cell content ranging from 5% to 95%. Additionally, the assay demonstrated high reproducibility across the six participating laboratories. The turnaround time of the OPA was notably shorter compared to traditional orthogonal methods, facilitating rapid molecular report generation. **Conclusions:** The OPA in combination with the Ion Torrent Genexus™ System allows for highly sensitive and specific detection of relevant *ERBB2* mutations. The assay's streamlined workflow, coupled with its automated data analysis pipeline, enables a fast

turnaround time for testing across a range of sample types. This includes samples with reduced tumor cell content and limited available input. This study demonstrates the future potential of using this assay in a clinical setting.

Keywords: NGS; molecular diagnostics; targeted therapy

1. Introduction

A considerable percentage of non-squamous non-small-cell lung cancers (nsNSCLCs) are associated with well-characterized oncogenic alterations [1]. An increasing number of targeted therapies have contributed to a meaningful reduction in mortality from nsNSCLC in recent years [1,2]. Mutations in the gene encoding erb-b2 receptor tyrosine kinase 2 (*ERBB2, HER2*), a member of the human epidermal growth factor receptor (*HER*) family, are observed in up to 2–7% of nsNSCLCs and are associated with the female sex, no smoking history, a younger age at diagnosis, a higher incidence of brain metastases, and a poor prognosis compared to nsNSCLC without *ERBB2* mutations or with other driven gene mutations [3–6]. Trastuzumab–deruxtecan (T-DXd) is an antibody–drug conjugate composed of an anti-*HER2* antibody, a tetrapeptide-based cleavable linker, and a topoisomerase I inhibitor payload. T-DXd has been approved in various countries for the treatment of patients with metastatic *HER2*-positive breast and gastric cancers based on the results from pivotal trials.

In a recent multicenter, international, phase 2 study (The DESTINY-Lung02 trial, NCT04644237 [7]), T-DXd was administered to patients who had metastatic *ERBB2*-mutant nsNSCLCs that were refractory to standard treatment. T-DXd showed durable anticancer activity in 91 patients with *ERBB2*-mutant nsNSCLC: a confirmed objective response was reported in 55% of the patients, the median duration of response was 9.3 months, the median progression-free survival was 8.2 months, and the median overall survival was 17.8 months [7]. These results highlight the durable anticancer activity of trastuzumab–deruxtecan in patients with previously treated *ERBB2*-mutant NSCLCs. Notably, most *ERBB2* mutations observed in this study were exon 20 insertions (86%). The results of this study led to the approval of T-DXd in the US, the EU, Latin America, and Asia for the treatment of adult patients with advanced non-small-cell lung cancer whose tumors have activating *ERBB2* mutations and who require systemic therapy following platinum-based chemotherapy with or without immunotherapy. Therefore, systematic testing for *ERBB2* mutations in patients with advanced nsNSCLC has become a standard of care.

In this study, we evaluated the analytical performance of a next-generation sequencing (NGS) panel compatible with the Ion Torrent™ Genexus™ System*, namely the Oncomine™ Precision Assay GX* (OPA). The OPA is a multi-cancer NGS panel, which detects genetic alterations across 50 genes, including *ALK*, *BRAF*, *EGFR*, *ERBB2*, *KRAS*, *MET*, *NTRK1/2/3*, *RET*, and *ROS1*, among others (Table 1). Among the detected alterations are single-nucleotide variants (SNVs), insertion and deletions (Indels), copy number variations (CNVs), gene fusions, and splice variants. Specifically, we assessed its performance in detecting *ERBB2* mutations in a cohort of NSCLC samples and assessed concordance with other orthogonal established methodologies across six European academic research centers. By thoroughly investigating the capabilities and limitations of the OPA, we aim to provide valuable insights into its potential use as a reliable tool for accurate *ERBB2* mutation detection.

Table 1. Oncomine Precision Assay gene list.

