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

L. Amrein, M. Loignon, R. Aloyz, L. Panasci. *MCETC, LDI, Department of Medicine, Cancer Pharmacology, Montreal, Canada*

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

H. Thomas[1], C. Calabrese[1], S. Canan[2], Z.K.S. Hostomsky[2], K. Maegley[2], D. Newell[1], D. Skalitzki[2], L. Wang[1], N. Curtin[1]. [1]*University of Newcastle upon Tyne, Northern Institute for Cancer Research, Medical School, Framlington Place, Newcastle upon Tyne, UK;* [2]*Pfizer GRD, La Jolla, CA, USA*

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

M.A. Sabatino[1], C. Geroni[2], M. Broggini[1]. [1]*Istituto di Ricerche Farmacologiche Mario Negri, Laboratory of Molecular Pharmacology, Milano, Italy;* [2]*Nerviano Medical Sciences, Oncology, Nerviano, Italy*

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

U. Raju, M. Koto, X. Lu, J. Ajani, W. Hittelman. *University of Texas M.D. Anderson Cancer Center, Houston, TX, USA*

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