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Inhibition of DNA repair vis-à-vis resistance in chronic lymphocytic leukemia

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Chronic lymphocytic leukemia (CLL) is an indolent leukemia in which there is an accumulation of malignant B-lymphocytes. While treatment with chlorambucil, a nitrogen mustard analogue, can control the disease, eventually all patients become resistant to chlorambucil. Chlorambucil cytotoxicity is mediated by induction of DNA interstrand crosslinks (ICLs). Chlorambucil (CLB) treatment is utilized in chronic lymphocytic leukemia but resistance to CLB develop in association with accelerated repair of CLB-induced DNA damage. This damage can be repaired by nonhomologous DNA end-joining (NHEJ) and/or homologous recombinational repair (HR) pathways. Key components of these two pathways are respectively the DNA-dependent protein kinase (DNA-PK) and the RecA-human homologue Rad51. Here we report that inhibition of either DNA repair pathways results in sensitization of CLL-lymphocytes to chlorambucil *in vitro* using the MTT assay. We utilize NU7026, a relatively specific DNA-PK inhibitor, and Dasatinib, a new c-abl and Src kinase inhibitor. We find that chlorambucil cytotoxicity is synergistically increased by sublethal doses of NU7026 (2−10 times) or Dasatinib (5−200 times). This effect was observed in both the CLL cell line I83 and in primary lymphocytes from CLL patients (sensitive and resistant to CLB). The effect of NU7026 was mediated by the inhibition of DNA-PK autophosphorylation (T2609) induced by CLB, which results in accumulation of DNA damage (quantified as the percentage of γH2AX positive cells). We are actually evaluating if Dasatinib mechanism of action is related to c-abl kinase inhibition since we recently reported that Gleevec sensitizes CLL lymphocytes to chlorambucil by inhibiting c-abl mediated Rad51 phosphorylation, HRR repair and increased apoptosis. Moreover we are assessing the effect of Dasatinib plus NU7026 in CLL cells to test the simultaneous inhibition of both DNA repair pathway on chlorambucil cytotoxicity. These exciting results should lead to new clinical trials testing the effect of inhibition of DNA repair vis-à-vis CLB sensitization in CLL patients.

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Identification of a PARP inhibitor for clinical trial: preclinical studies

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Background: Poly(ADP-ribose) polymerase-1 (PARP-1: EC 2.4.2.30) is a nuclear enzyme that promotes the repair of DNA breaks and can therefore compromise efficacy of DNA damaging anti-cancer therapies. Inhibition of PARP-1 enhances the efficacy anticancer agents, such as temozolomide (TMZ), topotecan (TP) and ionising radiation (IR).
Materials and Methods: To identify a PARP inhibitor for clinical trial superior to the previous lead, AG14361, we investigated the potency and biological properties of 42 potent PARP inhibitors from 4 structural classes by Ki determination using the recombinant human enzyme, in vitro chemo- and radiosensitisation growth inhibition assays and in vivo chemosensitisation assays. more potent *in vitro* and *in vivo* than the lead compound, selected on the basis of PARP-1 inhibitory potency (Ki $\leqslant$ 15 nM) to identify a compound for clinical use.
Results: Of the 42 compounds 25 were more potent inhibitors of the isolated enzyme than AG14361 (Ki < 5.8 nM) and 17 potentiated TMZ-induced cell growth inhibition more than AG14361 in Lovo cells (PF50 > 5.5). Eleven out of 38 and 21/24 potentiated TP-induced growth inhibition more than AG14361 in LoVo and SW620 cells, respectively. Potentially lethal IR damage recovery studies showed that 13 compounds were more potent radiosensitisers than AG14361. The most active compounds for evaluation of antitumour efficacy in combination with TMZ using a single dose schedule. Six of the 11 selected inhibitors increased TMZ-induced tumour growth delay more than AG14361. We confirmed the rank order of potency of the inhibitors in a conventional 5-day dosing schedule.
Conclusion: These studies identified a compound, AG14447, with outstanding in vivo chemosensitisation potency at tolerable doses, which was at least 10× more potent than the initial lead, AG14361, in both single dose and 5-day dosing schedules. The phosphate salt of AG14447 (AG014699), which has improved aqueous solubility, has been selected for clinical trial.

