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Human cancer cells are sensitized to voreloxin (formerly SNS-595) after modulation of DNA double strand break repair

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Voreloxin (formerly SNS-595) is a naphthyridine analog, structurally related to the quinolones, which have not previously been used clinically for cancer treatment. Voreloxin is a replication-dependent agent that induces DNA damage, irreversible G2 arrest and apoptosis by intercalation of DNA and poisoning of topoisomerase II (Stockett et al. and Hawtin et al., AACR 2008). Voleroxin is active in many preclinical models of human cancers, including drug resistant models. Sensitivity to voreloxin is related to the ability of cells to repair DNA double-strand breaks (DSBs) (Hawtin et al., AACR 2008). Therefore, we examined the impact of alterations in mismatch repair and microsatellite instability (MSI) on the activity of voreloxin in colorectal cancer cells in vitro. We further assessed the status of Mre11/Rad50/Nbs1 (MRN) complex proteins and examined the role of Rad51 activity, a component of homologous recombination DNA DSB repair mechanism (HRR), in determining sensitivity to voreloxin. The data reported here demonstrate that the mismatch repair (MMR)-defective colorectal cancer cell lines HCT116, LoVo and SW48 are more sensitive to voreloxin than are the MMR-competent colon cancer cell lines HT29 and SW480. This differential sensitivity may be attributable to the MSI in MMR-deficient cells causing inactivating mutations in critical components of DSB repair systems (Giannini et al., 2002). HCT116 cells, which are deficient for Mre11, are also defective in HRR as evidenced by minimal activation of Rad51 repair foci following voreloxin treatment, as compared to MRN-proficient HT29 cells. We evaluated the role of Rad51 and HRR in the MRN-proficient HT29 cells by generating stable cell lines with reduced Rad51 levels using shRNA. Reduction in the levels of Rad51 in this cancer cell line increased sensitivity to voreloxin. These data indicate a possible association of MRN mutations / MSI with increased sensitivity to voreloxin. The MRN proteins are mutated in a subset of colorectal as well as breast and ovarian cancers (Heikkinen et al., 2003; Hsu et al., 2007; Miquel et al 2007), and may identify patients who may benefit from treatment with voreloxin. Voreloxin is under clinical investigation in acute myeloid leukemia and ovarian cancer. Clinical responses have been observed in these indications (Lancet et al., ASH 2007; McGuire et al., SGO 2008), as well as in lung cancers (Burris et al., ECCO 2007).

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Acquired resistance to temozolomide in glioma cell lines: molecular mechanisms and potential translational applications

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Treatment for intractable Glioblastoma Multiforme (GBM) includes the alkylating agent temozolomide (TMZ) combined with ionising radiation. Persistent O6 methylation of guanine by TMZ, in O6 methylguanine methyl transferase (MGMT) negative tumours causes cytotoxic lesions recognised by DNA mismatch repair (MMR) triggering apoptosis. Intrinsic, or acquired resistance present severe obstacles to successful TMZ treatment, limiting drug efficacy and life expectancy.

The purpose of this study was to derive human glioma cell lines with acquired resistance to the alkylating agent TMZ and to characterise the mechanisms of acquired resistance. Two glioma cell lines, SNB19 and U373, initially sensitive to TMZ (GI50 values 36 μM and 68 μM respectively) were exposed to increasing concentrations of TMZ (1–100 μM). Variant cell lines SNBVR and U373VR were generated which display acquired resistance to TMZ (GI50 values 280 μM and 290 μM respectively) and cross-resistance to the ring-opened monomethyl triazeno imidazole carboxamide (MTIC). Resistance to mitozolomide (MTZ) was observed in U373VR cells only. O6-Benzylguanine significantly enhanced TMZ potency in U373VR cells indicating the mechanism of resistance involves re-expression of MGMT. Indeed, Western Blot analyses revealed MGMT protein expression in cell lysates. Furthermore, in clonogenic assays, depletion of MGMT using O6-benzylguanine sensitised U373VR cells to TMZ. In SNB19VR cells, loss of expression of MMR protein MSH6 confers resistance to TMZ. In conclusion, we have developed two model glioma cell lines whose distinct mechanisms of acquired resistance to TMZ, involving expression of MGMT, or inactivation of DNA MMR, are consistent with clinical observations.