DNA Hotspots			**CNV**	**Inter-Genetic Fusions**		**Intra-Genetic Fusions**
AKT1	*ESR1*	*MAP2K2*	*ALK*	*ALK*	*NTRK2*	*AR*
AKT2	*FGFR1*	*MET*	*AR*	*BRAF*	*NTRK3*	*EGFR*
AKT3	*FGFR2*	*MTOR*	*CD274*	*ESR1*	*NUTM1*	*MET*
ALK	*FGFR3*	*NRAS*	*CDKN2A*	*FGFR1*	*RET*	
AR	*FGFR4*	*NTRK1*	*EGFR*	*FGFR2*	*ROS1*	
ARAF	*FLT3*	*NTRK2*	*ERBB2*	*FGFR3*	*RSPO2*	
BRAF	*GNA11*	*NTRK3*	*ERBB3*	*MET*	*RSPO3*	
CDK4	*GNAQ*	*PDGFRA*	*FGFR1*	*NRG1*		
CDKN2A	*GNAS*	*PIK3CA*	*FGFR2*	*NTRK1*		
CHEK2	*HRAS*	*PTEN*	*FGFR3*			
CTNNB1	*IDH1*	*RAF1*	*KRAS*			
EGFR	*IDH2*	*RET*	*MET*			
ERBB2	*KIT*	*ROS1*	*PIK3CA*			
ERBB3	*KRAS*	*SMO*	*PTEN*			
ERBB4	*MAP2K1*	*TP53*				

2. Materials and Methods

2.1. Academic Clinical and Research Centers

This study was conducted in six European academic centers: Institute of Medical Genetics and Pathology, University Hospital of Basel, Basel, Switzerland; Istituto Nazionale Tumori "Fondazione Pascale" IRCCS, Naples, Italy; IHU RespirERA, University Côte d'Azur, Nice, France; Institute of Molecular Pathology; Immunology of the University of Porto, Porto, Portugal; University Federico II of Naples, Naples, Italy; and Hospital Universitario 12 de Octubre, Madrid, Spain.

2.2. Pre-Characterized Samples

A total of 108 biobanked nsNSCLC samples, which had undergone previous testing using locally validated methods, were included in the study (Table 2). Each participating center profiled a selection of samples from their own biobank, pre-characterized using their on-site validated orthogonal assay.

Table 2. Cohort description of non-squamous NSCLC samples assessed for ERBB2 testing using Oncomine Precision Assay.

Sample Characteristics	**No of Samples (%) Total = 108**
Biopsy site	
Primary tumor	77 (70)
Distant metastasis	21 (20)
Mediastinal lymph node	9 (8)
Not reported	1 (1)
Material type	
Biopsy (FFPE)	63 (58)
Excision	19 (18)
Cyto-block	17 (16)
Cyto-smear	7 (6)
Not reported	2 (2)

Table 2. *Cont.*

Sample Characteristics	No of Samples (%) Total = 108
Tumor cell content (tissue samples only)	
0–20	15 (14)
20–40	25 (23)
40–60	25 (23)
60–80	19 (18)
80–100	24 (22)
Orthogonal method used	
Next-generation sequencing	101 (94)
Sanger sequencing	4 (4)
qPCR	3 (3)

2.3. Genomic Profiling by Next-Generation Sequencing and Data Analysis

The OPA panel (Thermo Fisher Scientific, Waltham, MA, USA) was used to assess the presence of SNVs and Indels in *ERBB2*. For the detection of DNA in formalin-fixed and paraffin-embedded (FFPE) or cyto-smear-derived material, 10 ng of starting material was used whenever available, down to a minimum of 5 ng. Batches of 8 samples were sequenced per lane using the GX5™ chips* (Thermo Fisher Scientific, Waltham, MA, USA). NGS data analysis including *ERBB2* variant calling was performed using Genexus software version 6.6.2* (Thermo Fisher Scientific, Waltham, MA, USA), using either the default assay definition file or a custom assay definition file, allowing for the detection of complex non-hotspot variants. Initial quality control (QC), including chip loading density, median read length, and number of mapped reads, was carried out automatically with Genexus software version 6.6.2 based on pre-defined cut-offs for mapped reads and minimal average read length. Variant reporting was limited to *ERBB2* variants for the purpose of this study.

