Cross-Validation Study for Epidermal Growth Factor Receptor and *KRAS* Mutation Detection in 74 Blinded Non-small Cell Lung Carcinoma Samples

A Total of 5550 Exons Sequenced by 15 Molecular French Laboratories (Evaluation of the EGFR Mutation Status for the Administration of EGFR-TKIs in Non-Small Cell Lung Carcinoma [ERMETIC] Project—Part 1)

Michèle Beau-Faller, MD, PhD,†‡ Armelle Degeorges, PhD,§ Estelle Rolland, MSc,‖ Mounia Mounawar, PhD,¶ Martine Antoine, MD,‡# Virginie Poulot, LabTec,** Audrey Mauguen, MSc,‖ Véronique Barbu, MD, PhD,†† Florence Coulet, PharmD, PhD,‡‡ Jean-Luc Prétet, PhD,§§ Ivan Bièche, PharmD, PhD,‖‖ Hélène Blons, PharmD, PhD,¶¶ Jean-Christophe Boyer, PharmD, PhD,## Marie-Pierre Buisine, PharmD, PhD,*** Florence de Fraipont, PharmD, PhD,††† Sarab Lizard, PhD,‡‡‡ Sylviane Olschwang, MD, PhD,§§§ Patrick Saulnier, PhD,‖‖‖ Delphine Prunier-Mirebeau, MD, PhD,¶¶¶ Nicolas Richard, PharmD, MSc,### Claire Danel, MD,‡**** Elisabeth Brambilla, MD, PhD,‡†††† Christos Chouaid, MD, PhD,‡,‡‡‡‡ Gérard Zalcman, MD, PhD,‡§§§§ Pierre Hainaut, PhD,¶ Stefan Michiels, PhD,‖ and Jacques Cadranel, MD, PhD‡#‖‖‖‖‖‖*

Introduction: The Evaluation of the epidermal growth factor receptor (EGFR) Mutation status for the administration of EGFR-Tyrosine Kinase Inhibitors in non-small cell lung Carcinoma (NSCLC) (ERMETIC) project part 1 assessed the accuracy of *EGFR* and *KRAS* mutations detection in NSCLC among 15 French centers.

Methods: The 15 ERMETIC centers selected 74 NSCLC surgical specimens from previously untreated patients. Paraffin and paired frozen DNA were sequenced for *EGFR* exons 18 to 21 and *KRAS* exon 2 by an external molecular laboratory, yielding a gold standard. The 74 blinded paraffin DNAs were redistributed to the 15 ERMETIC laboratories for sequencing of a total of 5550 exons. Results were compared with the gold standard and between centers by discordance rates and kappa statistics.

Results: The gold standard included 39 mutated samples with 22 *EGFR* and 17 *KRAS* mutated samples. Kappa statistics showed that 10, 6, and 6 of the 15 ERMETIC centers had a moderate to good kappa score, when compared with external laboratory for *EGFR* exon 19, *EGFR* exon 21, and *KRAS* exon 2, respectively. Kappa statistics showed moderate score between centers which increased to good for *EGFR* exon 19 mutation when removing 16 poor-quality samples with high nonamplificable rates.

Conclusions: Paraffin-embedded specimens may represent a suitable source of DNA for sequencing analyses in ERMETIC centers. *EGFR* exon 19 deletions were most accurately detected by ERMETIC centers. Ease and accuracy of results, depended more on the quality of sample than on the difference in molecular sequencing procedures between centers, emphasize the need of preanalytical quality control programs.

*Laboratoire de Biologie Moléculaire, Hôpital de Hautepierre, Strasbourg; †EA4438 Faculté de Médecine, Strasbourg; ‡IFCT, French Thoracic Intergroup, Paris; §Unité de Pharmacologie, Département de Biologie des Tumeurs, Institut Curie, Paris; ‖Service de Biostatistique et d'Epidémiologie, Institut Gustave-Roussy, Villejuif; ¶International Agency for Research on Cancer, Lyon; #Service d'Anatomie pathologique, Hôpital Tenon, Paris; **Equipe de Recherche 2 de la Faculté de Médecine Pierre et Marie Curie, Université Paris VI, Hôpital Tenon, Paris; ††Laboratoire Commun de Biologie et Génétique moléculaires, Pôle de Biologie Imagerie, Assistance Publique-Hôpitaux de Paris, Hôpital Saint Antoine, Paris; ‡‡Laboratoire d'Oncogénétique et d'Angiogénétique moléculaires, Groupe Hospitalier Pitié-Salpétrière, Paris; §§EA3181, IFR133, Université de Franche-Comté, Laboratoire de Biologie Cellulaire et Moléculaire, CHU Besançon, Boulevard Fleming, Besançon; ‖‖Laboratoire d'Oncogénétique, Plateforme HUVEGEN, Centre René Huguenin, Saint-Cloud; ¶¶Service de Biochimie, UF de Pharmacogénétique et Oncologie moléculaire, Hôpital Européen Georges Pompidou, Paris; ##Unité de Toxicologie, Laboratoire de Biochimie, CHU Carémeau, Nîmes; ***Laboratoire Oncologie et Génétique Moléculaires, Pôle de Biochimie et de Biologie Moléculaire, Centre de Biologie Pathologie, CHRU de Lille, Lille; †††Université Joseph Fourier, INSERM U823, UF Cancérologie biologique et biothérapie, CHU Grenoble, Grenoble; ‡‡‡Unité de Biologie Moléculaire, Centre GF Leclerc, Dijon; §§§Département de Biopathologie, Institut Paoli Calmettes, Marseille; ‖‖‖Laboratoire de