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Investigating the role of Nucleotide Excision Repair (NER) in the antitumor activity of Nemorubicin

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Nemorubicin (3′-deamino-3′-[2(S)-methoxy-4-morpholinyl]doxorubicin hydrochloride) is a third generation anthracycline, currently undergoing Phase I/II trials as single agent or in combination with cisplatin (cDDP) in primary hepatocellular carcinoma patients. The drug has antiproliferative activity in experimental tumors and efficacy on P-gp- and MRP-positive multi-drug resistant tumors as well as on tumors resistant to platinum derivatives, alkylating agents, and topoisomerase I and II inhibitors. A cell line resistant to nemorubicin (L1210/MMDX) was selected and resulted specifically resistant to this class of molecules and highly sensitive to platinum derivatives and alkylating agents. In addition, L1210/MMDX cells were more sensitive (about 4–5 times) to UV light and not able to repair damage occurred on DNA transfected after UV exposure (Host Cell Reactivation), suggesting that defects in the NER could play a role in the mechanism of resistance to nemorubicin.
In this study nemorubicin was tested in several isogenic cell lines characterized by defects in the NER pathway. The antiproliferative effect of nemorubicin was evaluated by colony assay on:
– Chinese Hamster Ovary (CHO) cells wild type (AA8) vs two sublines defective in defined steps of the NER pathway (UV 96 for ERCC1 and UV 61 for ERCC6) and vs the restored UV96 subline transfected with human ERCC1 cDNA;
– murine leukaemia L1210/0 (containing a specific defect in XPG endonuclease activity) and L1210/cDDP (with functional XPG gene);
– human fibroblasts XPA −/− and XPA +/+ (transfected with XPA cDNA).
Results showed that nemorubicin was less active in ERCC1 and ERCC6-deficient cells than in control cells (IC_{50} 2.7, 2.9 and 1.3 nM, respectively) and the sensitivity to nemorubicin is restored in the UV96 cell line. Also L1210/cDDP cells were 2.5 times more sensitive to nemorubicin. Conversely, the drug showed comparable activity on XPA −/− and XPA +/+ cells. The results obtained on isogenic CHO, L1210 cells and on XPA −/− and XPA +/+ fibroblast cell lines clearly indicate that different defects in NER pathway compromise the activity of nemorubicin. This prompted us to study in details the molecular alterations that could be responsible for NER pathway defects in L1210/MMDX cells (compared to the parental L1210 subline).
At present, XPA and ERCC1 expression has been evaluated. Both proteins are equally expressed in L1210 and L1210/MMDX cells. Nemorubicin has a novel mechanism of resistance that involves the NER pathway, which plays a role in the repair of lesions caused by several anticancer grugs. These findings provide the rationale for clinical combination studies of nemorubicin with cisplatin or mitomycin C, anticancer agents commonly used for the treatment of hepatocellular carcinoma patients.

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Flavopiridol, a cyclin-dependent kinase inhibitor, enhances radiosensitivity of human esophageal adenocarcinoma cells by inhibiting DNA repair

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Background: Cyclins and cyclin-dependent kinases (cdks) are deregulated in cancer cells, which contributes to tumor resistance to cytotoxic agents, including radiation, making these molecules and their signaling pathways potential therapeutic targets. A number of cdk inhibitors, including flavopiridol, have been demonstrated to enhance the efficacy of chemotherapeutic drugs and radiation in a number of tumor cell types. The present study investigated whether flavopiridol enhances radiosensitivity of human esophageal cancer cells, and whether the enhancement is mediated by inhibition of cellular repair processes.
Methods: Seg-1 human esophageal adenocarcinoma cells were cultured *in vitro*, treated with graded doses (50 to 300 nM) of flavopiridol for 24 h and then exposed to 2 to 6 Gy ionizing radiation. The effect of the treatments was assessed on clonogenic cell survival, expression of proteins associated with cellular DNA repair (western blot analysis), electrophoretic mobility shift assay (EMSA) for Ku-DNA end binding activity, and expression of nuclear gamma-H2AX foci, a marker for DNA damage and repair (immunocytochemical analysis).
Results: Flavopiridol enhanced the radiosensitivity of Seg-1 cells in a dose-dependent manner. A dose of 300 nM was the most effective [radioenhancement factor (EF) of 1.7 at the cell survival level of 0.1], and it abolished the “shoulder” of the cell survival curve suggesting that cellular repair was inhibited. The EF at the clinically relevant dose of 2 Gy was