2.4. Statistical Analysis

Data analysis and visualization were performed using Microsoft™ Office Excel™ (Microsoft Corporation, Redmond, WA, USA) software and GraphPad Prism™ version 10.0.0 for Windows (GraphPad Software© Inc., Boston, MA, USA), respectively. The correlation between variables was evaluated by calculating the R^2 coefficient.

3. Results

3.1. Cohort Description

The pre-characterized sample cohort comprised a total of 108 nsNSCLC samples with a mutation in *ERBB2*, as assessed during routine workup of the samples at the corresponding centers. Each sample had specific characteristics related to biopsy site, material type, tumor cell content, histological subtype, and the orthogonal methods used for analysis (Table 2). The majority of samples were tissue biopsies (58%), followed by cytological specimens (22%) and tissue excisions (18%), and NGS was the primary orthogonal method used (94%), including amplicon- and hybrid capture-based enrichment methods, supplemented by quantitative PCR and Sanger sequencing in a smaller subset of samples (Table 2 and Figure 1A). The tumor cell content of the tissue samples ranged from 5% to 95% (Figure 1B).

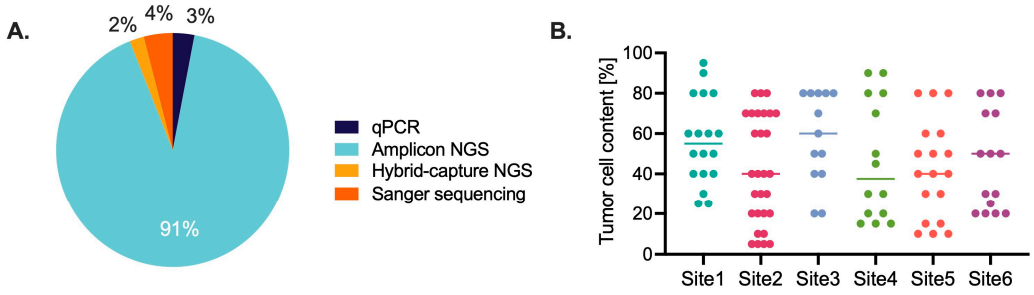

Figure 1. Orthogonal methods and tumor cell content across the sample cohort. (**A**): Overview and frequency of orthogonal methods used to characterize samples in Phase I of the study. qPCR = dark blue; Amplicon-based NGS = light blue; Hybrid-capture-based NGS = yellow; Sanger sequencing = orange. (**B**): Distribution of tumor cell content in Phase I samples, grouped by study site. Each site is highlighted by a different color.

3.2. Phase I: Testing Orthogonally Pre-Characterized Samples

All study nsNSCLC samples had been pre-characterized as part of the validated sample workup at each of the centers and were selected to represent a wide range of *ERBB2* oncogenic alterations in NSCLC (Figure 2A), with the majority of alterations located in exon 20 (99/108, 92%). In line with the literature, the most common *ERBB2* mutation was Y772_A775dup [6]. The collected tissue samples were subjected to analysis using the OPA, a comprehensive genetic profiling tool designed to identify a wide spectrum of oncogenic alterations. In most cases (90/108), the same extracted and purified DNA was used in the initial sample workup as well as for testing with the OPA. For the remaining 18 samples, new DNA was extracted from the same FFPE blocks that were used for orthogonal testing. The assay workflow from DNA to variant reporting, including library preparation, sequencing, and data analysis, was performed in a fully automated manner on the Genexus System.

