Prediction of lung cancer genotype noninvasively using droplet digital PCR (ddPCR) analysis of cell-free plasma DNA (cfDNA)

Adrian G. Sacher M.D.1,3,4, Geoffrey R. Oxnard M.D.1,3, Stacy Mach1, Melissa M. Messineo2, David M. Jackman M.D.1,3, Pasi A. Jänne M.D. Ph.D.1,2,3, Cloud P. Paweletz Ph.D.2

1Lowe Center for Thoracic Oncology, Dana-Farber Cancer Institute, Boston, United States
2Belfer Institute for Applied Cancer Science, Dana-Farber Cancer Institute, Boston, United States
3Brigham & Women’s Hospital, Harvard Medical School, Boston, United States
4Division of Medical Oncology, Princess Margaret Cancer Center, University of Toronto, Toronto, Canada

Background: Noninvasive plasma genotyping has the potential to accelerate delivery of targeted therapies to genotype-defined cancer populations and obviate repeat biopsies, particularly following drug resistance. We recently reported on a new assay for plasma genotyping using ddPCR of cfDNA (Oxnard et al., Clinical Cancer Research, 2014). Here, we aimed to predict the genotype of tumor biopsies using plasma genotyping.

Methods: We identified patients (pts) with advanced NSCLC and acquired resistance to erlotinib that underwent rebiopsy and plasma collection on three IRB-approved protocols. Rebiopsy specimens underwent clinical EGFR genotyping. Plasma was collected in EDTA tubes, cfDNA extracted, and EGFR genotype quantified using ddPCR assays for L858R, exon 19 del and T790M. Serial plasma genotyping on treatment was performed for a subset of pts. This assay was then piloted in pts with advanced NSCLC who had not yet undergone tumor genotyping.

Results: A total of 32 pts undergoing rebiopsy had plasma available for analysis. Sensitivity of plasma genotyping for EGFR exon 19 del & L858R was 57%; sensitivity increased to 91% among 11 pts with symptomatic bone or visceral metastases. One additional pt with no tumor EGFR sensitizing mutation but response to erlotinib had high levels of plasma exon 19 del, suggesting that plasma genotyping may identify mutations missed by tumor genotyping. T790M was detected on rebiopsy in 16 pts (50%); plasma genotyping was concordant with rebiopsy T790M status in 27 of 32 pts (84%). Post-treatment plasma specimens were available for 12 pts; the 5 pts with a partial response on imaging had a significant decrease in plasma concentration compared to the 7 pts without a response (median 895 copies/mL decrease vs 2 copies/mL increase, p=0.01). In 17 NSCLC pts without a known genotype, we identified EGFR mutations in 3 pts with a median 3 day turnaround time, up to 19 days before tumor genotyping confirmed the result.

Conclusions: Plasma genotyping of cfDNA with ddPCR can predict tumor genotype rapidly and quantitatively with a high degree of accuracy, potentially obviating the need for re-biopsy in some circumstances. Clinical development of this assay is ongoing.