Introduction The protein p53 is activated in response to DNA damage. The active protein induces cell cycle arrest and DNA repair program and thus protects the organism against carcinogenic events. Inactivation of p53 gives the cancer cell a profit of genetic instability, resulting in enhanced cancer cell microevolution. Apart from gaining direct mutations in p53-coding gene, overexpression of Mdm2 protein, which is a natural negative regulator of p53, is the most common method utilised by cancer to keep p53 inactive. Mdm2 binds to p53, blocks its transactivation domain and directs p53 to degradation. The re-activation of p53 protein by forced dissociation of Mdm2-p53 complexes using small molecule Mdm2 antagonists is currently believed to be a promising therapeutic strategy of the treatment of cancers expressing wild-type p53. Several representatives of this class of molecules, such as Idasanutlin (RG7388), are currently evaluated in clinical trials. However no data concerning the generation of secondary resistance is available for those modern, optimised compounds.

Material and methods Human osteosarcoma U-2 OS cells were treated with Idasanutlin, followed by the analysis of viability (MTT assay), p53 activation (western blotting), the induction of apoptosis (annexin V staining) and cell cycle distribution (double staining with BrdU/FITC-anti-BrdU and propidium iodide). Homogenous populations of U-2 OS cells were established by expanding the monoclonal populations from single U-2 OS cells. For the generation of drug-resistant subpopulations the cells were treated with 5 μM Idasanutlin for 12–20 days. The resistant populations were visualised by in situ staining of the growing cells with BrdU/FITC-anti-BrdU and Hoechst 33342.

Results and discussions The treatment of U-2 OS cells with Idasanutlin leads to a strong induction of p53 expression level and the expression of p53-target gene encoding p21 protein. As a result Idasanutlin induces tremendous cell cycle arrest. However no apoptosis is induced and Idasanutlin-treated U-2 OS cells can be cultured for long time periods in the presence of the drug. This prolonged treatment of U-2 OS cells leads to de novo generation of cell populations that escape from the cystatic effect evoked by Idasanutlin.

Conclusion Although modern Mdm2 antagonists have much improved activities compared to their precursors (i.e. nutlin-3), they suffer from similar weaknesses, which are limited elimination of cancer cells and the generation of p53-mutated drug resistant subpopulations.

Omic Technologies

PO-491 SINGLE-CELL PHENOTYPIC PROFILING OF BREAST CANCER PATIENT-DERIVED TUMOUR XENOGRAFTS USING MASS CYTOMETRY

D Georgopoulos*, M Callari, A Martin, OM Rueda, W Greenwood, M Lawson, IC Carneville, SC Coskun, IA Bruna, IC Caldas, University of Cambridge, Cancer Research UK Cambridge Institute, Cambridge, UK; AstraZeneca, Oncology IMED, Cambridge, UK

Material and methods Human osteosarcoma U-2 OS cells were treated with Idasanutlin, followed by the analysis of viability (MTT assay), p53 activation (western blotting), the induction of apoptosis (annexin V staining) and cell cycle distribution (double staining with BrdU/FITC-anti-BrdU and propidium iodide). Homogenous populations of U-2 OS cells were established by expanding the monoclonal populations from single U-2 OS cells. For the generation of drug-resistant subpopulations the cells were treated with 5 μM Idasanutlin for 12–20 days. The resistant populations were visualised by in situ staining of the growing cells with BrdU/FITC-anti-BrdU and Hoechst 33342.

Results and discussions The treatment of U-2 OS cells with Idasanutlin leads to a strong induction of p53 expression level and the expression of p53-target gene encoding p21 protein. As a result Idasanutlin induces tremendous cell cycle arrest. However no apoptosis is induced and Idasanutlin-treated U-2 OS cells can be cultured for long time periods in the presence of the drug. This prolonged treatment of U-2 OS cells leads to de novo generation of cell populations that escape from the cystatic effect evoked by Idasanutlin.

Conclusion Although modern Mdm2 antagonists have much improved activities compared to their precursors (i.e. nutlin-3), they suffer from similar weaknesses, which are limited elimination of cancer cells and the generation of p53-mutated drug resistant subpopulations.

Drug Resistance

PO-492 ESTABLISHMENT OF NEW DRUG-RESISTANT GASTRIC CANCER CELL LINES

P Brebi*, I Cartas, B Mora, K Buchegger, I Viscarra, L Zanella, I Riquelme, C Li, Universidad de La Frontera, Scientific and Technological Bioresource Nucleus, Temuco, Chile

Introduction Gastric cancer (GC) is an important public health problem in Chile, because constitutes the first cause of death for cancer in man and the third in women. Most GC patients are diagnosed at advanced stages where surgery is not effective. In these cases, the GC treatment is mainly based in chemotherapy with Cisplatin (CDDP) or 5-fluorouracil (5-FU). However, tumour cells usually develop chemotherapy resistance, increasing the rate of recurrence. The establishment of drug resistant cell lines could serve as an initial screen for agents that might modulate drug resistance in gastric cancer.

Material and methods Two gastric cancer cell lines AGS and MKN-28 were treated with incremental doses of cisplatin or 5-