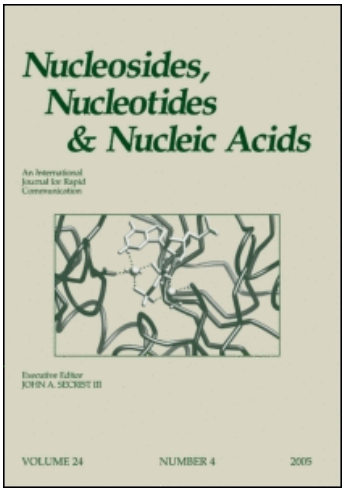

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THE ACTIVITY OF THE LIPOPHILIC NUCLEOSIDE DERIVATIVES ELACYTARABINE AND CP-4126 IN A PANEL OF TUMOR CELL LINES RESISTANT TO NUCLEOSIDE ANALOGUES

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□ *The clinical activity of pyrimidine analogues (araC and gemcitabine) is impaired by different mechanisms of resistance and several efforts to overcome this problem have been undertaken. Elacytarabine (CP-4055, araC-5′elaidic acid ester) and CP-4126 (gemcitabine-5′elaidic acid ester) are lipophilic fatty acid derivatives of the nucleoside analogues araC and gemcitabine, respectively, that are currently investigated in clinical trials in solid tumors and hematological malignancies. Here, we present results on the activity of elacytarabine and CP-4126 in a panel of tumor cell lines that are resistant to araC and gemcitabine and we discuss the potential use of these agents in the treatment of patients with drug resistance phenotypes. We conclude that elacytarabine and CP-4126 are active in cells with deficient nucleoside membrane transport and altered mismatch repair. These results should be taking into consideration for future clinical development of elacyatrabine and CP-4126.*

KEYWORDS Pyrimidine analogues; tumor cells; lipophilic fatty acids; elacytarabine

INTRODUCTION

The clinical activity of pyrimidine analogues (araC and gemcitabine) is impaired by the appearance of resistance.[1] A primary mechanism arises from an insufficient intracellular concentration of nucleoside analogue triphosphates, which may result from inefficient cellular uptake of the nucleoside analogues, reduced levels of activating enzymes, increased degradation by increased 5′-nucleotidases or cytidine deaminase activity, or expansion of the deoxynucleoside triphosphate (dNTP) pools. Resistance may also be related to an overexpression of ribonucleotide reductase (RNR) leading to increased dNTP pools preventing dFdCTP to be incorporated, or leading to

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an increased DNA repair. Furthermore, increased RNR can not be inhibited adequately by dFdCDP. Finally, drug resistance may be a consequence of a defective induction of apoptosis.

Different efforts have been made to overcome the drug resistant problem with nucleoside analogues, such as strategies aiming to increase the intracellular concentrations of active compounds. Clavis Pharma has developed fatty acid derivatives of araC (elacytarabine) and gemcitabine (CP-4126). Once inside cells, these compounds are metabolized to nucleoside analogues by hydrolytic cleavage of the lipid vector and are then phosphorylated by different kinases to their triphosphate derivatives which exert their cytotoxic activity. Lipid vector-nucleoside analogue derivatives have the ability to cross cell membranes independently of nucleoside transport proteins. Therefore, their administration results in higher cellular levels of the active compound for longer periods, causing prolonged inhibition of DNA synthesis when compared with the parent molecule.[2] The derivatives are not deaminated prior to hydrolysis. Elacytarabine was investigated in a Phase I clinical study in patients with solid tumors[3] with a favourable safety profile, and it is currently in Phase II trials both in haematology and solid tumors. CP-4126 is currently undergoing clinical trials with both oral (Phase I) and intravenous administration (Phase II).

Here, we will describe the potential of elacytarabine and CP-4126 to overcome resistance of cancer cells to araC and gemcitabine.