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MP-470, a novel multi-targeted tyrosine kinase inhibitor targeting rad51 is not toxic to human primary marrow stem cells at clinically relevant concentrations

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Background: MP-470 is being studied in a five-arm Phase 1b clinical trial in combination with various chemotherapy regimens in solid tumors. One of the chemotherapy regimens is carboplatin (Pt)+ etoposide (Et). As both these drugs are myelosuppressive, there is a concern MP-470 combination would exacerbate the myelosupression. This *ex vivo* human primary bone marrow stem cells study examined if MP-470, alone or in combination, is toxic to the stem cells.

Materials and Methods: Normal human bone marrow cells obtained from healthy donors were diluted in Iscove's modified Dulbecco's medium (IMDM 2% FBS) and washed by centrifugation. Cell pellets were resuspended, and cell count and viability were assessed. MP-470, carboplatin and etoposide in DMSO were added to a methylcellulose-based medium to provide final test concentrations: MP-470 at 0.005–10 μg/mL; Pt at 0.1−20 μg/mL and Et at 0.01–1.0 μg/mL. For combination toxicity, MP-470 at 0.5 μg/mL was added to cultures containing Pt or Et at above concentrations. To examine whether MP-470 affects recovery, the cells after 2-h incubation with these drugs were washed and then incubated with MP-470 for 14 d. The clonogenic progenitors of erythroid (CFU-E and BFU-E), myeloid (CFU-GM) and multi-potential (CFU-GEMM) lineages were monitored and scored based on size and morphology.

Results: Pt, Et and MP-470 as single agents and in combination inhibited erythroid and myeloid colony formation in a concentration-dependent manner resulting in IC50s as shown in the table.

Groups	IC50 (μg/mL)	
	Erythroid	Myeloid
Pt	0.31	0.30
Et	0.04	0.02
MP-470	18.43	8.00
Pt + MP-470	0.34	0.23
Et + MP-470	0.04	0.02

When cells, after a 2-h incubation with either Pt or Et, were exposed to MP-470 (0.5 μg/mL) for 14 days, there was a decrease of total colony forming cell (CFC) progenitors compared to controls (no drug) but there was no significant difference due to addition of MP-470 to either drug; total CFC were 4203±332, 4935±390 and 5004±522 for MP-470, MP-470+Pt and MP-470+Et, respectively. MP-470 did not significantly affect recovery of stem cells previously exposed to Pt or Et, there were minor morphological changes with MP-470.

Conclusion: Compared to carboplatin and etoposide, MP-470 is ~25–400-fold less toxic to human bone marrow stem cells; simultaneous exposure to MP-470 with Pt or Et did not significantly exacerbate the toxicity. Furthermore, MP-470 did not significantly affect the recovery of stem cells previously exposed to Pt or Et.

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Expression of genes involved in DNA damage response pathways in ovarian cancers

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The cellular response to DNA lesions is orchestrated in such a way that the detection of the damage activates a number of signal transduction pathways leading to cell cycle arrest and thus allowing repair, or if the damage is too heavy, induction of apoptosis. DNA represents the cellular target of many currently used anticancer agents and the repair activity of the cell is an important determinant of cell sensitivity to anticancer agents. Indeed, it has been reported that resistance to DNA-damaging agents can be associated with increased cellular repair activities, while defects in DNA repair pathways result in hypersensitivity to these agents. In particular, both the "Fanconi Anemia-BRCA" pathway (FA-BRCA) and the Nucleotide Excision Repair (NER) have been shown to be required for the cellular response to DNA interstrand crosslinks and bulky lesions, such as the ones induced by cisplatinum and mytomicin C. FA/BRCA pathway has been reported to be inactivated in sporadic cancers by epigenetic silencing,

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)

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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

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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

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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.