the mutations detected by sequencing were also identified by Cast-PCR. In addition, Cast-PCR detected four samples carrying mutations and was able to clearly identify two mutations of uncertain interpretation by Sanger sequencing. Cloning and sequencing of the clones was used to confirm mutations. The limit of detection of the two techniques were evaluated by constructing dilution curves of BRAFV600E and BRAFV600K mutated clinical samples mixed with a not-mutated specimens. Mutations could be detected until a 1:100 mutated/not mutated ratio for CAST-PCR but only at 1:25 ratio for Sanger sequencing. Among the technical issues related to BRAF mutation detection in melanoma, melanin content is overlooked. Indeed, melanin coisolates with DNA and it is a strong inhibitor of DNA polymerase. Thus we compared the performance of Cast-PCR and Sanger analyses before and after GC-purification. Melanin content strongly affected the number of cases successfully analysed by Sanger sequencing, and interpretable electropherograms were obtained only in 4% of not purified cases. Likely due to the use of less amount of starting DNA, Cast-PCR was less affected, however, DCt values were higher in not purified samples (Mean±SD 2.55±1.61) than in purified ones (Mean±SD 3.60±2.19) (p value = 0.14), and in 1 case a BRAFV600E mutation was only detected after purification. With respect to intratumour heterogeneity Cast-PCR showed concordant results in all samples for which different regions on the same section were available. Whereas, in one case Sanger sequencing was unable to detect the mutation in 1 out 4 regions.

Conclusions: We demonstrated that the use of highly sensitive techniques such as Cast-PCR is able to improve the ability to correctly genotype melanoma patients. Our results strongly suggest that diagnostic laboratories should evaluate whether their pre-analytical procedures are able to reduce the impact of melanin content on subsequent analytical methods.

No conflict of interest.

582 Molecular determinants of sensitivity and resistance to FGFR inhibition in *FGFR2*-amplified gastric cancer

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Background: Despite improvements in diagnostics and chemotherapy regiments in gastric cancer, there is still an urgent need for novel biomarkers and second-line treatment interventions. High-level FGFR2 amplification is found in ~5% gastric cancers, and responses in FGFR2-amplified gastric cancer have been observed in a phase II study of FGFR inhibitor. In this study we used patient-derived xenografts (PDX) to understand the mechanisms of sensitivity and resistance in FGFR2-amplified gastric cancer.

Methods: PDX models were generated from the baseline biopsies of two Caucasian patients with junctional FGFR2-amplified tumors who had durable clinical responses to AZD4547. To investigate adaptive molecular changes to AZD4547 treatment, PDX tumors and PDX-derived spheroid cultures were profiled using various immunodetection approaches. Resistant PDX models were generated via continuous treatment with AZD4547.

Results: Both PDX recapitulated the histology of the original cancers, and whole exome sequencing demonstrated 85−90% similarity in mutations between the patient biopsies and the PDX tumors. Comparable to the patients, both models were highly sensitive to AZD4547, with regression of ~60% seen after 10 days of treatment and subsequent stability on chronic dosing.

FGFR inhibition resulted in prolonged MAPK signaling suppression in primary spheroids, with initial acute PI3K-mTOR pathway suppression. However chronic exposure to the drug resulted in an increase in phospho-S6 and phospho-4EBP1. Similarly, phospho-S6 expression was elevated in PDX tumours after continuous exposure to AZD4547. Adapted stable PDX tumours additionally demonstrated increased phosphorylation of alternative RTKs, increased ERBB3 and insulin receptor phosphorylation, and elevated levels of MCL1 compared to vehicle controls. This data implicated restoration of mTOR signaling as a key adaptive mechanism in the persistent PDX tumours.

Chronically treated residual PDX tumours acquired resistance to AZD4547 after a median of 180 days. Resistant PDX demonstrated an increase in FGFR2 copy number and reproducible loss of 4p and 9p. Whole exome sequencing of individual resistant tumours suggested independent acquisition of resistant genotypes in xeno-patients, demonstrating convergent evolution from stable residual disease into acquisition of resistance.

Conclusion: We show that re-activation of mTOR signaling limits sensitivity of FGFR2-amplified tumors to AZD4547. Subsequent acquired resistance originates stochastically from the stable adapted disease, emphasizing the importance of therapeutic strategies to deepen response and reduce persistence of tumour cells available to acquire resistance.

