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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,