Antimetabolite Treatment for Pancreatic Cancer

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Abstract

Pancreatic cancer is a deadly and aggressive disease. Less than 1% of diagnosed patients survive 5 years with an average survival time of only 4–8 months. The only option for metastatic pancreatic cancer is chemotherapy where only the antimetabolites gemcitabine and 5-fluorouracil are used clinically. Unfortunately, efforts to improve chemotherapy regimens by combining, 5-fluorouracil or gemcitabine with other drugs, such as cisplatin or oxaliplatin, have not increased cell killing or improved patient survival. The novel antimetabolite zebularine shows promise, inducing apoptosis and arresting cellular growth in various pancreatic cancer cell lines. However, resistance to these antimetabolites remains a problem highlighting the need to discover and develop new antimetabolites that will improve a patient’s overall survival.

Keywords

Chemotherapy; Pancreatic cancer; Gemcitabine; Zebularine; 5FU; Platinum

Introduction

In the United States, pancreatic cancer is the 4th leading cause of cancer death aggressively and silently attacking the patient [1–3]. Pancreatic cancer is only identified in more advanced stages when the patient is symptomatic, as there are no screening tests for this disease [4]. At the time of diagnosis, approximately 85% of the patients have advanced pancreatic cancer resulting in a short median survival time of 4–8 months where less than 1% survive more than 5 years [5,6]. Currently, the best treatment is surgical resection where approximately 20% of patients increase their life span by approximately 2 years [7]. For metastatic pancreatic adenocarcinoma, chemotherapy using gemcitabine (GEMZAR) is currently the only first-line FDA approved treatment [8]. Antimetabolite drugs are designed to stop DNA replication and normal cellular metabolic processes by different mechanisms and have been investigated for almost 70 years [9,10]. Currently, efforts to improve the
treatment for metastatic pancreatic cancer explore using combinations of therapeutic agents as well as searching for new antimetabolite drugs. This review will discuss the different antimetabolite agents used to treat pancreatic cancer, both clinically approved and experimental, their mechanisms of action, and therapy resistance.

5-Fluorouracil

The pyrimidine 5-fluorouracil (5FU) has been under investigation for the treatment of human cancers since 1954 when it was observed that uracil is utilized more efficiently by tumor cells than normal cells [11]. The knowledge that fluorine substitutions of hydrogen in metabolites often resulted in a toxic compound inspired the design of 5FU (Figure 1) and testing as a tumor-inhibiting compound [11–13]. Since its discovery, 5FU has been used as a treatment for many solid tumors such as colon, breast, head and neck cancers, and advanced pancreatic cancer. For 20 years, 5FU was regarded as the only effective drug against advanced pancreatic cancer. However, despite numerous efforts to improve therapy outcomes, the best response rate was approximately 20% [12,14,15].

Mechanism of action

Like uracil, 5FU is salvaged to form 5-fluorouridine and then phosphorylated by nucleoside and nucleotide kinases as well as reduced by ribonucleotide reductase forming three different active metabolites (Figure 2). After incorporation of 5-fluorouridine triphosphate (FUTP) into cellular RNA, RNA processing and post-transcriptional modification can be inhibited [15,16]. During DNA synthesis, 5-fluoro-2'-deoxyuridine monophosphate (FdUMP) inhibits thymidylate synthase resulting in an imbalanced pool of deoxynucleotide triphosphates, particularly decreased deoxythymidine triphosphate (dTTP) and increased deoxyuridine triphosphate (dUTP). Absent dTTP, stalled DNA polymerases can incorporate 5-fluorodeoxyuridine triphosphate (FdUTP) or dUTP which are subsequently recognized as damaged DNA setting up a futile cycle of misincorporation and repair [15,16]. When DNA damage exceeds a cells ability to repair misincorporated FdUTP or dUTP, single strand and double strand breaks accumulate favoring cell death. Given these cellular actions of 5FU, its toxicity is generally considered a function of transport into the cell and metabolism to active metabolites, particularly FdUMP, while resistance occurs when 5FU metabolism is decreased or DNA repair is efficient.