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Primary lung cancer accounts for the highest number of cancer deaths worldwide,[1] with, a 5-year survival between 10 and 15% in France.[2,3] More than 80% of lung cancers are non-small cell lung cancer (NSCLC), which are subdivided into squamous cell carcinoma (SCC), adenocarcinoma (ADC), and large cell carcinoma.[4]

In ADC, epidermal growth factor receptor (*EGFR*) is found to be mutated in 10 to 15% tumors from white patients[5] and in more than 40% of tumors from Asian patients.[6] Inhibiting EGFR signaling using tyrosine kinase inhibitors (TKIs), gefitinib or erlotinib, is an effective treatment for patients with tumors expressing *EGFR*-sensitizing mutations.[7–10] Molecular selection should be performed because clinical characteristics were shown to be insufficient to accurately select patients harboring *EGFR* mutations and because EGFR-TKI resistance may be conferred by mutations in *KRAS* (~30% of ADC cases) or in *EGFR* exon 20.[11,12]

Many diagnostic methods are available for *EGFR* and *KRAS* mutation analysis, but standardized procedures are lacking,[13] although gefitinib recently obtained restrictive European Medicines Agency/Food and Drug Administration approval for first-line treatment of patients with *EGFR* mutated NSCLC.[14] In 2005, the French National Cancer Institute granted a nationwide 2-years multicenter prospective project to address the standardization of mutation analysis. The project entitled Evaluation of the EGFR Mutation status for the administration of EGFR-TKIs in NSCLC (ERMETIC) involves 15 French clinical/pathological/biological centers. The project has three consecutive objectives: (i) validate the widespread use of sequencing as a screening method for *EGFR* and *KRAS* molecular diagnosis on fixed paraffin-embedded tissues; (ii) select and rank clinical, pathological, and biological predictors of EGFR-TKI response and clinical benefit in a large prospective clinical cohort; and (iii) determine the most cost-effective strategy to prescribe EGFR-TKIs, i.e., based on EGFR biomarkers. This study focuses on part 1 of the ERMETIC project.

PATIENTS AND METHODS

The ERMETIC project part 1 was subdivided into two phases, phase A and phase B. The phase A addressed the question of the discordances that could be observed between results of paraffin and frozen samples. It consisted in the comparison of direct sequencing analysis of paraffin-embedded samples from 74 patients with NSCLC and their snap-frozen counterparts by an external molecular laboratory (P. Hainaut, IARC, Lyon, France). The phase B addressed the cross-validation of paraffin-embedded samples analysis among the 15 ERMETIC French molecular laboratories.

Description of the ERMETIC Tumor Bank

Each of the 15 ERMETIC centers selected 1 to 10 NSCLC surgical specimens from previously untreated patients, according to French regulations. For each deidentified specimen, a fixed paraffin-embedded block containing more than 50% tumor cells and a snap-frozen counterpart was required. Samples were selected for clinical features linked to a high probability of *EGFR* mutation: female, ADC, and nonsmoker.[6] Each center also selected one patient with SCC, with a high probability of being wild type for *EGFR* and *KRAS*. Tumor paraffin blocks were sent to the coordination center for centralized review by three pathologists (M.A., E.B., and C.D.) based on 2004 World Health Organization classification and to determine the proportion of tumor cells assessed on a slide performed at the end of block sections and stained by hematoxylin-eosin-safran coloration.

DNA Preparation

Each ERMETIC centers prepared, without macrodissection, 16 × 3 sections (15 μm thick) from their own paraffin blocks to perform 16 extractions for each sample. All centers extracted DNA using similar principles, i.e., affinity-column-based protocols excepted for two centers using protocols based on magnetic particles, with extraction controls (water) in each series (see supplementary data, http://links.lww.com/JTO/A89; Table Si, http://links.lww.com/JTO/A90). DNAs from the 16 extractions were pooled. The median quantity of pooled DNA by sample was 13 μg (range: 0.7–127 μg) by Nano Drop (Wilmington, DE) (see supplementary data, Table Sii, http://links.lww.com/JTO/A91). Quality of pooled DNA samples was evaluated by a ladder amplification technique analysis[15] (see supplementary data, Table Sii, http://links.lww.com/JTO/A91). The 74 pooled DNA sam-

Recherche Translationnelle, Institut Gustave Roussy, Villejuif; ¶¶¶Laboratoire de Biochimie et Biologie moléculaire, CHU Angers; ###Laboratoire de Génétique moléculaire, CHU de Caen, Caen; ****Service d'Anatomie pathologique, Hôpital Européen Georges Pompidou, Paris; ††††Département de Pathologie, INSERM U823/Université Joseph Fourier, CHU de Grenoble, Grenoble; ‡‡‡‡Service de Pneumologie, Hôpital Saint-Antoine, APHP, Faculté de Médecine Pierre et Marie Curie, Université Paris VI, Paris; §§§§Service de Pneumologie, CHU de Caen, Caen; ||||||Service de Pneumologie, Assistance Publique Hôpitaux de Paris, Hôpital Tenon, Faculté de Médecine Pierre et Marie Curie, Université Paris VI, Paris, France.

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Address for correspondence: Jacques Cadranel, MD, PhD, Service de Pneumologie, Hôpital Tenon, 4 rue de la Chine 75970, Paris Cedex 20, France. E-mail: jacques.cadranel@tnn.aphp.fr

Armelle Degeorges contributed equally for this study.

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