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Do We Know What Is in Our Samples?

To the Editors:

Molecular testing to personalize therapy is an essential part of the investigation of cancer to personalize anticancer therapy.[1] Availability of sufficient tissue is important, and in some settings, for example, lung cancer diagnosed by bronchoscopic biopsy or ultrasound guided cytology, this may be challenging.

For cytological specimens, residual material remaining after the initial diagnostic slide can be made into a cell block. In most pathology laboratories, these are prepared by using plasma to form a clot entrapping the cells followed by embedding into paraffin wax. Sections from this allow better morphological assessment of the malignant cell population and permits immunohistochemsitry.[2] This is the preferred method to prepare cell blocks worldwide.[2–4] Current molecular pathology guidelines from the College of the American Pathologists, International Association for the Study of Lung Cancer, and the Association for Molecular Pathology recommend the use of cell blocks for molecular testing and therapy selection criteria for epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors and anaplastic lymphoma kinase inhibitors.[1]

Plasma of course contains cell-free DNA.[5] Plasma sources vary between laboratories (human, rabbit, etc.) as does the volume of plasma used to form cell blocks.[3,4] To identify the level of DNA contamination in cell blocks, we have analyzed DNA extracted from cell-free plasma thrombin cell blocks and blank (tissue free) histological paraffin blocks using routine molecular techniques including an in-house PCR/Pyrosequencing and a Qiagen Therascreen EGFR RGQ PCR Kit. DNA product was detected in the elute extracted from the empty cell blocks; however, no DNA was detected in the control paraffin blocks.

We conclude that plasma is a source of contaminating DNA. Fortunately, a review of our molecular assays performed on cell blocks found no evidence of false-positive or false-negative results. As high throughput Next Generation Sequencing platforms make their way into routine diagnostic practice, their superior sensitivity in DNA mutation detection in the presence of any extraneous DNA might generate false-positive results and impact on the molecular diagnosis. Given the potential for extraneous DNA to affect molecular assays, we believe that the plasma thrombin clot technique should no longer be used when molecular testing is to be undertaken, and that pathology laboratories should change their processing techniques accordingly.

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Successful AZD9291 Therapy Based on Circulating T790M

To the Editor:

The epidermal growth factor receptor (EGFR) gatekeeper mutation T790M is associated with resistance against first- and second-generation EGFR tyrosine kinase inhibitors in patients with advanced non–small-cell lung cancer.[1] The third-generation inhibitor AZD9291 can overcome T790M-mediated resistance in patients, as shown by a recent clinical trial.[2] T790M can be detected in tumor tissue, circulating tumor cells, and circulating tumor DNA.[3–5] Here, we report on a first patient with AZD9291 therapy based on circulating T790M.

In 2015, we established an IRB-approved research collaboration with NEO New Oncology AG (Cologne,

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Germany), to implement their circulating tumor DNA-based assay NEOLiquid. This assay covers clinically relevant genomic alterations, such as mutations, fusions, and copy number alterations of 31 genes. One of the first patients tested was a 53-year-old never-smoker with metastatic lung adenocarcinoma and EGFR exon 19 deletion diagnosed in 2012. He had been extensively pretreated with EGFR- tyrosine kinase inhibitors, chemotherapy, neurosurgery, and brain radiation. Previous attempts to detect T790M in tumor rebiopsies by conventional sequencing failed. The patient consented to testing, and T790M was found in plasma (allele frequency, AF = 39%), along with the exon 19 deletion (AF = 28%). We received permission from AstraZeneca and Swissmedic for compassionate use of AZD9291, and the patient started on a daily dose of 80 mg. Within a few days, his clinical condition improved. After 6 and 12 weeks, ^{18}F-deoxyglucose positron emission tomography with computer tomography showed tumor response (Fig. 1*A–F*). Further plasma samples drawn every 2 weeks were analyzed, showing gradual decrease of the T790M, and complete molecular remission after 10 weeks (Fig. 2). Retrospective analysis of the brain metastasis revealed T790M, but with very low AF of 0.2%. Currently, AZD9291 therapy is ongoing, tolerability is excellent, and there is no clinical, radiological, or genomic evidence of tumor progression.

We conclude that blood genomic tests can complement tissue biopsy, are suitable for therapy monitoring, and deserve consideration as companion diagnostics for mutation-selective cancer drugs.