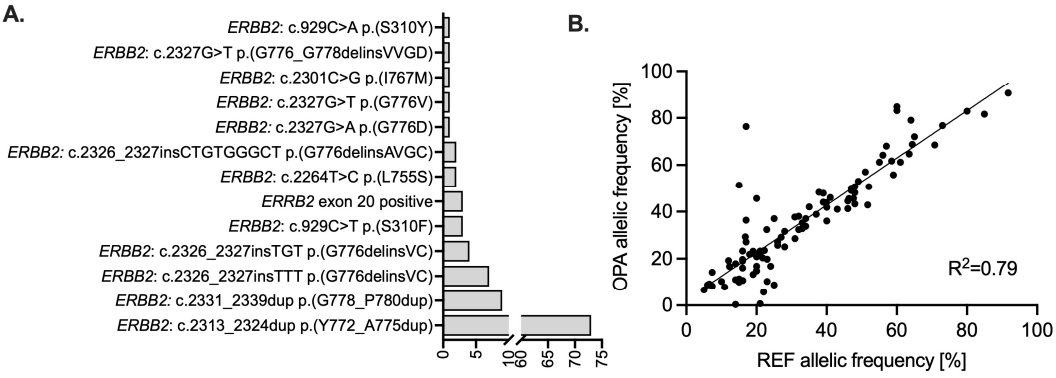

Figure 2. Detection of known *ERBB2* variants using Oncomine Precision Assay. (**A**): List and prevalence of different *ERBB2* variants included in the cohort, based on pre-characterization with validated orthogonal methods. (**B**): Correlation of observed allelic frequencies between OPA and orthogonal methods.

A total of 10ng DNA was used as input for sequencing. Overall, the OPA exhibited a 100% detection rate in the analysis of the 108 pre-characterized nsNSCLC tissue samples, based on manufacturer-provided QC metrics such as the total number of mapped reads and the minimum mean read length per sample. The resulting QC-passing reads were used as input for variant calling in the automated data analysis pipeline with the Genexus instrument.

To determine the performance of the OPA in detecting *ERBB2* variants, a comparison was made with orthogonal methods. *ERBB2* status determination included identification

of SNVs, insertions, deletions, and CNVs. The assay demonstrated a concordance of 100% for the detection of *ERBB2* variants across the 108 samples. The allelic frequencies at which the *ERBB2* mutations were detected showed a very high correlation (R2 = 0.79, Figure 2B) with orthogonal methods. Overall, these results highlight the precision and reliability of the results obtained from the OPA.

3.3. Phase II: Reproducibility Assessment Across the Six Centers

A set of eight deidentified DNA samples were sent from the study's lead coordinating center to the remaining five centers for analysis, with the aim of assessing the inter-site reproducibility of *ERBB2* mutation detection. The aim was to evaluate the consistency of variant calls (i.e., presence and allelic frequency) across the participating laboratories and determine the overall sequencing success rate in diverse laboratory settings.

Out of the eight DNA samples, three were confirmed to lack any *ERBB2* alteration by all centers, resulting in a negative percent agreement (NPA) of 100%, demonstrating the highest degree of concordance in identifying non-mutated samples.

For the samples known to harbor *ERBB2* mutations, the variant calls were consistent among all centers, with a positive percent agreement (PPA) of 100% (Table 3, Figure 3). Overall, total agreement across the eight samples was 100%, highlighting the robustness of the mutation detection methodology.

Table 3. Phase II results on inter-laboratory reproducibility.