RESISTANCE TO NUCLEOSIDE ANALOGUES DUE TO DEFICIENT DRUG UPTAKE OR DRUG METABOLISM

The cytotoxic activity of elacytarabine and CP-4126 was tested in cell lines with deficiencies in the uptake and metabolism of araC and gemcitabine, known to be resistant to both drugs. In cancer cells with a deficiency in nucleoside transport (the CEM-araC/8C cell line model), elacytarabine and CP-4126 were able to reverse drug resistance to their respective parental compounds.[4] These tumor cells are highly resistant to araC and gemcitabine (IC_{50} values were in the high μM/ low mM range for both compounds), but were sensitive to elacytarabine and CP-4126 with IC_{50} values in the nM range. The increase in cytotoxic activity observed after treatment of CEM/araC/8C cells with elacytarabine and CP-4126, relative to their parental drugs, was principally due to an increase in apoptosis induction compared to the parental drugs. These results were further confirmed by inhibition of the equlibrative nucleoside transport activity in CCFR-CEM parental cells with nitrobenzylthioinosine (30 μM for 30 minutes), a known inhibitor of nucleoside transport and inducer of resistance to araC and gemcitabine. As expected, NBTI treatment minimally affected the growth inhibitory activity of elacytarabine and CP-4126, since the IC_{50} values were still in the nM range

compared to μM values obtained with araC and gemcitabine. Similar results were seen in a range of cell lines.[5] When using cell line models with deficient expression of the deoxycytidine kinase enzyme, no major reversion of the resistant phenotype was observed.[2] In fact, elacytarabine and CP-4126 will release the nucleoside analogues inside cells and these needs to be phosphorylated firstly by dCK to the corresponding nucleoside monophosphate and subsequently to triphosphates in order to induce the cytotoxic activity.

RESISTANCE TO GEMCITABINE DUE TO ALTERED INTRACELLULAR DRUG TARGETS

It was previously reported that gemcitabine also induces cell cytotoxicity by inhibition of ribonucleotide reductase (RNR) and that over-expression of the enzyme is related to resistance to this agent.[6] Unpublished data demonstrated that the MCF7–1K, overexpressing the M1 subunit of RNR and resistant to gemcitabine,[7] are slightly more sensitive to CP-4126 indicating that tumor cell cytotoxicity induced by this compound is less influenced by overexpression of RNR-M1. Elacytarabine is equally active in the MCF7 parental line and the MCF-1K subline, indicating an independence of the ribonucleotide reductase pathway for the activity of this compound.

RESISTANCE TO GEMCITABINE DUE TO ALTERED DNA REPAIR

The sensitivity profiles for elacytarabine, CP-4126, araC, and gemcitabine were firstly evaluated in a panel of human mismatch DNA repair (MMR) proficient and deficient colorectal cancer cell lines including SW480 (wildtype for hMLH1 and hMSH2 genes), Caco-2 (wildtype for hMLH1 and hMSH2 genes), HT-29 (wildtype for hMLH1 and hMSH2 genes), SW48 (mutation in the hMLH1 gene), Lovo (mutation in hMSH2 gene), and LS174T (deficient expression of hMLH1 and hMSH2 genes). The studies demonstrated that MMR-deficient cells (SW48, HCT116, LS174T, and Lovo) were significantly more sensitive to the action of the four compounds than MMR-proficient cells (SW480, Caco-2, and HT29; Table 1). The mean IC_{50} values for each compound in MMR-deficient cells were in the low nM range while in MMR-proficient cells these IC_{50} values were in the μM range for araC and elacytarabine, but still in the nM range for gemcitabine and CP-4126 (Table 1). In order to confirm these results, we used an isogenic model in which the HCT116 cell line was compared with a variant with chromosome 3 containing the hMLH1 gene (kindly donated by Dr D. Boothman). HCT116-chr3+ cells were more resistant to drug treatment than HCT116 cells (Table 1), with IC_{50} values that were 3- to 7.5-fold higher than those observed in HCT116 cells (Table 1). No differences between the cell lines were observed when they were treated with CP-4126. Moreover, cell cycle (Figure 1a) and apoptotic (Figure 1b) studies demonstrated that the HCT-116chr3+

TABLE 1 Sensitivity profiles of different MMR-proficient and MMR-deficient tumor cell lines and one p53 deficient cell line to elacytarabine and CP-4126

	araC[a]	Elacytarabine	Gemcitabine	CP-4126
SW480	1.6 ± 0.7[a]	2.4 ± 0.7	0.015 ± 0.005	0.04 ± 0.005
Caco-2	>100	35 ± 7	>100	30 ± 14
HT29	>100	36 ± 8	0.075 ± 0.03	0.08 ± 0.03
SW48	0.04 ± 0.01	0.04 ± 0.01	0.005 ± 0.0008	0.008 ± 0.001
LS174T	0.066 ± 0.04	0.04 ± 0.02	0.0026 ± 0.0004	0.0024 ± 0.0008
LOVO	0.014 ± 0.0008	0.15 ± 0.05	0.008 ± 0.0009	0.003 ± 0.001
HCT116	0.027 ± 0.008	0.055 ± 0.006	0.0058 ± 0.001	0.004 ± 0.001
HCT116-chr3+	0.21 ± 0.07	0.28 ± 0.1	0.017 ± 0.01	0.003 ± 0.001
HCT116-p53-/-	0.56 ± 0.2	0.58 ± 0.2	0.063 ± 0.06	0.043 ± 0.03