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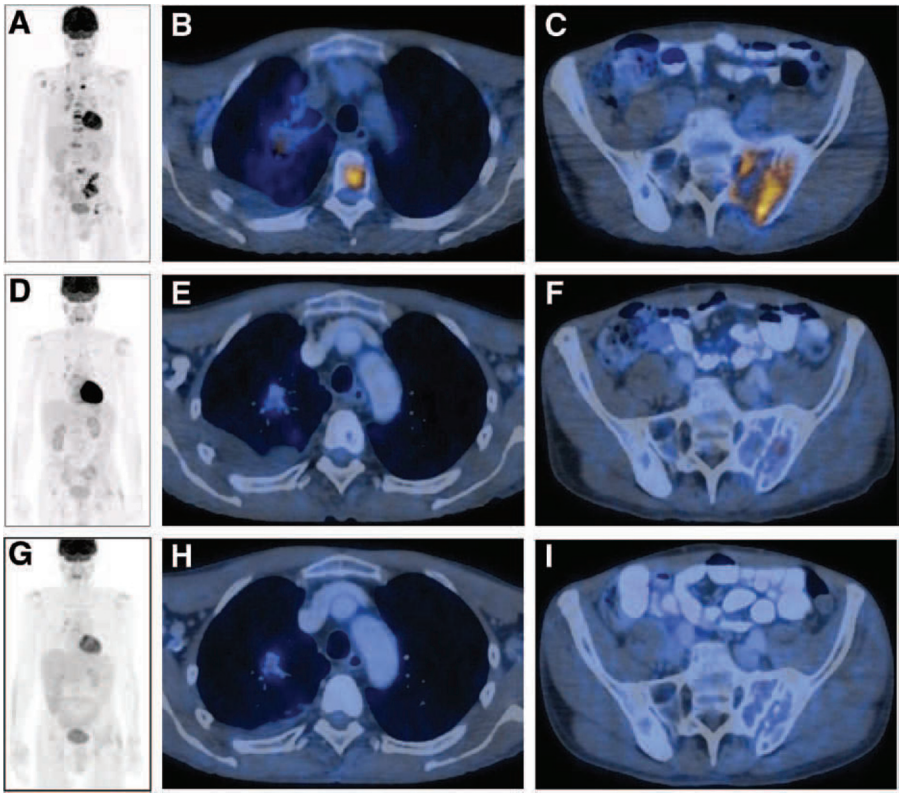

FIGURE 1. *A–C*, Pretreatment ^{18}FDG-PET/CT showing the primary tumor in the right lung, disseminated nodal and skeletal metastases, with strong uptake in the fourth thoracic vertebrae (*B*), and in the left sacrum and ilium (*C*). ^{18}FDG-PET/CT at 6 weeks (*D–F*) and 12 weeks (*G–I*) weeks after start of treatment with AZD9291, showing metabolic and morphological tumor response, and persistent pleural effusion (*E*). ^{18}FDG-PET/CT, ^{18}F-deoxyglucose positron emission tomography with computer tomography.

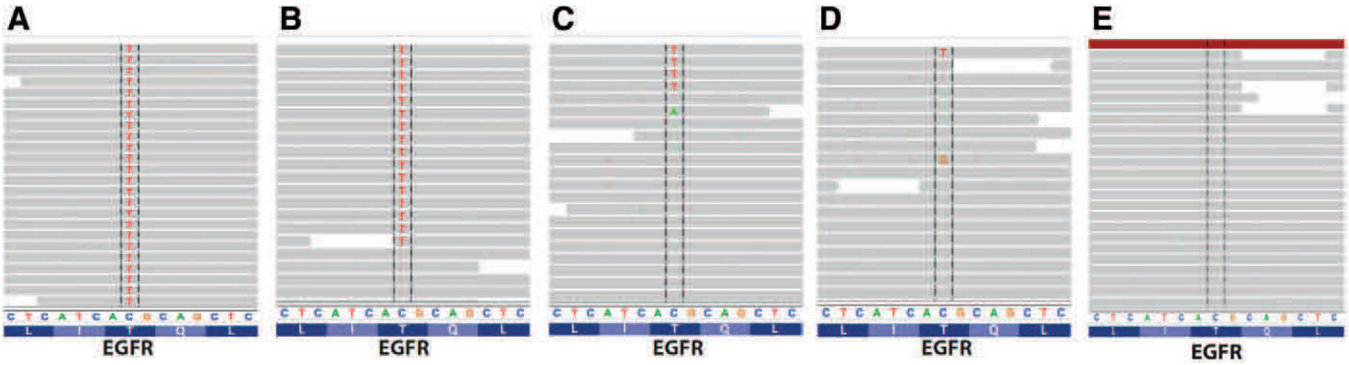

FIGURE 2. Allele frequency of EGFR T790M in the plasma by NEOLiquid (NEO New Oncology AG, Cologne, Germany) before the start of AZD9291 treatment (*A*), and after 2 weeks (*B*), 4 weeks (*C*), 6 weeks (*D*), and 12 weeks (*E*). The *vertical dashed black line* depicts the positional nucleotide change of C>T in exon 790 of EGFR, resulting in the T790M mutation.