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Poster Sessions

Antimetabolites

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The role of thymidine kinase and thymidylate synthase in the response of tumor cells to the suicide prodrug 2′-F-ara-deoxyuridine

P. Phatak[1], C.M. Daniels[1], A.F. Shields[2], J.M. Collins[2], P.M. LoRusso[3], A.M. Burger[1]. [1]*Karmanos Cancer Institute Wayne State University, Pharmacology, Detroit, USA;* [2]*National Cancer Institute, Developmental Therapeutics Program, Rockville, USA;* [3]*Karmanos Cancer Institute Wayne State University, Internal Medicine Division of Hematology and Oncology, Detroit, USA*

Prodrug approaches in cancer drug development are aimed to enhance the tumor selectivity of anticancer agents. 2′-F-ara-deoxyuridine (FAU) is a suicide prodrug which has to be phosphorylated by thymidine kinase (TK1), and methylated by thymidylate synthase (TS) in tumor cells before it can be incorporated into their DNA causing cancer cell-specific death. Therapeutic approaches exist which target the enzyme TS such as 5-fluorouracil (5-FU). Resistance to 5-FU stems from an up-regulation in TS. FAU could offer a targeted treatment to patients with high levels of TS. Studies of TS protein and mRNA revealed an inverse relationship between TS expression and a cell's responsiveness to 5-FU.

To assess whether sensitivity of cancer cells to FAU is related to TK1 or TS levels, we analyzed protein expression by immunohistochemistry and mRNA expression by RT-PCR in a panel of eight human tumor cell lines including breast (MCF-7, MDA-MB-231, Hs578T), colon (HT29, HCT116), lung (H23), leukemia (K562) and ovarian (AG6000) cancers. TK1 and TS activity data were extracted from the National Cancer Institute's Developmental Therapeutics Program target database. mRNA expression of TK1 and TS was quantified in relation to the housekeeping gene hypoxantine phosphoribosyl-transferase and relative expression intensities (RI) were determined. Protein expression was analyzed by assigning an intensity score (0 to 3+). TK1, TS expression and enzyme activity were compared to FAU's cytotoxic activity as determined by 5 day methyltetrazolium (MTT) assays.

We found that those cells which had low TS levels were resistant to FAU, while those with high amounts proved sensitive. E.g. H23 cells that strongly express TS mRNA (RI 2.6) and protein (3+), they also exhibit exquisitely high TS activity and are very sensitive to FAU. The inhibitory concentration (IC) 50% of FAU in H23 was 800 nM, whereas all lines with low TS protein or mRNA such as Hs578T (RI 1.6) and MCF-7 (1+) were insensitive (IC50 >100 μM).

In contrast, TK1 levels appear not to be a major rate limiting determinant of FAU cytotoxicity because FAU sensitive H23 cells exhibit low TK1 (RI 0.5) activity, whereas cell lines with higher TK1 activity such as HCT116 (RI 3) cells were not responsive to FAU. However, K562 leukemia cells with the highest TK activity (RI 5.8), high TS protein levels (2+) and intermediate TS activity were also partially responsive to FAU (IC50 = 10 μM). These results indicate that low TK1 expression is sufficient for effective phosphorylation of FAU, but that high TK1 levels can increase FAU cytotoxicity.

Our data suggest that FAU may be useful as an alternative therapy for cancer patients over expressing TS (e.g. when resistant to 5-FU) and that both TK and TS at the protein and mRNA level should be evaluated as markers of response in early clinical trials aimed to investigating the therapeutic potential of FAU.

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The nucleoside analogue sapacitabine (CYC682) synergises with histone deacetylase inhibitors in multiple tumour types

I.N. Fleming[1], A.K. Choudhary[1], R.H. Mackay[1], S.R. Green[1], S. Davis[1]. [1]*Cyclacel Ltd, Translational Biology, Dundee, United Kingdom*

Background: CNDAC (1-(2-C-cyano-2-deoxy-b-D-arabino-pentofuranosyl)-cytosine) is a 2′-deoxycytidine analogue that hinders DNA synthesis by inhibiting DNA polymerase and strand elongation. In contrast to gemcitabine and cytarabine which block cells in S-phase, CNDAC induces an S-phase delay, followed by arrest in G2. This is a function of the cyano group incorporated into the ribose moiety of CNDAC, which promotes the induction of single strand DNA breaks after it has been integrated into DNA, making CNDAC unique among this class of agents. Sapacitabine (CYC682) is a palmitoyl-derivative of CNDAC that is orally administered to patients and undergoing phase II clinical trials. Addition of the fatty-acid group to CNDAC reduces inactivation of the nucleoside analogue by deamination, and enhances activity as shown in preclinical studies.