3.0. Flavopiridol highly reduced the expression of Ku70 protein, a major participant of DNA repair, as well as Ku-DNA end binding activity, in the nucleus. Gamma-H2AX foci analysis showed that the foci in cells treated with radiation only (2 Gy) can be visualized for 4 h following radiation after which their number rapidly declined, the foci in cells exposed to flavopiridol and radiation were present for 24 h after radiation, indicating the prolonged presence of radiation-induced DNA damage in flavopiridol-treated cells.
Conclusions: Treatment with flavopiridol strongly enhanced sensitivity of Seg-1 esophageal adenocarcinoma cells to radiation, involving inhibition of DNA repair as an underlying mechanism. These findings suggest that flavopiridol has the potential to increase the efficacy of radiotherapy for esophageal cancer.
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Small inhibitory DNA (siDNA) enhancing tumor sensitivity to radiotherapy by baiting DNA-PK repair proteins

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Background: Radiotherapy, used alone or in association with surgery and/or chemotherapy, remains in 2006 one of the main anticancer therapies. Unfortunately, the cumulative toxicity of the combined therapies frequently limits their efficacy. In this work, we present a novel strategy to enhance the effect of radiotherapy on radioresistant tumors without directly increasing damage to genetic material.
Materiel and Methods: Small inhibitory DNA (siDNA) molecules – called Dbait – that mimic DNA double-strand breaks (DSB) were designed and synthesized. They were tested for their ability to interfere with various functions of DNA-PK in cell extracts and in transfected cells with the goal of inhibiting the DSB repair pathway in irradiated tumors and so promoting tumor regression. The efficacy of these molecules in sensitizing tumor cells to irradiation was evaluated in nude mice xenografted with several radioresistant human tumor cell line.
Results: In vitro, Dbait specifically activates DNA-PK's kinase activity and inhibits non-homologous recombination and DNA repair by non-homologous end joining (NHEJ), thereby increasing cell death in response to irradiation. The requirements for Dbait activity were similar in all the assays (activation of the protein kinase, inhibition of DNA fragment ligation in a cell-free assay, inhibition of plasmid integration and enhanced sensitivity to γ-irradiation in cultured cells). We found that the optimal Dbait molecule was a double-stranded DNA, at least 32-bp long and with at least one free end. The sequence had no influence on the activities tested indicating that the effects of Dbait were due to the molecular structure as a substrate mimetic rather than to targeting of a specific sequence. In vivo, a combination of Dbait treatment and radiotherapy induces regression of tumors in nude mice xenografted with various radioresistant tumors, in a dose-dependent manner.
Conclusion: The use of siDNA as a DSB substrate mimetic which baits and hijacks the enzyme complexes that repair DSBs is a novel and original pathway-targeting approach. This work provides evidence of a potential new adjunct molecular therapy to radiotherapy for treating radio-resistant malignant tumors. Further work is required to confirm whether our DNA bait strategy presents a paradigm shift from single gene/protein targeting to multi-gene/protein targeting (pathway), in order to fight against treatment-resistant cancer.

