through methylation of promoter region of critical genes and defects in NER have been reported to associate with an extreme sensitivity to platinum-based therapy. Considering the clear emerging role of the DNA repair proteins and the cell cycle checkpoints proteins as predictive, prognostic and therapeutic targets in cancer there is the need to better characterize human tumours to define sub-sets of patients that would better benefit of a particular treatment modality. With this aim we analysed the mRNA expression of different genes involved in the cellular response to anticancer agents in a cohort of ovarian tissue samples obtained from patients with both stage I-II and stage III-IV ovarian carcinoma by RT-PRC. In particular we focused in genes involved in NER pathway (ERCC1, XPA, XPG), in the FA/BRCA pathway (FANCA, FANC, FANF, FANCD2), in BER pathway (PARP), cell cycle checkpoint (Chk1, Claspin). XPA, XPG and claspin were found to be statistically significantly more expressed in Stage I versus Stage III tumor samples, while claspin was significantly more expressed in Stage III tumor samples. No stastistically significant levels of mRNA were found in all the other genes analized between stage I and stage III. Studies are in progress to correlate the gene expression levels with patient survival.

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Pre-clinical pharmacology of the novel PARP inhibitor, AZD2281 (KU-0059436)

A.N. Cranston[1], S. Moore[1], L. Beaudoin[1], A. Lau[2], L. Lewis[3], L. Copsey[3], M.J. O'Connor[2], K.A. Menear[4], G.C.M. Smith[5], N.M.B. Martin[3]. *[1]KuDOS Pharmaceuticals, Pharmacology, Cambridge, United Kingdom; [2]KuDOS Pharmaceuticals, Translational Science, Cambridge, United Kingdom; [3]KuDOS Pharmaceuticals, Discovery Biology, Cambridge, United Kingdom; [4]KuDOS Pharmaceuticals, Chemistry, Cambridge, United Kingdom; [5]KuDOS Pharmaceuticals, Discovery Research, Cambridge, United Kingdom*

Background: Poly(ADP-ribose) polymerase-1 activation is an immediate cellular response to metabolic, chemical or ionizing radiation-induced DNA damage. Due to its central role in modulating DNA repair processes PARP-1 represents a new and interesting target for cancer therapy.
Materials & Methods: Medicinal chemistry was used to identify and optimize a lead series of substituted benzyl phthalazinones, from which the compound AZD2281 was identified. Leads from this series were tested for potency against isolated enzyme and for in vitro cell activity in human cancer cell lines. The efficacy of AZD2281 was examined in combination with alkylating agents and topoisomerase poisons in human cancer cell line xenograft tumours and also as a single-agent in BRCA-deficient cell line xenograft tumours implanted subcutaneously in the flank of nude mice. Anti-tumour activity was assessed primarily by tumour growth delay. Tolerability was assessed by a combination of body weight loss, clinical signs and blood parameters.
Results: AZD2281 showed excellent potency, good pharmacokinetic profiles and oral bioavailability in both rodent and non-rodent species as well as activity in both human cancer cell lines and xenograft models of cancer. AZD2281 was efficacious in terms of its ability to potentiate clinically-relevant chemotherapies in in vivo cancer xenograft models. In addition, AZD2281 displayed in vivo anti-tumour activity when given as a single agent therapy in BRCA-deficient cancer xenograft models. At the doses, schedules and, in the combinations given, AZD2281 was well tolerated in vivo.
Conclusion: AZD2281 has in vivo anti-tumour activity when given (i) in combination with DNA-damaging agents and, (ii) as a single agent in BRCA-defective pre-clinical human cancer cell line xenograft models. This potent, orally bioavailable, novel PARP inhibitor is currently undergoing clinical development for the therapeutic treatment of solid tumours including breast and ovarian cancer.

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Immunohistochemical DNA repair expression profile breast cancer: correlation with clinical-pathological features

S. Costa[1], F. Milanezi[2], M. Duarte[3], J. Paredes[2], A.L. Correia[2], A. Longatto-Filho[1], F. Schmitt[2]. *[1]Life and Health Sciences Research Institute (ICVS), School Health Sciences Minho University, Braga, Portugal; [2]IPATIMUP, Porto University, Porto, Portugal; [3]Cebal, Cebal, Beja, Portugal*