No conflict of interest.

583 Effects of doxorubicin-loaded dextran coated magnetic nanoparticles: on gene and protein expression profile of p53, survivin and bcl-2 in doxorubicin sensitive/resistant MCF-7 cell lines

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Background: In the present study, Dextran coated magnetic nanoparticles (Dox-Dex-MNPs) were prepared to obtain an effective targeted delivery system for Doxorubicin on breast cancer cell line.

Material and Methods: Dox-Dex-MNPs were synthesized and characterized by TEM, SEM, FTIR, VSM and TGA analyses. Drug loading efficiencies and release characteristics were investigated. On the other hand, drug-sensitive and drug-resistant MCF-7 cell lines were grown in RPMI 1640 medium supplemented with 10% FBS at 37oC and 5% CO2. Total RNA content was isolated by TRI Reagent according to the manufacturer's instructions. cDNA was synthesized from 1 ug of total RNA and random hexamer primers. The expression levels of p53, survivin and bcl-2 genes related to cell survival and apoptosis were shown using qRT-PCR. In order to confirm the results obtained by qRT-PCR analyses, the protein levels of Survivin, p53 and bcl-2 were demonstrated by immunocytochemistry(ICC).

Results: According to SEM, TEM, VSM and TGA results, the synthesized Dox-Dex-MNPs possess desired shape and size range (10−15 nm) as they can be internalized into the cell and also have superparamagnetic properties. FTIR analysis confirmed that Doxorubicin was successfully loaded on dextran coated MNPs. On the other hand, Dox-Dex-MNPs have pH-responsive release characteristics. Nanoparticles were efficiently taken up by both sensitive and Doxorubicin resistant MCF-7 cell lines (MCF/Dox) and this increases the efficacy of the drug and maintains overcoming the resistance of Doxorubicin in MCF-7/Dox cells. According to the gene expression results, survivin expression was 3 fold upregulated in MCF-7/Dox cells. However, it was 3 fold and 6 fold downregulated due to the application of free Doxorubicin and Dox-Dex-MNPs respectively. The anti-apoptotic Bcl-2 gene was approximately 10 fold downregulated in response to Dox-Dex-MNPs with respect to the application of free Doxorubicin on MCF-7/Dox cell lines. The immunoreactivity of p53 levels of MCF-7/Dox cells increased up to 95% with 3+ intensity after treatment with Dox-loaded Dex-MNPs.

Conclusion: The results of this study demonstrated that Dox-Dex-MNPs can be a potential targeted therapeutic agent to overcome drug resistance.

No conflict of interest.

584 miR-625-3p regulates oxaliplatin resistance by directly targeting MAP2K6/MKK6 in human colorectal adenocarcinoma

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Introduction: Oxaliplatin resistance in colorectal cancer (CRC) is a major medical problem, and predictive markers are urgently needed. Recently, we reported miR-625-3p as a candidate biomarker. We showed in two independent cohorts of patients with metastatic CRC that patients with a high miR-625-3p transcript level had a more than 6 fold higher risk of not responding to oxaliplatin than other patients. Now, we provide evidence that miR-625-3p is directly and functionally involved in resistance to oxaliplatin.

Material and Method: In order to investigate the role of miR-625-3p in modulating oxaliplatin sensitivity in CRC cells in vitro we constructed a transposon-based doxycycline inducible vector. This was used for generation of three different stable and inducible CRC cell line models. The models were constructed in HCT116 (mitch-match repair deficient (MMR) and p53wt), SW620 and HCC2998 (both MMR proficient and p53mut). These models, as well as additional CRC cell lines, were used to dissect the mechanism of action for miR-625-3p in oxaliplatin resistance by use of reporter systems, rescue experiments, functional assays, siRNA knockdown, chemical inhibitor experiments, microarray profiling, and advanced proteomics.

Results and Discussion: Our results indicate that miR-625-3p induces resistance by abrogating the stress and DNA damage-response signaling-axis MAP2K6-p38, which then in turn leads to increased cell viability by decreasing apoptosis. We show that miR-625-3p directly binds and down-regulates MAP2K6 and that this primes blockage of the central kinase p38. Inhibiting the pathway both at and downstream of MAP2K6 closely mimicked the effect of the miRNA. Rescue experiments showed that the sensitivity towards oxaliplatin could be restored by expression of a miR-625-3p insensitive MAP2K6 variant.