Sample	Variant Type	Expected *ERBB2* Alteration	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
			Observed AF	Observed AF	Observed AF	Observed AF	Observed AF	Observed AF
USB01	SNV	p.(L755S)	49.0%	44.0%	47.4%	46.9%	46.8%	41.5%
USB02	Insertion	p.(G778_P780dup)	36.0%	25.5%	32.8%	27.8%	28.5%	28.2%
USB03	Insertion	p.(Y772_A775dup)	27.0%	37.0%	31.8%	28.8%	37.0%	27.9%
USB04	Delins	p.(G776delinsVC)	46.0%	47.0%	45.9%	39.0%	46.6%	39.6%
USB05	Delins	p.(Gly776_G778delinsVVGD)	10.0%	9.0%	15.0%	11.9%	10.5%	13%
USB06	n/a	WT	WT	WT	WT	WT	WT	WT
USB07	n/a	WT	WT	WT	WT	WT	WT	WT
USB08	n/a	WT	WT	WT	WTs	WT	WT	WT

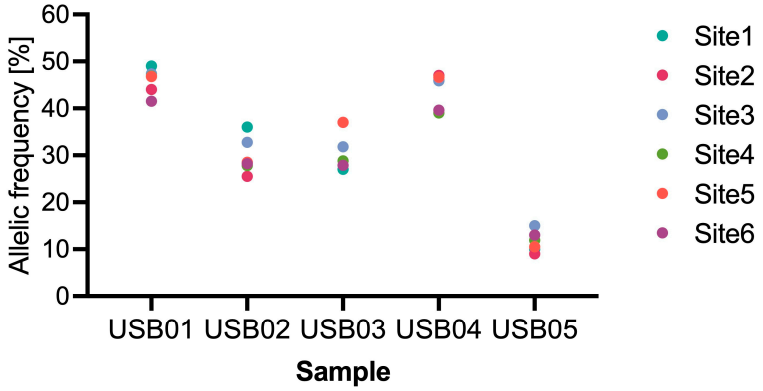

Figure 3. Allelic frequency of *ERBB2* mutations across the six laboratories in Phase II of the study.

4. Discussion

This multicenter observational retrospective study aimed to evaluate the technical feasibility of analyzing *ERBB2* mutations using pre-characterized nsNSCLC samples collected during routine molecular workup with an available NGS assay, namely the Oncomine Precision Assay (OPA).

ERBB2 is a member of the EGFR family of receptor tyrosine kinases and has been found to be frequently mutated in lung cancers [8]. Genetic alterations in *ERBB2*, particularly exon 20 insertions, have been extensively reported in NSCLC [3]. T-DXd has recently been approved by the FDA and EMA for the treatment of nsNSCLC patients harboring oncogenic *ERBB2* mutations, including exon 20 insertions. Furthermore, other agents, such poziotinib and pyrotinib, have been tested in prospective randomized interventional clinical trials [9]. Despite differences in clinical outcomes among the tested drugs and their combinations, *ERBB2* has emerged as a clear promising novel therapeutic target for nsNSCLC [10]. Thus, comprehensive molecular profiling, including *ERBB2* mutation testing, is required to help identify nsNSCLC patients who may benefit from biomarker-directed therapies such as T-DXd [11,12].

Another critical concern for cancer patients, particularly those diagnosed with stage IV disease, is the turnaround time for generating the molecular profile. As cancer can progress rapidly at this stage, treatment decisions can profoundly impact a patient's prognosis [13]. Molecular profiling aids in the identification of specific genetic alterations or biomarkers within a patient's tumor, which in turn guides treatment choices. The quicker this information becomes available, the sooner a tailored treatment plan can be initiated, thereby enhancing the likelihood of treatment success. Moreover, expedited results from molecular profiling empower patients and may alleviate their anxiety while waiting for treatment decisions. Finally, in certain instances, molecular profiling results may indicate a patient's eligibility for participation in clinical trials targeting their specific mutation. Timely access to this information can expand the overall pool of patients eligible for clinical trials.

