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Background: The identification of activating epidermal growth factor receptor (*EGFR)* mutations is essential for deciding therapy of non-small cell lung cancer (NSCLC) patients. Circulating cell-free tumor DNA (cftDNA) holds promise as a non-invasive methodology for tumor monitoring in solid malignancies. Among advanced NSCLC patients with an acquired resistance to EGFR-tyrosine kinase inhibitors (TKIs), about 50% carry *T790M* mutation, but its frequency in EGFR-TKI-naive patients and dynamic change during therapy remains unclear. We hypothesized that *EGFR*mutation analysis detection in cftDNA for NSCLC may be feasible for monitoring treatment response to EGFR-TKIs and also predict drug resistance.

Methods: *EGFR* sensitive mutations and *T790M* were analyzed using digital PCR (d-PCR) (Quant studio 3D, life technologies) in longitudinally (at baseline, at 4, 8, 20, 60, 120, 180, 270, 360 days) collected plasma samples (n=50) from 8 tissue-confirmed *EGFR*-mutant NSCLC patients treated with an EGFR-TKI (Gefitinib N = 4; Erlotinib N = 1; Afatinib N = 3). DNA extracted from plasma of 8 healty blood donors were used to detect the specificity of *EGFR* mutant assay. Tumor assessment was performed according to RECIST criteria 1.1 every two months.

Results: The sensitivity of d-PCR in plasma versus tissue was 71.4%. No *EGFR* mutation was present in the 8 control cases (specificity of 100%). Of four patients who developed progression disease (PD), in the samples of progression, *T790M* was detected in 75% of cases. The frequency of *T790M* in pre-TKI plasma samples was of 37.5%. *EGFR* sensitive mutations decreased at PD while *T790M* mutation increased in 75% of patients. Patients with concomitant pre-TKI *EGFR* 19 deletion and *T790M* showed a PD before of 12 months compared to those with *L858R*. *T790M* was frequently detected when new lesions were developed. Four patients had *T790M* level decreased to undetectable level with longer PFS than those with detectable *T790M* in blood.

Conclusion: Our results indicated that d-PCR was a highly sensitive and useful method for detecting the *T790M* mutation. Moreover, dynamically monitoring *T790M* change might help determining EGFR-TKI resistance. We thank Italian Association for Cancer Research (AIRC) for supporting the study.

Keywords: cftDNA, advanced NSCLC, digital PCR, resistance to EGFR-TKIs

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Plasma and Tissue Inflammatory and Angiogenic Biomarkers to Explore Resistance to EGFR-TKIs and Association with VeriStrat Status

Topic: EGFR Biomarkers

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Background: VeriStrat is a proteomic test validated to be prognostic and predictive of overall survival (OS) to EGFR Tyrosine Kinase Inhibitors (TKIs) in EGFR wild type patients[1]. Serum Amyloid A1 (SAA1) and its two truncated forms are responsible for 4 of the 8 peaks overexpressed in Veristrat (VS) Poor classified patients. The aim of the study was to explore if the microenvironment, with its complex network mediated by inflammation and angiogenesis could represent the base of the aggressive disease behavior of VS Poor subjects. Moreover, the activity of the immune escape axis PD-1/PD-L1 was explored.

Methods: Plasma biomarkers analyses were retrospectively performed on plasma baseline samples of 244 patients enrolled in the PROSE trial[1], while exploratory tissue analysis was performed on 37 available histological specimens. Circulating levels of HGF, VEGF, FGF, Cromogranin A (CgA) and its pro- and anti-angiogenic fragments (fragment 1-373 and 1-76, respectively) were determined by an ELISA assay. PD-L1 expression both on tumor cells and inflammatory elements of the tumor microenvironment, and the tissue expression

(T) of HGF and its receptor c-MET were measured by immunohistochemistry.