[a]Mean $\pm$ SD of IC_{50} values expressed in μM. IC_{50} values were obtained from dose response curves assessed by MTT assay after exposure to the drugs for 72 hours and are means of three separate experiments each of which were performed in triplicate.

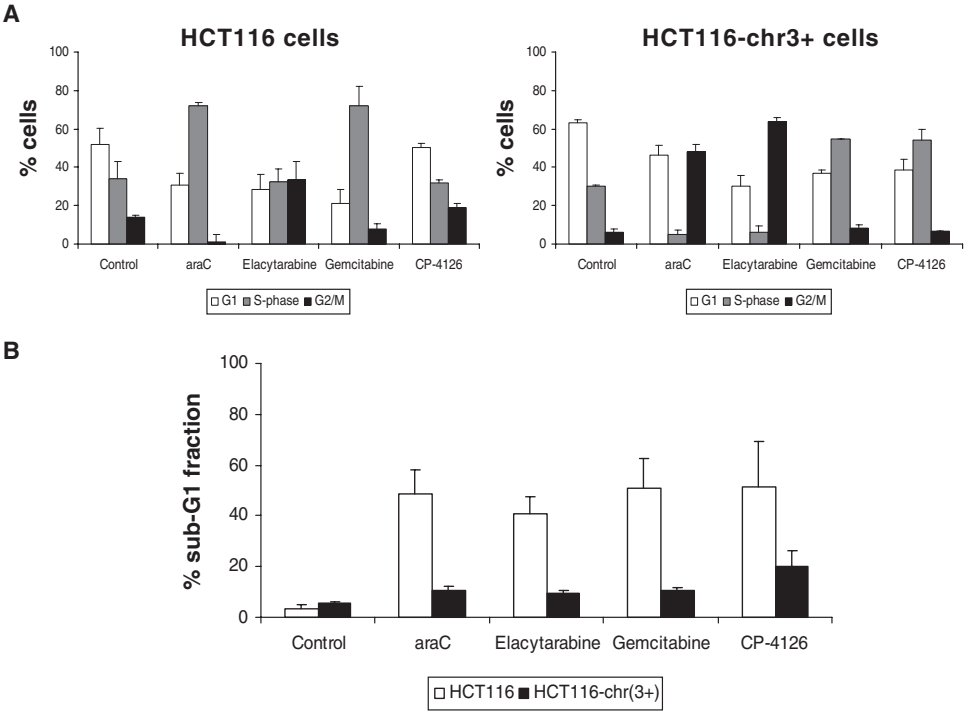

FIGURE 1 Flow cytometric detection of cell cycle distribution (A) and apoptosis (B) (sub-G1 evaluation) after 48 hours exposure to araC, elacytarabine, gemcitabine and CP-4126 in a MMR-deficient (HCT116 colorectal cell line) and a MMR-proficient (HCT116-chr3 + colorectal cell line. For analysis of cell cycle distribution and apoptotic sub-G1 peak, HCT116 and HCT-chr3 + cells were treated with 1 μM of araC, gemcitabine, CP-4055 and CP-4126 for 48 hours. After drug-exposure, 10^6 cells/ml were resuspended in 2 ml of propidium iodide solution (50 μl/ml), incubated at 4°C for 1 hour and then analyzed by flow cytometry in a FACScalibur (Becton Dickinson, San Jose, U.S.A.) using FlowJo 7.2.2 (Treestar, Ashland, TX, USA).

cell line showed lower cell cycle induced perturbations and apoptotic rates compared to HCT116 parental cells, in line with the generally lower sensitivity observed in the MMR-proficient cell lines. Interestingly, the S-Phase cell cycle arrest persisted for 48 hours after treatment with CP-4126. Moreover, CP-4126 induced the highest degree of apoptosis of the compounds tested, confirming the higher sensitivity observed in the cell growth inhibitory tests.