Here, we demonstrate the feasibility of using a fully automated NGS solution for comprehensive testing of *ERBB2* variants in NSCLC samples. The OPA covers variants across 50 genes relevant in NSCLC, melanoma, colorectal cancer, breast cancer, and other cancer types. Our data show that this assay, in combination with the Ion Torrent Genexus System, allows for the highly sensitive and specific detection of relevant *ERBB2* mutations, with a minimal sample input of only 10 ng of DNA. Notably, in this study, various types of samples were tested, including cytological specimens. NGS testing on cytological specimens, such as fine needle aspirates or pleural effusion samples, plays a crucial role in the context of NSCLC. Our data suggests that the OPA can also be used to reliably detect *ERBB2* variants on cytological specimens. Additionally, while not directly addressed in this study, one of the significant features of the employed technology is its remarkably rapid turnaround time for molecular profiling of samples. This was previously demonstrated in several studies where turnaround times from specimen to molecular reporting of as little as 48–72 h were reported when using amplicon-based automated next-generation sequencing technology [14,15].

An emerging complementary approach to tissue-based molecular profiling is liquid biopsy. This minimally invasive method enables the detection of tumor-derived genetic alterations, including *ERBB2* mutations, from circulating cell-free DNA (cfDNA) in a patient's blood. Liquid biopsy may serve as a viable alternative in scenarios where tissue availability is limited or biopsy procedures are not feasible due to clinical constraints [16]. Moreover, the use of liquid biopsy allows for serial sampling to monitor treatment response or emerging resistance mutations in real time. While this study focused on tissue-based testing, the integration of liquid biopsy into routine clinical practice, particularly in advanced NSCLC, could further streamline patient management, reduce time to treatment, and improve access to targeted therapies.

Nonetheless, our study has some limitations, including its retrospective design. Additionally, while the overall tested cohort of over 108 samples for Phase I of this study (testing orthogonally pre-characterized samples) can be considered sufficient to draw some initial

but solid conclusions, the number of selected cases for Phase II (reproducibility assessment across the six centers) was limited to only 8 samples, and therefore interpretation of the data warrants adequate caution. A larger prospective study or, as an alternative, the collection of real-world data in the range of several hundreds of samples across multiple sites should be used in the future to draw definitive conclusions. In summary, our study provides initial data on the feasibility of OPA as a potential solution for the detection of *ERBB2* variants in nsNSCLC samples across multiple specimen types.

* For research use only, not for diagnostic use: Ion Torrent Genexus System; Oncomine Precision Assay GX; and GX5 chips.

Author Contributions: I.A.: Writing—original draft preparation, investigation, formal analysis, visualization. M.D.: Investigation, formal analysis. P.W.: Supervision, coordination, original draft preparation. S.H.: Formal analysis. F.L.-R.: Supervision, coordination, original draft preparation. O.B.: Formal analysis. C.B.: Formal analysis. P.H.: Supervision, coordination, original draft preparation. C.D.L.: Formal analysis. G.T.: Supervision, draft review. L.R.: Formal analysis. U.M.: Supervision, coordination, original draft preparation. R.S.d.S.: Formal analysis. L.C.: Formal analysis. F.S.: Supervision, coordination, original draft preparation. E.K.: Investigation, formal analysis. P.M.J.: Resources, draft review. J.L.: Resources, draft review. L.B.: Conceptualization, supervision, writing—original draft preparation. All authors agreed to the content of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This study is sponsored by AstraZeneca. In March 2019, AstraZeneca entered into a global development and commercialization collaboration agreement with Daiichi Sankyo for trastuzumab–deruxtecan (T-DXd; DS-8201). This study was supported in part by Thermo Fisher Scientific in the form of reagents.

Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki, and the study was approved by the Ethical Committee Nordwest und Zentralschweiz in Basel, Switzerland (EKNZ 2023-00843), on 4-December-2023.

Informed Consent Statement: Global informed consent was obtained from the patients.

Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon justified request.

Acknowledgments: We thank AstraZeneca and Thermo Fisher Scientific for their support. The funding parties helped designing the study but had no role in the collection of data and in the decision to submit the article for publication. P.M.J. (Thermo Fisher) was involved in data analysis and review of the manuscript, and J.L. (Astra Zeneca) in the review of the manuscript.

