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Downregulation of EZH2 is associated with estrogen receptor upregulation and favourable outcome to tamoxifen in advanced breast cancer

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Background: We identified previously a gene signature for resistance to first-line tamoxifen therapy in advanced breast cancer. The aim of this study is to investigate Enhancer of Zeste Homologs (EZH), one of these genes, for its prognostic and predictive value and to evaluate functional involvement in treatment response in vitro.
Materials and Methods: EZH2 mRNA levels were measured with quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) in 1318 primary breast cancer specimens. Levels were related to clinicopathologic factors and disease outcome. EZH2 expression was downregulated with siRNAs in the estrogen sensitive breast cancer cell line MCF7. Cell numbers were determined in controls and after treatment with (the anti-estrogen) ICI164.384. Expression levels of the estrogen receptor (ESR1) were also measured.
Results: EZH2 levels showed a significant inverse correlation with ESR1 levels in 1318 patients ($R = -0.33$; $P < 0.001$). In 688 lymph node negative patients who did not receive adjuvant systemic therapy, EZH2 levels were not correlated with metastasis free survival (HR = 1.14, 95% CI: 0.98–1.32; $P = 0.10$). In 278 patients with advanced disease treated with first-line tamoxifen monotherapy, high EZH2 levels were significantly associated with treatment resistance. The tertile with highest EZH2 levels was associated with no clinical benefit (OR = 0.48, 95% CI: 0.26–0.89; $P = 0.02$) and with shorter progression free survival (PFS) (HR = 1.80, 95% CI: 1.32–2.46; $P < 0.001$). In multivariate analysis including traditional predictive factors, highest EZH2 levels were independently related with a shorter PFS (HR = 1.58, 95% CI: 1.10–2.26; $P = 0.01$).
To test whether EZH2 has an effect on anti-estrogen therapy, EZH2 downregulation in MCF7 cells and its influence on response to ICI164.384 was determined. EZH2 silencing caused a significant decrease in cell numbers (38%, range 17–53%, N = 3) whereas ICI164.384 treatment resulted in decrease of 25% (range 12−30%, N = 3) compared to controls. Combining EZH2 silencing with ICI-treatment reduced cell numbers with 67% (range 54−75%, N = 3, $P < 0.001$) compared to untreated cells. Interestingly, EZH2 downregulation was associated with an almost 2-fold upregulation of ESR1 (N = 6, $P = 0.001$).
Conclusion: The favourable outcome tot tamoxifen in patients with low EZH2 expression may be explained by the upregulation of ESR1 as found in vitro. Further validation is needed to confirm that downregulated EZH2 leads to an upregulation of the ESR1 and as a consequence to a better response to tamoxifen.

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EGFR mutations based on circulating free DNA (cfDNA) in the subset of Japanese patients (pts) from IPASS (IRESSA Pan ASia Study), a Phase III study of first-line gefitinib (G) vs carboplatin/paclitaxel (C/P) in clinically selected patients with advanced non-small-cell lung cancer (NSCLC)

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Background: IPASS demonstrated superiority of G vs C/P for progression-free survival (PFS) in 1217 chemonaïve, never/light ex-smoker pts with advanced NSCLC in Asia. Different outcomes were seen in pts with EGFR mutation (M)+ (G benefit) and M− (C/P benefit) tumors. A preplanned analysis of outcome by cfDNA EGFR M status for the subset of Japanese pts is reported here.
Materials and Methods: Serum samples were obtained from the subset of Japanese pts before study treatment. cfDNA was extracted and assayed for 21 EGFR mutations (exon 19 deletions, L858R and T790M) by the amplification refractory mutation system (ARMS). Correlation of cfDNA EGFR M status with PFS (the primary endpoint; by Cox proportional hazards model), objective response rate (ORR; by logistic regression), and tumor EGFR M status were evaluated.
Results: Of 233 pts randomized in Japan, 194 provided an evaluable pre-dose serum sample, of which 46 (24%) were EGFR M+ and 148 (76%) EGFR M−. Of 91 pts with an evaluable tumor sample, 56 (62%) were EGFR M+ and 35 (38%) EGFR M−. A significant interaction between cfDNA EGFR M status and treatment was evident for PFS (interaction test $p = 0.0448$). PFS was significantly longer with G than C/P in cfDNA EGFR M+ pts (HR 0.29, 95% CI 0.14, 0.60, $p = 0.0009$). In cfDNA EGFR M− pts, PFS HR slightly favored G (HR 0.88, 95% CI 0.61, 1.28, $p = 0.5013$). ORR by cfDNA EGFR M status was generally consistent with PFS (EGFR M+ ORR 75.0% for G and 63.6% for C/P; EGFR M− 27.1% and 21.8%, respectively). All 22 pts identified as cfDNA EGFR M+ (and known tumor M result) were tumor M+ (specificity and positive predictive value 100%); however, 29 (57%) of 51 pts who were tumor M+ (and known cfDNA M result) were cfDNA M− (sensitivity 43%; negative predictive value 55%).
Conclusion: The proportion of M+ pts was lower when assessed by cfDNA compared with tumor tissue. The treatment effect for the cfDNA EGFR M+ subgroup was similar to the tumor EGFR M+ subgroup of the overall IPASS population. The specificity and positive predictive value was 100% for cfDNA, but the 43% sensitivity highlighted that over half of tumor M+ pts were not detected by cfDNA testing (possibly due to limits of technology currently available and the amount of cfDNA in a sample). These data suggest that cfDNA EGFR M status should only be used as a positive predictor of treatment outcome to gefitinib, and that a negative cfDNA EGFR M result is inconclusive and warrants further investigation.