Resistance—One mechanism of 5FU resistance may result from high levels of thymidylate synthase expression in pancreatic cancer patients. Head and neck [17] and gastric [18] cancer patients with low tumoral thymidylate synthase expression exhibited increased sensitivity to 5FU treatment, while a lack of response was seen in advanced colorectal patients [19] with high thymidylate synthase expression. Interestingly, the opposite was observed where node-positive breast [20] and Dukes’ B and C rectal [21] cancer patients with high expression levels of thymidylate synthase responded well to 5FU therapy. It is not currently known why this phenomenon was seen, but 5FU therapy-outcome may be associated with the tumor type that is being treated or with the biome of stress-associated molecules expressed and/or induced. One retrospective study of pancreatic cancer patients found that 5FU resulted in longer survival for patients with low thymidylate synthase expression [22]. Further translational studies are needed to better understand the
role of thymidylate synthase expression and therapy outcome [10,16]. These and other studies on the mechanism of resistance continue and may prove instrumental in understanding resistance leading to better therapeutic design and combinations.

An additional mechanism of resistance is decreased expression 5FU transport into pancreatic cancer cells. In human pancreatic cancer cell lines, the sensitivity to 5FU directly correlated with the expression level of the human equilibrative nucleoside transporter 1 (hENT1) [23]. However, increased median survival time in pancreatic cancer patients treated with 5FU was not significantly different [24]. Additional studies are needed to understand the differences in resistance to 5FU in cell lines as opposed to pancreatic cancer patients.

**Gemcitabine**

Gemcitabine (2’, 2’-difluoro-2’-deoxycytidine, dFdC) was originally considered as an antiviral drug [25], but was later shown to demonstrate anti-cancer activity in both *in vivo* and *in vitro* models of solid and hematological cancers [14,25,26]. Today, gemcitabine is the only FDA approved single chemotherapy agent against metastatic pancreatic cancer, showing a better 1-year survival rate, median survival, and clinical benefit when compared to 5FU [8].

**Mechanism of action**—Gemcitabine is a 2’-deoxycytidine analogue with fluorine substituted for hydrogen at the 2’ position of the furanose ring (Figure 3). Gemcitabine is a broad-spectrum agent, which has different mechanisms of action, depending upon its phosphorylation state (Figure 4) [8,25]. Uptake of Gem into the cell uses both human equilibrative nucleoside transporters (hENTs) and human concentrative nucleoside transporters (hCNTs) [27,28]. Inside the cell, gemcitabine is phosphorylated by deoxycytidine kinase into gemcitabine monophosphate (dFdCMP), which is further converted into its active di- and triphosphate (dFdCDP and dFdCTP) states by nucleotide kinases [29]. Ribonucleotide reductase is inhibited by dFdCDP leading to a reduction in dCTP levels. Reduced dCTP lessens the negative feedback regulation of deoxycytidine kinase and favors the efficient phosphorylation of gemcitabine [30]. The cytotoxic activity of gemcitabine leading to apoptosis is mainly the result of its triphosphate form. DNA polymerase activity is inhibited when dFdCTP is incorporated into the DNA strand leading to a termination of the DNA chain synthesis and single strand breakage [31–33]. Consequently, a depletion of dCTP levels, due to inhibition of ribonucleotide reductase activity, results in the competition of dFdCTP with dCTP leading to an increased incorporation of dFdCTP into the DNA strand [30]. In addition, high intracellular levels of dFdCTP also strongly inhibited dCMP deaminase activity, by directly inhibiting the deaminase as well as indirectly because of the decreased Dctp:dTTP ratio [34].

**Resistance**—It has been shown *in vitro* that low levels of hENT1, leading to limited gemcitabine intracellular uptake, is a mechanism of chemoresistance [23,35,36]. In pancreatic cancer patients, the levels of hENT1 were recently observed to correlation with overall median survival time, where patients with higher levels of hENT1 have better survival rates [24]. Further mechanisms of resistance to gemcitabine observed in cell lines from multiple cancer types resulted from decreases in deoxycytidine kinase activity and
increased ribonucleotide reductase activity [37]. Implications for pancreatic cancer patients regarding activity and expression of these enzymes, however, are still unknown [38].