Conflicts of Interest: Lukas Bubendorf has received personal fees (as consultant and/or speaker) from Amgen, Astra Zeneca, Janssen, Boehringer Ingelheim, Eli Lilly, Takeda, Thermo Fisher Scientific, and Merck and financial research support from Roche, Novartis, and Thermo Fisher Scientific unrelated to the current work. Umberto Malapelle has received personal fees (as consultant and/or speaker bureau) from Boehringer Ingelheim, Roche, MSD, Amgen, Thermo Fisher Scientific, Eli Lilly, Diaceutics, GSK, Merck and AstraZeneca, Janssen, Diatech, Novartis, and Hedera unrelated to the current work. Giancarlo Troncone reports personal fees (as speaker bureau or advisor) from Roche, MSD, Pfizer, and Bayer, unrelated to the current work. No relevant conflicts of interest were declared by Caterina De Luca and Luisella Righi.

Abbreviations

The following abbreviations are used in this manuscript:

CNVs	Copy number variations
FFPE	Formalin-fixed and paraffin-embedded
NGS	Next-generation sequencing

NSCLC	Non-small-cell lung cancer
OPA	Oncomine Precision Assay
PPA	Positive percent agreement
QC	Quality control
SNVs	Single-nucleotide variants
T-DXd	Trastuzumab–deruxtecan