We also tested the HCT116-p53-/- subline with no expression of the p53 protein. In this cell line, both alleles of p53 have been deleted through homologous recombination. HCT116-p53-/- cells displayed 2- to 10-fold resistance to the four compounds compared to the HCT116 cells (Table 1).

RESISTANCE DUE TO EXPRESSION OF THE MDR PHENOTYPE

Although the expression of efflux pumps is not related to resistance to nucleoside analogues the lipophilic prodrugs might be a substrate for these pumps. Therefore elacytarabine was tested in cells expressing a multidrug resistant phenotype including CCRF-CEM (T-lymphoblastic leukaemia) and its subline CEM/VLB100 (over-expression of P-glycoprotein)[8] NCI-H69/P (small cell lung cancer cell) and its subline NCI-H69/LX4 (overexpression of P-glycoprotein),[9] and COR-L23/P (large cell lung cancer), and its subline COR-L23/R (overexpression of MRP1 protein). As expected, no differences were observed between parental and MDR sublines (Table 2).

DISCUSSION

In this article, we describe the cytotoxic activity of elacytarabine and CP-4126 in different drug resistant tumor models in comparison to araC and gemcitabine. We observed that both compounds were able to overcome drug resistance to nucleoside analogues in two main settings: deficiency of nucleoside transport and altered DNA repair.

TABLE 2 Sensitivity profiles of different MDR tumor cell lines to elacytarabine and araC

	MDR phenotype	araC[a]	Elacytarabine
CEM		9 ± 7	10 ± 2
CEM/VLB	P-Gp	8 ± 7	11 ± 2
COR-L23/P		29 ± 25	65 ± 20
COR-L23/R	P-Gp	53 ± 25	105 ± 30
H69P		177 ± 66	296 ± 2
H69/LX4	MRP1	119 ± 41	237 ± 59

[a]Mean $\pm$ SD of IC_{50} values expressed in nM. IC_{50} values were obtained from dose response curves assessed by MTT assay after 6 days of drug exposure, and are means of three separate experiments each of which were performed in triplicate.

In the first setting, elacytarabine and CP-4126 retain cytotoxic activity even when the nucleoside transporter hENT1 is absent or inhibited chemically, a condition known to confer resistance to other pyrimidine analogues. This higher cytotoxic activity was also reflected by an increase in cell cycle perturbations and apoptotic rates induced by drug treatment. Moreover, the formation of active triphosphate of gemcitabine or araC is not affected in transport deficient cells after drug treatment.[10] The clinical relevance of hENT1 expression for the prediction of non-response to araC or gemcitabine containing treatments has been reported for pancreatic cancer,[11–13] non-small cell lung cancer,[14,15] and acute myeloid leukemia (AML).[16,17] In these clinical subsets, the expression of hENT1 has been shown to be low in 30% to 50% of the patients. Hence, we can expect that this finding is important for the use of CP-4126 and elacytarabine as replacements of araC and gemcitabine, respectively, in the treatment of patients with nucleoside transporter deficient tumours.

Concerning DNA repair, MMR-deficient cells are clearly more sensitive to the action of nucleoside analogues than the MMR-proficient cell liens. This was seen in a panel of three proficient and four deficient MMR phenotype cell lines. Since differences in sensitivity might also be due to altered patterns of drug-activating or -inactivating enzymes, we also tested a pair of isogenic cell lines differing in the status of MMR repair system, in which a similar difference was found. Interestingly, the cell growth inhibition activity of CP-4126 was not affected by the MMR-status in this specific model as observed by MTT assays, apoptosis and cell cycle measurements. DNA repair pathways have been shown to be important for resistance to platinum compounds both in the preclinical and clinical setting,[18] in which the mismatch repair deficiency is important and can both be inherited or developed by epigenetic silencing. This has been shown in ovarian, endometrial, gastric and colorectal carcinoma, among others. Mismatch repair status and in particular hMLH1 deficiency plays a role in the radiosensitization effects observed with gemcitabine.[19] The loss of MMR function has also been suggested to contribute to refractory and relapsed AML.[20] Loss of MMR function induces resistance to a certain number of chemotherapeutic drugs. In combination studies in vitro, gemcitabine was found to affect both the platinum-adduct formation and repair in cell lines with differences in mismatch repair systems.[21] SNPs of MMR genes have a potential value as predictors for clinical response to chemoradiotherapy and as prognostic markers for tumor resectability and overall survival of patients with resectable pancreatic cancer.[22] Thus, our results showing that MMR deficient cells, known to be resistant to a number of chemotherapeutic agents, are more sensitive to nucleoside analogues present a new therapeutic opportunity to stratify and treat this specific subset of patients with this drug family. Moreover, the finding that CP-4126 also remains active in mismatch repair proficient cells

seems to be even more relevant, since this compound would kill cells with both MMR phenotypes.