**Platinum**

Platinum agents are used today in combination therapy regimes with gemcitabine as second line chemotherapy for metastatic pancreatic cancer. Cisplatin (diamminedichloroplatinum, CDDP, PtCl2(NH3)2) is shown in Figure 5 and is an inorganic platinum complex composed of a doubly charged platinum ion, and four ligands-two chloride ions and two amines. Cisplatin is a potent chemotherapy drug discovered in the 1960’s. It is widely used today against a variety of tumors including head and neck, non-small cell lung, stomach and bladder cancers, non-Hodgkin’s lymphoma and sarcomas [39,40], Oxaliplatin (trans-l,1,2-diaminocyclohexane oxalatoplatinum) (Figure 6) is a new platinum agent that is more potent in vitro and has a better toxicity profile compared to cisplatin, as it only needs a small number of DNA adducts to attain the same cytotoxicity profile as cisplatin. In preclinical studies, oxaliplatin shows efficacy in a number of cancer cell lines, which also includes cell lines that are cisplatin resistant [41,42]. This provides hope that with minor modification of these platinum compounds, not only will efficacy increase, but resistance will decrease as well.

**Mechanism of action**—Once taken up into the cells, the chloride ions are lost and replaced with water molecules transforming cisplatin into a reactive species. Loosely bound, the water molecules easily fall off, exposing the platinum ion which readily forms bonds with DNA bases, forming DNA-DNA cross-links and DNA-protein cross-links. These cross-links between bases are usually formed at sites where adenosine and guanine are adjacent on the same DNA strand. It has been speculated that the cis-geometry of cisplatin is important to its anti-tumor activity, as the trans-isomer of cisplatin, transplatin, is inactive [43]. Unlike 5FU, cisplatin chemotherapy arrests cells at the G1, S or G2-M phase of the cell cycle, making this drug efficient in killing cells that are in all stages of the cell cycle [39,44–46].

In oxaliplatin, the two amines and two chloride ions of cisplatin are replaced with diaminocyclohexane and carboxylate compounds, respectively (Figure 6). Similar to cisplatin, once inside the cell, the carboxylate compound is displaced, transforming oxaliplatin into a reactive compound that forms DNA intra-strand cross-links, DNA interstrand cross-links, and DNA-protein cross-links [45]. DNA lesions induced by intrastrand cross-links are formed when the drug binds to two adjacent guanine bases, and to a lesser extent, to adjacent adenosine and guanine bases. Binding of the mismatch repair protein complex to the DNA becomes more difficult due to the conformation of adducts, which may result in poor repair of the lesion. Oxaliplatin has been reported to inhibit TS activity, much like 5FU [44,45].

**Resistance**—There are several mechanisms whereby tumor cells become resistant to both cisplatin and Oxaliplatin. The toxicity of cisplatin and oxaliplatin is reduced in cells with an efficient repair of damaged DNA where enzymes involved in nucleotide excision repair remove the platinum-DNA adducts [39]. The relationship between enhanced platinum
resistance, a decrease in drug sensitivity, and increased DNA repair protein levels has been described [39, 47, 48]. Another mechanism is through a decrease in intracellular platinum concentration resulting from a reduction in drug uptake and an increase of platinum expulsion out of the cell or detoxification by glutathione and metallothionein and an increased level of glutathione and metallothionein has been shown in some cases to correlate with cisplatin resistance [49]. This resistance is not due to only one mechanism, but on a variety of mechanisms targeting various systems [39, 44, 45]. The mechanisms of resistance for cisplatin and oxaliplatin differ from the mechanisms of resistance for gemcitabine resulting in a benefit from combining these agents in a therapeutic regimen.

**Combination therapy with platinum agents**

Cisplatin and oxaliplatin are not used as single agents against pancreatic cancer, but rather, in combination with either gemcitabine or 5FU when treatment with gemcitabine alone has failed. There have been multiple studies on the effects of cisplatin used in combination with gemcitabine. One phase III study showed that compared to patients treated with gemcitabine alone, the overall median survival and progression-free survival of patients on the Gemcitabine-cisplatin combination therapy improved, but did not reach statistical significance [50]. Furthermore in another study, comparable results in patients treated with Gemcitabine alone or in combination with cisplatin were observed [46]. However, they also noted that the combination therapy was more toxic than gemcitabine alone. Nevertheless, studies do show favor for a Gem-cisplatin combination, where disease progression and the median 1-year event-free survival is encouraging [42]. Oxaliplatin has been used in combination with both Gemcitabine and 5FU. One study has shown that patients with inoperable pancreatic cancer tolerated the combination of Gemcitabine with oxaliplatin well and was recorded to be highly effective [51] while a phase II trial showed moderate activity [41]. When in combination with 5FU, clinical benefits were recorded and toxicity levels were acceptable [52]. These platinum agents, when combined with Gemcitabine or 5FU, may be a promising treatment regime for pancreatic cancer patients.