References

1. Tan, A.C.; Tan, D.S.W. Targeted Therapies for Lung Cancer Patients with Oncogenic Driver Molecular Alterations. *J. Clin. Oncol.* **2022**, *40*, 611–625. [CrossRef] [PubMed]
2. Hendriks, L.E.; Kerr, K.M.; Menis, J.; Mok, T.S.; Nestle, U.; Passaro, A.; Peters, S.; Planchard, D.; Smit, E.F.; Solomon, B.J.; et al. Oncogene-addicted metastatic non-small-cell lung cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann. Oncol.* **2023**, *34*, 339–357. [CrossRef] [PubMed]
3. Wei, X.W.; Gao, X.; Zhang, X.C.; Yang, J.J.; Chen, Z.H.; Wu, Y.L.; Zhou, Q. Mutational landscape and characteristics of ERBB2 in non-small cell lung cancer. *Thorac. Cancer* **2020**, *11*, 1512–1521. [CrossRef] [PubMed]
4. Pillai, R.N.; Behera, M.; Berry, L.D.; Rossi, M.R.; Kris, M.G.; Johnson, B.E.; Bunn, P.A.; Ramalingam, S.S.; Khuri, F.R. HER2 mutations in lung adenocarcinomas: A report from the Lung Cancer Mutation Consortium. *Cancer* **2017**, *123*, 4099–4105. [CrossRef] [PubMed]
5. Kim, E.K.; Kim, K.A.; Lee, C.Y.; Shim, H.S. The frequency and clinical impact of HER2 alterations in lung adenocarcinoma. *PLoS ONE* **2017**, *12*, e0171280. [CrossRef] [PubMed]
6. Hong, L.; Patel, S.; Drusbosky, L.M.; Xiong, Y.; Chen, R.; Geng, R.; Heeke, S.; Nilsson, M.; Wu, J.; Heymach, J.V.; et al. Molecular landscape of ERBB2 alterations in 3000 advanced NSCLC patients. *Npj Precis. Oncol.* **2024**, *8*, 217. [CrossRef] [PubMed]
7. Li, B.T.; Smit, E.F.; Goto, Y.; Nakagawa, K.; Udagawa, H.; Mazières, J.; Nagasaka, M.; Bazhenova, L.; Saltos, A.N.; Felip, E.; et al. Trastuzumab Deruxtecan in Her2-Mutant Non–Small-Cell Lung Cancer. *N. Engl. J. Med.* **2022**, *386*, 241–251. [CrossRef] [PubMed]
8. Shigematsu, H.; Lin, L.; Takahashi, T.; Nomura, M.; Suzuki, M.; Wistuba, I.I.; Fong, K.M.; Lee, H.; Toyooka, S.; Shimizu, N.; et al. Clinical And Biological Features Associated with Epidermal Growth Factor Receptor Gene Mutations in Lung Cancers. *J. Natl. Cancer Inst.* **2005**, *97*, 339–346. [CrossRef] [PubMed]
9. Uy, N.F.; Merkhofer, C.M.; Baik, C.S. HER2 in Non-Small Cell Lung Cancer: A Review of Emerging Therapies. *Cancers* **2022**, *14*, 4155. [CrossRef] [PubMed]
10. Nützinger, J.; Lee, J.B.; Low, J.L.; Chia, P.L.; Wijaya, S.T.; Cho, B.C.; Lim, S.M.; Soo, R.A. Management of HER2 alterations in non-small cell lung cancer—The past, present, and future. *Lung Cancer* **2023**, *186*, 107385. [CrossRef] [PubMed]
11. Aggarwal, C.; Marmarelis, M.E.; Hwang, W.T.; Scholes, D.G.; McWilliams, T.L.; Singh, A.P.; Sun, L.; Kosteva, J.; Costello, M.R.; Cohen, R.B.; et al. Association Between Availability of Molecular Genotyping Results and Overall Survival in Patients with Advanced Nonsquamous Non–Small-Cell Lung Cancer. *JCO Precis. Oncol.* **2023**, *7*, e2300191. [CrossRef] [PubMed]
12. Qi, Z.; Ha, T.; Feng, W.; Karnoub, M.; Pereira, K.; Shiga, R.; Smit, E.F.; Goto, Y.; De Langen, A.J.; Goto, K.; et al. Analytical and Clinical Validation of the Oncomine Dx Target Test to Assess HER2 Mutation Status in Tumor Tissue Samples From Patients with Non-Small Cell Lung Cancer Treated with Trastuzumab Deruxtecan in the DESTINY-Lung01 and DESTINY-Lung02 Studies. *Arch. Pathol. Lab. Med.* **2024**, *149*, 542–549. [CrossRef] [PubMed]
13. Scott, J.A.; Lennerz, J.; Johnson, M.L.; Gordan, L.N.; Dumanois, R.H.; Quagliata, L.; Ritterhouse, L.L.; Cappuzzo, F.; Wang, B.; Xue, M.; et al. Compromised Outcomes in Stage IV Non–Small-Cell Lung Cancer with Actionable Mutations Initially Treated Without Tyrosine Kinase Inhibitors: A Retrospective Analysis of Real-World Data. *JCO Oncol. Pract.* **2024**, *20*, 145–153. [CrossRef] [PubMed]
14. Sheffield, B.S.; Beharry, A.; Diep, J.; Perdrizet, K.; Iafolla, M.A.; Raskin, W.; Dudani, S.; Brett, M.A.; Starova, B.; Olsen, B.; et al. Point of Care Molecular Testing: Community-Based Rapid Next-Generation Sequencing to Support Cancer Care. *Curr. Oncol.* **2022**, *29*, 1326–1334. [CrossRef] [PubMed]
15. Ilié, M.; Hofman, V.; Bontoux, C.; Heeke, S.; Lespinet-Fabre, V.; Bordone, O.; Lassalle, S.; Lalvée, S.; Tanga, V.; Allegra, M.; et al. Setting Up an Ultra-Fast Next-Generation Sequencing Approach as Reflex Testing at Diagnosis of Non-Squamous Non-Small Cell Lung Cancer; Experience of a Single Center (LPCE, Nice, France). *Cancers* **2022**, *14*, 2258. [CrossRef] [PubMed]
16. Hofman, P. Matched tissue and liquid biopsies for advanced non-small cell lung cancer patients A potentially indispensable complementary approach. *Transl. Oncol.* **2023**, *35*, 101735. [CrossRef] [PubMed]