Results and discussions We uncovered an oncogenic RAS-dependent ‘safeguard’ mechanism in order to evade the cytotoxic effect of HDACi and thereby apoptosis. Cells harbouring oncogenic RAS were observed to undergo a reversible senescence-like growth arrest in G2 phase, allowing for re-entry into cell cycle following a withdrawal of the inhibitor. This mechanism is implemented as a consequence of the inhibition of the RAS deacetylase, namely HDAC2, which in turn result in the generation of (hyper)acetylated RAS with increased binding affinity to BRAF and CRAF. This translates to a further amplification in MAPK-signalling and thus an increase in the priming of c-MYC for ubiquitin-mediated pro teaseomal degradation, thereby enabling the cells to exit the cell cycle and enter the defined protective state of G2 arrest. The prospect of HDACi treatment was effectively improved using current MAPK-targeted therapy and senolytic drugs by effectively preventing the observed pro-oncogenic effect of the HDACi treatment alone.

Conclusion Our study reveals an oncogenic RAS-dependent resistance mechanism, enabling cells harbouring oncogenic RAS, to establish a favourable cellular state of prolonged pharmacological hideout - a phenomenon that is replicated in patient-derived 3D cell culture models of CRC. This highlights the potential clinical relevance of our findings and thus the importance of a rational mechanism-based combinatorial therapeutic design in order to realise the true therapeutic potential of HDACi.

Introduction EGFR alterations (overexpression and mutations) are frequent events in non-small cell lung cancer (NSCLC). In the last years, second-generation EGFR-targeted therapies, such as afatinib such as allitinib were designed, having a potent irreversible inhibitor action of EGFR and other ErbB family members. Besides the EGFR mutation status, there are no predictive biomarkers of response to this new generation of inhibitors. On this context, the aim of our study was to compare the cytotoxic effects of two irreversible anti-EGFR inhibitors in a panel of NSCLC cell line and assess the impact of KRAS mutations in the response to these agents.

Material and methods Total of 15 NSCLC cell lines were used. Cytotoxicity was assessed by (MTS). According to GI50 score, cell lines were classified into three groups: highly sensitive (HS), moderate sensitive (MS) and resistant (R). Mutational status of EGFR, KRAS, BRAF and PIK3CA was determined by direct sequencing. Sensitive H292 cell line was transfected with KRAS mutations (p.G12D and p.G12S), then profile of MAPK phospho-protein was assessed by RPPA. Subsequently, cytotoxicity, colony formation, migration and invasion were measured. In vivo chorioallantoic membrane assay (CAM) was used to evaluate the impact of KRAS mutations on tumour proliferation.

Results and discussions We found 2 cell lines with EGFR and 5 with KRAS mutations. GI50 score show that 2 cell lines treated with afatinib and 7 treated with allitinib exhibited a HS phenotype. We observed IC50 values of 0.95±0.17 μM for H292 wild-type, a six times increase (6.56±0.23 μM) in the H292-KRAS-G12D and an eight times increase (8.47±0.15 μM) in the H292-KRAS-G12S cells. Protein array of KRAS mutant cell lines exhibited an AKT, CREB, HSP27, JNK and TOR activation. In addition, colony formation, migration and invasion potential were increased. Cam assay revealed increased tumour perimeter in KRAS mutant cell lines.

Conclusion We demonstrated that allitinib is most potent anti-EGFR agent. Importantly KRAS mutations increase aggressive behaviour and can constitute a potential predictive biomarker of therapy response. Activation of intracellular protein, revealed by array represent a potential target for therapies combination. In additional, KRAS mutant cell lines increased the proliferative potential measured by CAM in vivo model.