**Zebularine**

Epigenetic changes accompany pancreatic tumorigenesis as well as the acquisition of resistance to chemotherapy [53, 54]. Therapeutic agents that alter the epigenetic state of pancreatic cancer cells are under investigation as cytotoxic agents as well as agents to reverse acquired resistance to first-line agents. Lacking an amino group on the C-4 position of the pyrimidine ring, zebularine ((1-β-D-Ribofuranosyl)-2(1H)-pyrimidinone), a cytidine analogue (Figure 7), was originally developed as a cytidine deaminase inhibitor. It is also a novel DNA methyltransferase (DNMT) inhibitor and unlike other DNMT inhibitors, zebularine is more stable in aqueous solution and is less toxic in vitro and in vivo [55–57]. Continuous exposure of numerous cancer cell lines to zebularine slowed tumor cell growth as compared to normal human fibroblast cell lines indicating its promise as a chemotherapy agent for cancer treatment [58].

**Mechanism of action**—Once inside cells, zebularine is phosphorylated by uridine-cytidine kinase. Nucleotide kinases phosphorylate zebularine monophosphate to form zebularine triphosphate, which is then incorporated into DNA. The 2(1H)-pyrimidinone ring
is important as its incorporation into the DNA strand leads to DNMT1 depletion and DNA methylation inhibition. When zebularine replaces cytosine in a CpG dinucleotide and a DNA methyltransferase attempts to methylate zebularine, an irreversible covalent complex is formed thus inhibiting DNA methylation [58]. In a transgenic mouse model of breast cancer, zebularine slows tumor growth and induces cell death by both necrosis and apoptosis [55]. Other studies show that zebularine decreases levels of DNMT1, DNMT3a, and DNMT3b in breast cancer cell lines [59] as well as DNMT1 and partially DNMT3b in bladder cancer cells [58]. A reduction in DNMT1 and DNMT3b was also shown in the mammary tumors in transgenic mice [55]. The growth inhibition property of zebularine may be due to drug incorporation into the DNA. However, the amount of zebularine in DNA was low in normal cells and growth was minimally affected, while the opposite was seen in cancer cells [58]. Understanding incorporation aspects of this agent may prove useful in developing more effective analogues.

**Zebularine and pancreatic cancer**

Studies have shown that zebularine effectively slows cellular growth in CFPAC-1, a pancreatic cancer cell line, by inducing the p21 and/or p16 genes [58]. The p21 protein in response to DNA damage, directly stops DNA replication and arrests cellular growth. They have also shown a decrease in DNMT1 through the incorporation of the 2(1H)-pyrimidinone ring, as stated above [58]. In addition, studies also showed that zebularine, as a single agent, induced apoptosis and growth arrest by inhibition of DNMT1 in three pancreatic cancer cell lines: YAP C, DAN G and Panc-89 [60]. Though there are minimal studies showing the potential use of zebularine in pancreatic cancer, initial reports show promise for the use of zebularine in treating pancreatic cancer. More studies, however, are needed to fully test the full potential of zebularine *in vivo*.

**Conclusion**

The only effective treatment option available for patients with advanced metastatic pancreatic adenocarcinoma remains the antimetabolite gemcitabine. Despite efforts to improve therapy regimens by using 5FU or Gem in combination with alkylating agents, the prognosis for treating metastatic pancreatic cancer remains bleak. Therefore, it is imperative to continue studying and developing novel antimetabolite agents, such as zebularine, to improve treatment options and improve overall survival rates.