In conclusion, our results demonstrate that elacytarabine and CP-4126 are active in cells with deficient nucleoside transport and altered MMR phenotypes. These facts should be taken into consideration in the future clinical development of elacytarabine and CP-4126.

REFERENCES

1. Galmarini, C.M.; Mackey, J.R.; Dumontet, C. Nucleoside analogues: mechanisms of drug resistance and reversal strategies. *Leukemia* **2001**, 15, 875–890.
2. Bergman, A.M.; Kuiper, C.M.; Noordhuis, P.; Smid, K.; Voorn, D.A.; Comijn, E.M.; Myhren, F.; Sandvold, M.L.; Hendriks, H.R.; Fodstad, O.; Breistol, K.; Peters, G.J. Antiproliferative activity and mechanism of action of fatty acid derivatives of gemcitabine in leukemia and solid tumor cell lines and in human xenografts. *Nucleosides Nucleotides Nucleic Acids* **2004**, 23, 1329–1333.
3. Dueland, S.; Aamdal, S.; Lind, M.J.; Thomas, H.; Sandvold, M.L.; Gaullier, J.M.; Rasch, W. Intravenous administration of CP-4055 (ELACYT) in patients with solid tumours. a phase I study. *Acta Oncol* **2009**, 48, 137–145.
4. Galmarini, C.M.; Myhren, F.; Sandvold. M.L. CP-4055 and CP-4126 are active in ara-C and gemcitabine-resistant lymphoma cell lines. *Br. J. Haematol.* **2009**, 144, 273–275.
5. Breistol, K.; Balzarini, J.; Sandvold, M.L.; Myhren, F.; Martinsen, M.; De Clercq, E.; Fodstad, O. Antitumor activity of P-4055 (elaidic acid-cytarabine) compared to cytarabine in metastatic and s.c. human tumor xenograft models. *Cancer Res.* **1999**, 59, 2944–2949.
6. Bergman, A.M.; Eijk, P.P.; Ruiz van Haperen, V.W.; Smid, K.; Veerman, G.; Hubeek, I.; van den Ijssel, P.; Ylstra, B.; Peters, G.J. In vivo induction of resistance to gemcitabine results in increased expression of ribonucleotide reductase subunit M1 as the major determinant. *Cancer Res.* **2005**, 65, 9510–9516.
7. Jordheim, L.P.; Guittet, O.; Lepoivre, M.; Galmarini, C.M.; Dumontet, C. Increased expression of the large subunit of ribonucleotide reductase is involved in resistance to gemcitabine in human mammary adenocarcinoma cells. *Mol. Cancer Ther.* **2005**, 4, 1268–1276.
8. Beck, W.T.; Cirtain, M.C.; Lefko, J.L. Energy-dependent reduced drug binding as a mechanism of Vinca alkaloid resistance in human leukemic lymphoblasts. *Mol. Pharmacol.* **1983**, 24, 485–492.
9. Twentyman, P.R.; Fox, N.E.; Wright, K.A.; Bleehen, N.M. Derivation and preliminary characterisation of adriamycin resistant lines of human lung cancer cells. *Br. J. Cancer* **1986**, 53, 529–537.
10. Adema, A.; Smid, K.; Losekoot, N.; Myhren, F.; Sandvold, M.L.; Peters, G.J. Fatty acid derivatives of cytarabine and gemcitabine, CP-4055 and CP-4126, show a prolonged cellular retention compared to the parent drug. Proceedings of the 99th Annual Meeting of the American Association for Cancer Research. Abstract nr 5740, **2008**.
11. Giovannetti, E.; Del Tacca, M.; Mey, V.; Funel, N.; Nannizzi, S.; Ricci, S.; Orlandini, C.; Boggi, U.; Campani, D.; Del Chiaro, M.; Iannopollo, M.; Bevilacqua, G.; Mosca, F.; Danesi, R. Transcription analysis of human equilibrative nucleoside transporter-1 predicts survival in pancreas cancer patients treated with gemcitabine. *Cancer Res.* **2006**, 66, 3928–3935.
12. Marechal, R.; Mackey, J.R.; Lai, R.; Demetter, P.; Peeters, M.; Polus, M.; Cass, C.E.; Young, J.; Salmon, I.; Deviere, J.; Van Laethem, J.L. Human Equilibrative nucleoside transporter 1 and human concentrative nucleoside transporter 3 predict survival after adjuvant gemcitabine therapy in resected pancreatic adenocarcinoma. *Clin. Cancer Res.* **2009**, 15, 2913–2919.
13. Farrell, J.J.; Elsaleh, H.; Garcia, M.; Lai, R.; Ammar, A.; Regine, W.F.; Abrams, R.; Benson, A.B.; Macdonald, J.; Cass, C.E.; Dicker, A.F.; Mackey, J.R. Human Equilibrative nucleoside transporter 1 levels predict response to gemcitabine in patients with pancreatic cancer. *Gastroenterology* **2009**, 136, 187–195.
14. Hubeek, I.; Giovannetti, E.; Broekhuizen, A.J. Pastor-Anglada, M.; Kaspers, G.J.; Peters, G.J. Immunocytochemical detection of hENT1 and hCNT1 in normal tissues, lung cancer cell lines, and NSCLC patient samples. *Nucleosides Nucleotides Nucleic Acids* **2008**, 27, 787–793.