Results: CgA, HGF, VEGF, and FGF plasma levels were statistically significantly higher in VS Poor subjects. High plasma HGF levels were associated with lower PFS (3.4 versus 2.0 months, HR 1.67; 95% CI 1.25-2.23; $p<0.001$) and OS (11.2 versus 6.4 months, HR 1.64; 95% CI 1.12-2.23 $p=0.002$). High PD-L1- tumor expression was associated with worse PFS (5.9 versus 1.9 months, HR 2.28; 95% CI. 1.14-4.57; $p<0.020$) and a trend for lower OS (14.6 versus 6.7 months, HR 1.47; 95% CI 0.85-2.53; $p=0.165$), but not significantly associated with VS status ($p=0.656$). At the multivariate analysis, CgA, HGF and VEGF were independently associated with VS Poor status. When clinical variables were also included (histology and PS), multivariate analysis evidenced VEGF as the only independent biomarker associated with the VS Poor classification ($p=0.0013$). Plasma HGF levels (HR 2.083; 95% CI. 1.306-3.321; $p=0.0021$) and tumor PD-L1 expression (HR 2.579; 95% CI 1.036-6.421; $p=0.0417$) remained independent prognostic biomarkers for shorter PFS.

Conclusion: Inflammation and angiogenesis appear to be associated with the complex processes at the base of the Veristrat signature. Plasma HGF levels and tumor tissue PD-L1 are prognostic in terms of a worse PFS, but VeriStrat remains the only highly reproducible clinically relevant biomarker associated with OS. [1]V Gregorc et al, The Lancet Oncology, p713, 15(7), 2014.

Keywords: NSCLC, VeriStrat, EGFR-TKIs

P3.02b-010
Urine Detection of EGFR T790M Mutation in Non-Small-Cell Lung Cancer: An Outcomes and Total Cost of Care Analysis

Topic: EGFR Biomarkers

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Background: Third-generation tyrosine kinase inhibitors (3rd-TKIs) have proven effective in patients with *EGFR* T790M who progress on prior EGFR TKI therapy. Median progression-free survival (PFS) on a 3rd-TKI was 9-10 months for T790M+ patients compared to 2-4 months for T790M- patients. PFS is similar regardless of the specimen used to assess T790M (tissue, plasma, or urine). Using simulation analytics, the primary study aim was to assess the cost effectiveness of a urine-testing strategy (UTS) versus a tissue-testing strategy (TTS) for T790M detection in patients with *EGFR*-positive lung adenocarcinoma and progression on prior TKI therapy.

Methods: Analytics followed International Society for Pharmacoeconomics and Outcomes Research (ISPOR) and Society for Medical Decision Making (SMDM) guidelines for Good Modeling Practices and Consolidated Health Economic Evaluation Reporting Standards (CHEERS) for reporting findings. Outcomes and economic implications were assessed from the perspective of a third-party US payer, stratified by government versus commercial fee rates. Endpoints were PFS, overall survival (OS), direct medical resources used (biopsies, chemotherapy, post-progression) and related costs. Data sources were published reports of randomized drug trials and current data, which includes accuracy results of tissue versus urine testing (Trovagene, San Diego, CA), Medicare fee schedules, and available adjustments for fees in commercial markets. A state-transition analysis and Markov model tracked patients from stable disease, progression, and to death. Full univariate and multivariate sensitivity analyses were performed to assess the robustness of findings and factors that most influenced outcomes and costs.

Results: Median PFS after treatment with 3rd-TKI was 3.4 months if tumor testing is T790M- versus 9.7 months if T790M+. Because urine testing can be used in patients for whom biopsy cannot be performed or when tissue testing reveals indeterminate results, PFS and OS were slightly increased using the UTS. UTS resulted in avoidance of a biopsy procedure, potential complications, and tissue-based molecular testing in approximately 48% of patients, leading to a 2- to 10-fold total cost savings relative to the unit cost for a urine test. Within the robust variations in input parameters, the cost of a biopsy procedure/complications and tissue-based molecular testing were the most influential factors.

Conclusion: UTS is a dominant scenario to TTS by saving costs and improving patient experience (e.g., PFS/OS, and reduction in biopsy related complications). This result is based on LEVEL I evidence from a large, randomized trial that showed PFS is similar among patients regardless of urine versus tissue testing for T790M mutation status.

Keywords: NSCLC, T790M, liquid biopsy, Health Economics