Histone deacetylase (HDAC) inhibitors are a relatively new class of anti-cancer therapeutics that induce apoptosis by modulating gene expression of proteins in multiple pathways. In this study we investigated whether HDAC inhibitors could enhance the pro-apoptotic effect of CNDAC.

Methods: In vitro synergy was assessed using the Chou and Talalay median effect model and determined for both sequential and concomitant treatments. The molecular basis for these synergistic interactions was explored using flow cytometry-based techniques and western blot analysis.

Results: Synergistic cell killing was observed in a variety of tumour types, including acute myeloid leukaemia (AML), non-hodgkins lymphoma, cutaneous T-cell lymphoma and non-small cell lung cancer. Mode of action analysis in AML cells demonstrated that combining CNDAC with suberoylanilide hydroxamic acid (SAHA) or valproate induced a synergistic time- and dose-dependent increase in apoptotic cells. The schedule of administration was not critical, since different treatment regimes produced synergistic increases in apoptosis. Western blotting analysis identified changes in the levels of select pro- and anti-apoptotic proteins that are consistent with increased apoptosis.

Conclusions: CNDAC and HDAC inhibitors demonstrated synergy in several cell lines derived from diverse tumour types. CNDAC-induced apoptosis was enhanced by HDAC inhibitors due to selective modulation of proteins that regulate apoptosis. These agents have non-overlapping toxicities and represent a combination with significant promise that is worthy of investigation in the clinic.

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Target identification permits rational development of the prodrug GMX1777 for the treatment of melanoma

M. Watson[1], A. Roulston[1], H. Chan[1], D. Goulet[1], D. Bedard[1], E. Turcotte[1], G. Shore[1], J. Viallet[2], P. Beauparlant[1]. [1]*Gemin X Pharmaceuticals, Biology, Montreal, Quebec, Canada;* [2]*Gemin X Pharmaceuticals, Clinical Affairs, Malvern, PA, USA*

GMX1777 is a prodrug of the cyanoguanidinopyridine GMX1778 and demonstrates strong anti-tumor activity *in vitro* and *in vivo* across a variety of human tumor cell lines and in mouse xenograft models. Based on pre-clinical data GMX1777 was tested in a Phase I trial as a 24 hour infusion every three weeks in patients with solid tumors. Recently, we identified that GMX1778 is a potent and specific inhibitor of nicotinamide phosphoribosyltransferase (NAMPRT), the rate limiting enzyme in the biosynthesis of nicotinamide adenine dinucleotide (NAD^+), and results in NAD^+ depletion in tumor cells. Because DNA damage repair pathways are extremely dependent on NAD^+ levels, we tested a wide spectrum of DNA-damaging agents for synergistic cell killing in combination with GMX1778 *in vitro*. The DNA-alkylating agent temozolomide exhibited the greatest synergy with GMX1778 treatment. The clinical development of GMX1777 in combination with temozolomide is currently being explored for the treatment of melanoma patients. To this end, pre-clinical development of a new dosing schedule that would accommodate a combination treatment with temozolomide resulted in a dosing schedule of five daily 3 hour infusions of GMX1777. This dosing schedule was as effective in mouse xenograft models as the previous 24 hour infusion schedule. Additional pre-clinical results indicate that cells with high melanin content exhibit increased retention of GMX1778. *In vitro* data suggests that this characteristic can be exploited in order to provide increased sensitization of melanin-containing melanoma cells to temozolomide treatment. Overall, these results illustrate the impact that rational drug combinations utilizing targeted agents can have on the clinical development of novel cancer therapeutics.

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Plasma pharmacokinetics of CP-4055 in patients with acute myeloid leukaemia at the recommended phase II dose

S. Hagen[1], S.M. O'Brien[2], T.F. Jacobsen[3], H.M. Kantarjian[4], M.L. Sandvold[5], F. Giles[6]. [1]*Clavis Pharma, CMC, Oslo, Norway;* [2]*UT MD Anderson Cancer Center, Dept. of Leukemia, Houston, USA;* [3]*Clavis Pharma, Clinical Hematology, Oslo, Norway;* [4]*Ut MD Anderson Cancer Center, Dept. of Leukemia, Houston, USA;* [5]*Clavis Pharma, Preclinical R&D, Oslo, Norway;* [6]*UTSCSA, Division of Hematology and Medical Oncology, San Antonio, TX, USA*

Background: CP-4055 (ELACYT™, cytarabine 5′-elaidic acid ester) is a novel cytotoxic nucleoside analogue. While CP-4055 has similar mechanisms of action to cytarabine, it is, unlike cytarabine (ara-C) independent of nucleoside transporters for cellular uptake. The study was conducted as part of a phase I dose escalation study. The aim was to determine the plasma levels of CP-4055 and its metabolites, ara-C and