15. Oguri, T.; Achiwa, H.; Muramatsu, H.; Ozasa, H.; Sato, S.; Shimizu, S.; Yamazaki, H.; Eimoto, T.; Ueda, R. The absence of human equilibrative nucleoside transporter 1 expression predicts nonresponse to gemcitabine-containing chemotherapy in non-small cell lung cancer. *Cancer Lett.* **2007**, 256, 112–119.
16. Wiley, J.S.; Jones, S.P.; Sawyer, W.H.; Paterson, A.R. Cytosine arabinoside influx and nucleoside transport sites in acute leukemia. *J. Clin. Invest.* **1982**, 69, 479–489.
17. Hubeek, I.; Stam, R.W.; Peters, G.J.; Broekhuizen, R.; Meijerink, J.P.; van Wering, E.R.; Gibson, B.E.; Creutzig, U.; Zwaan, C.M.; Cloos, J.; Kuik, D.J.; Pieters, R.; Kaspers, G.J. The human equilibrative nucleoside transporter 1 mediates in vitro cytarabine sensitivity in childhood acute myeloid leukaemia. *Br. J. Cancer* **2005**, 93, 1388–1394.
18. Martin, L.P.; Hamilton, T.C.; Schilder, R.J. Platinum resistance: the role of DNA repair pathways. *Clin. Cancer Res.* **2008**, 14, 1291–1295.
19. Flanagan, S.A.; Robinson, B.W.; Krokosky, C.M.; Shewach, D.S. Mismatched nucleotides as the lesions responsible for radiosensitization with gemcitabine: a new paradigm for antimetabolite radiosensitizers. *Mol. Cancer Ther.* **2007**, 6, 1858–1868.
20. Mao, G.; Yuan, F.; Absher, K.; Jennings, C.D.; Howard, D.S.; Jordan, C.T.; Gu, L. Preferential loss of mismatch repair function in refractory and relapsed acute myeloid leukemia: potential contribution to AML progression. *Cell Res.* **2008**, 18, 281–289.
21. Peters, G.J.; Van Moorsel, C.J.; Lakerveld, B.; Smid, K.; Noordhuis, P.; Comijn, E.C.; Weaver, D.; Willey, J.C.; Voorn, D.; Van der Vijgh, W.J.; Pinedo, H.M. Effects of gemcitabine on cis-platinum-DNA adduct formation and repair in a panel of gemcitabine and cisplatin-sensitive or -resistant human ovarian cancer cell lines. *Int. J. Oncol.* **2006**, 28, 237–244.
22. Dong, X.; Jiao, L.; Li, Y.; Evans, D.B.; Wang, H.; Hess, K.R.; Abbruzzese, J.L.; Li, D. Significant associations of mismatch repair gene polymorphisms with clinical outcome of pancreatic cancer. *J. Clin. Oncol.* **2009**, 27, 1592–1599.