In vitro cytotoxicity of allitinib, an irreversible anti-EGFR agent, in a large panel of human cancer cell lines: *KRAS* mutation status as a predictive biomarker

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BACKGROUND: The epidermal growth factor receptor (EGFR) is a member of HER family that activates several intracellular signaling pathways promoting proliferation and cell survival. The overexpression of EGFR is frequently associated with gene mutation or amplification, constituting a major target for molecular therapies. Recently, a novel generation of EGFR inhibitors has been developed with the properties of pan-HER and having irreversible action. Allitinib® (AST1306), is an orally active, highly selective irreversible inhibitor of the HER family receptor tyrosine kinases, with interesting efficacy data.

HYPOTHESIS: In the present study we aimed to investigate the cytotoxicity of allitinib in a large panel of human cancer cell lines and correlate its efficacy with the mutational status of *EGFR*, *KRAS*, *BRAF*, *PI3KCA*, and *PTEN* cancer related genes. In addition, we evaluate the functional role of *KRAS* mutations in response to this new inhibitor.

METHODS: Were used in total of 76 different types of tumor cell lines, representing 11 tumor types. Cytotoxicity was assessed by (MTS) and classified into three groups: highly sensitive (HS-efficiency> 60%), moderate sensitive (MS-efficiency 40-59%) and resistant (R-efficiency <39%). Mutational status of EGFR, KRAS was determined by direct sequencing and NRAS, PIK3CA and PTEN mutational status was collected in Roche Cancer Genome Database (Mutome, DB). Sensitive H292 cell line was transfected with *KRAS* mutations (p.G12D and p.G12S), then viability and cytotoxicity were measure by ApoToxGlo assay.

RESULTS: We observed that 28/76 (36.8%) cancer cell lines exhibited a HS phenotype, 19/76 (25.0%) MS and 29/76 (38.1%) were classified as resistant. Allitinib showed a stronger cytotoxicity in head and neck 4/7 (57.1%), esophageal 4/4 (100%), melanoma 5/9 (55.6%) and lung cancer cell lines 7/15 (46.6%). We observed that *KRAS* mutations were significantly associated with allitinib-resistant phenotype 14/20 (70%). Functional analyses showed that both activating *KRAS* mutations revert the sensitive phenotype to allitinib in H292 cells. The present study represents the largest *in vitro* assessment of allitinib cytotoxicity. We identified the tumor types that could potentially benefit from this drug, and importantly our findings suggest that *KRAS* mutations constitute a potential predictive biomarker of allitinib response.