Background: Breast cancer cells, as most of the cancer cells, have high genomic instability, representing a critical feature to enable tumor initiation and progression. In this way, it is of extremely importance to maintain a DNA damage/repair balance, through the perfect function of DNA repair pathways. Our aim was to evaluate XRCC1 and XRCC3 protein expression profiles in a breast tissue series, from normal to invasive carcinoma, assessing the possible correlations between expression and clinical-pathological features of invasive breast carcinomas.
Materials and Methods: One hundred and eighty one breast tissue samples were obtained from the Hospital São João Porto, Portugal. These included normal-like parenchyma samples adjacent to the tumor, benign breast lesions, ductal carcinomas in situ and invasive ductal carcinomas. Clinical-pathological features were obtained from hospital records when it was available (as family history, histological grade, lymph nodes metastasis and estrogen receptor status. XRCC1 and XRCC3 immunostaining was carried out using the streptavidin-biotin-peroxidase technique in each set of glass slides containing the TMAs (tissue microarrays). The expression of XRCC1 and XRCC3 was classified by the absence (negative, 0) or presence of nuclear staining (positive, 1). Pearson's Chi-square test (χ^2) test or Fisher's exact test (when $n < 5$) was used to analyse the relationship of protein expressions with clinical-pathological parameters.
Results: The predominant pattern for XRCC1 and XRCC3 protein expression observed in all breast tissue samples was nuclear; although, XRCC3 expression was also detected in the cytoplasm. Regarding XRCC3 nuclear expression, the percentage of positive cases increases with the malignity of the tissues. There is no statistical significant relationship between XRCC1 nuclear staining and any of the clinical-pathological parameters considered. On the other hand, positive XRCC3 nuclear staining showed correlation with lower tumor grade (grade I and II) ($p = 0.070$), as well as estrogen receptor positivity status in the tumor ($p = 0.060$), with relative statistically significant differences.
Conclusions: Our study demonstrated that XRCC3 nuclear expression correlates with well/moderate differentiated tumors and with positivity to estrogen receptors. Thus, cancer cells with nuclear XRCC3 expression are relatively associated with features of a better prognostic.

Drug resistance and modifiers

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EGFR mutations and gefitinib affinity: molecular insights from in silico experiments

S. Pricl[1], G.M. Pavan[1], M. Fermeglia[1], E. Tamborini[2], M.A. Pierotti[3], S. Pilotti[2]. *[1]University of Trieste, MOSE – DICAMP, Trieste, Italy; [2]IRCSS Istituto Tumori Milano, Experimental Molecular Pathology, Milan, Italy; [3]IRCSS Istituto Tumori Milano, Scientific Direction, Milan, Italy*

Background: Somatic mutations in the epidermal growth factor receptor (EGFR) have been identified in patients with advanced non-small cell lung cancer who achieve dramatic clinical and radiographic response to the EGFR tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib. These mutations in EGFR are found most frequently in patients with adenocarcinomas, nonsmokers, patients of Asian ethnicity, and in females: the same populations that are most likely to have a clinical response when treated with EGFR TKIs. Although retrospective studies comparing the outcomes of patients with and without EGFR mutations treated with TKIs show a significant clinical benefit of EGFR TKIs in patients with EGFR mutations, the molecular mechanisms underlying these effects are still obscure. Moreover, beyond the promise of a tailored medicine and as frequently observed in target therapy, the presence secondary mutations resulting in gefitinib-resistant variants have been detected.
Methods: Extensive parallel molecular dynamics (MD) calculations, in the so-called Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA) framework of theory were employed to characterize the structural details of the wild-type (WT) and a series of mutant EGFRs in complex with gefinitib. The set of EGFR mutations considered included several missense substitutions (e.g., L858R and R831H, and T790M) and, for the first time, three deletion mutations (i.e., del747–753, del746-A750, and del747–752). The affinity of the WT and its mutated variants towards gefitinib was estimated, and the molecular factors at the basis of the favorable/unfavorable binding of the TK toward the inhibitor analyzed in details.
Results: Our in silico experiments revealed that the L858R mutated receptor interacts more efficiently with the inhibitor, as observed in the clinic, whilst for the R831H mutant isoform the affinity for gefitinib is comparable to that of the corresponding WT counterpart. In the case of the deletion mutants, the calculations clearly reveal how a difference of one single aminoacid in this set can dramatically change the affinity of the TK for its inhibitor. Indeed, while the binding of EGFR del747–753 to gefitinib shows an improvement in drug affinity, the well known del746–750 isoform is substantially less affine to the drug. Finally, the deletion mutant del747–752 seems to induce an effect similar to that caused by del747–753, enhancing the affinity of the receptor towards the TKI.
Conclusions: Our computational investigation contributes, for the first time, a piece of knowledge, at the molecular level, of the main structural and energetical factors underlying the different sensitivities of wt and mutated EFGR TK towards one of its most successful inhibitors, gefitinib.