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Phase I pharmacokinetic (PK) and pharmacodynamic (PD) evaluation of an oral small molecule inhibitor of Poly ADP-Ribose Polymerase (PARP), Ku in patients (p) with advanced tumours

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Background: PARP is a DNA strand break and damage repair enzyme. Ku inhibits PARP-1 with a mean IC50 of 2 nM. Inhibition of PARP leads to defective DNA repair and induces selective cytotoxicity in cells with defective homologous recombination through, for example, loss of BRCA 1/2 function (Farmer et al, Nature 2005; 434(7035): 917−21). This is a first in human Phase I trial of Ku. PD studies included functional evaluation of PARP-1 activity in surrogate and tumor tissue.
Methods: Ku was administered daily for 14 of every 21 days to p with advanced solid tumors refractory to standard treatment. Cohorts of 3–6 p were treated, with a starting dose of 10 mg/day. The dose was doubled in the absence of drug related grade 2 CTC-AE toxicity. Drug related toxicity in cancer patients known to carry a BRCA mutation is being compared to toxicity in other patients.
Results: To date 21 p (mean age 55y [25−82y; 12 females]) with solid tumours have received 54 courses (range 1–8). 3 of these p have either a known BRCA mutation (2 p) or a strong family history suggesting BRCA mutation (1 p; refused BRCA testing). Dose levels evaluated to date include 10, 20, 40, and 80 mg once a day; and then 60 and 100 mg twice a day. No dose limiting toxicity has been reported with only grade 1 drug related toxicity being observed to date. PK support dose proportionality with a mean elimination half-life of 6.7 hours (Range: 6.3–6.9), a mean clearance of 4.37 L/h (Range: 3.1−6.3) and a mean volume of distribution of 41.0 L (Range: 29.5−60.6). PD studies indicate inhibition of PARP functional activity in peripheral blood mononuclear cells with increasing inhibition observed with increasing dose of Ku. Initial studies in tumor biopsies performed pre-treatment and on day 8 revealed PARP inhibition of around 50% at doses above 40 mg/day. A p with metastatic ovarian carcinoma, with previously platinum-responsive disease but became platinum-resistant later and a strong family history suggesting BRCA mutation has had an objective partial response by RECIST criteria with a CA125 fall of >70%. Two p having soft tissue sarcoma and renal carcinoma respectively and progressing disease pretreatment achieved stable disease for 24 weeks.
Conclusions: Dose escalation continues with more BRCA carriers planned. PARP inhibition in both surrogate and tumor tissue is achievable with minimal toxicity in cancer patients, and has not resulted in any short-term toxicity difference in BRCA mutation carriers.

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Centrosome abnormalities occur early and coexist with genomic instability during cancer progression in Barrett's esophagus

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Centrosomes play important roles in processes that ensure proper segregation of chromosomes and maintain the genomic stability of human cells. Centrosome defects have been found in aggressive carcinoma of multiple origins. The contribution of centrosome defects to esophageal adenocarcinoma (EadCA) and its precursor Barrett's esophagus (BE) has not been evaluated. We have previously shown that genomic instability (GIN) precedes alterations in tumor suppressor p53 and APC in BE-associated tumorigenesis. The aim of this study was to determine centrosome alterations during cancer progression in BE.
We analyzed specimens from endoscopic biopsies or esophagectomies in patients with BE (10 cases) or with BE-associated esophageal adenocarcinoma (10 cases), with normal gastro-esophageal junction (5 cases) as controls. A mouse monoclonal γ-tubulin antibody or a rabbit polyclonal pericentrin antibody was used for centrosome staining. Chromosomal enumeration probe Cep 7, 11, 12, 17 and 18 were detected by fluorescence in situ hybridization (FISH). In normal controls, centrosomes appeared uniform in size. In contrast, centrosomes showed structurally and numerically abnormal in the majority (90%) of EadCA. In pre-cancerous lesions, centrosome abnormalities were observed in 57% of non-dysplastic Barrett's epithelium, 67% of low-grade dysplasia (LGD), and 83% of high-grade dysplasia (HGD), respectively. Interestingly, centrosome abnormalities coexisted with GIN.
These results, for the first time, demonstrate that centrosome abnormalities occur early and coexist with GIN during cancer development and progression in BE. These findings suggest that the centrosome may be a biomarker for predicting patients at risk for cancer and a potential therapeutic target.

Formulation research

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Improved effectiveness of nab-paclitaxel versus docetaxel in various xenografts as a function of HER2 and SPARC status

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Background: Docetaxel (Taxotere®) showed improved survival and time to progression over paclitaxel (Taxol®) in a randomized phase 3 study in metastatic breast cancer, but toxicity was greater for docetaxel *[Jones,*