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**References**

1. Bockman DE, Büchler M, Beger HG. Interaction of pancreatic ductal carcinoma with nerves leads to nerve damage. Gastroenterology. 1994; 107:219–230. [PubMed: 8020665]

*Chemotherapy (Los Angel)*. Author manuscript; available in PMC 2015 July 07.
2. Li D, Xie K, Wolff R, Abbruzzese JL. Pancreatic cancer. Lancet. 2004; 363:1049–1057. [PubMed: 15051286]

3. Warshaw AL, Fernández-del Castillo C. Pancreatic carcinoma. N Engl J Med. 1992; 326:455–465. [PubMed: 1732772]

4. Moss RA, Lee C. Current and emerging therapies for the treatment of pancreatic cancer. Onco Targets Ther. 2010; 3:111–127. [PubMed: 20856847]

5. Galloway NR, Aspe JR, Sellers C, Wall NR. Enhanced antitumor effect of combined gemcitabine and proton radiation in the treatment of pancreatic cancer. Pancreas. 2009; 38:782–790. [PubMed: 19506533]

6. Palmer DH, Stocken DD, Hewitt H, Markham CE, Hassan AB, et al. A randomized phase 2 trial of neoadjuvant chemotherapy in resectable pancreatic cancer: gemcitabine alone versus gemcitabine combined with cisplatin. Ann Surg Oncol. 2007; 14:2088–2096. [PubMed: 17453298]

7. Kleeff J, Michalski C, Friess H, Büchler MW. Pancreatic cancer: from bench to 5-year survival. Pancreas. 2006; 33:111–118. [PubMed: 16868475]

8. Toschi L, Finocchiaro G, Bartolini S, Gioia V, Cappuzzo F. Role of gemcitabine in cancer therapy. Future Oncol. 2005; 1:7–17. [PubMed: 16555971]

9. Shewach DS, Lawrence TS. Antimetabolite radiosensitizers. J Clin Oncol. 2007; 25:4043–4050. [PubMed: 17827452]

10. Kaye SB. New antimetabolites in cancer chemotherapy and their clinical impact. Br J Cancer. 1998; 78(Suppl 3):1–7. [PubMed: 9717984]

11. Rutman RJ, Cantarow A, Paschkis KE. Studies in 2-acetylaminofluorene carcinogenesis. I. The intracellular distribution of nucleic acids and protein in rat liver. Cancer Res. 1954; 14:111–114. [PubMed: 13126944]

12. Thomas DM, Zalcberg JR. 5-fluorouracil: a pharmacological paradigm in the use of cytotoxics. Clin Exp Pharmacol Physiol. 1998; 25:887–895. [PubMed: 9807659]

13. Heidelerberger C, Chaudhuri NK, Danenberg P, Moooren D, Griesbach L, et al. Fluorinated pyrimidines, a new class of tumour-inhibitory compounds. Nature. 1957; 179:663–666. [PubMed: 13418758]

14. Burris HA 3rd, Moore MJ, Andersen J, Green MR, Rothenberg ML, et al. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. J Clin Oncol. 1997; 15:2403–2413. [PubMed: 9196156]

15. Noordhuis P, Holwerda U, Van der Wilt CL, Van Groeningen CJ, Smid K, et al. 5-Fluorouracil incorporation into RNA and DNA in relation to thymidylate synthase inhibition of human colorectal cancers. Ann Oncol. 2004; 15:1025–1032. [PubMed: 15205195]

16. Longley DB, Harkin DP, Johnston PG. 5-fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer. 2003; 3:330–338. [PubMed: 12724731]

17. Johnston PG, Mick R, Recant W, Behan KA, Dolan ME, et al. Thymidylate synthase expression and response to neoadjuvant chemotherapy in patients with advanced head and neck cancer. J Natl Cancer Inst. 1997; 89:308–313. [PubMed: 9048835]

18. Lenz HJ, Leichman CG, Danenberg KD, Danenberg PV, Groshen S, et al. Thymidylate synthase mRNA level in adenocarcinoma of the stomach: a predictor for primary tumor response and overall survival. J Clin Oncol. 1996; 14:176–182. [PubMed: 8558194]

19. Leichman LH, Leichman CG, Groshen S, Danenberg S, Danenberg K, et al. Quantitation of intratumoral thymidylate synthase expression predicts for resistance to protracted infusion of 5-fluorouracil and weekly leucovorin in disseminated colorectal cancers: preliminary report from an ongoing trial. Eur J Cancer. 1995; 31:1306–1310. [PubMed: 7577041]

20. Pestalozzi BC, Gelber RD, Goldhirsch A, Gusterson BA, Trihia H, et al. Prognostic importance of thymidylate synthase expression in early breast cancer. J Clin Oncol. 1997; 15:1923–1931. [PubMed: 9164203]

21. Johnston PG, Fisher ER, Rockette HE, Fisher B, Wolmark N, et al. The role of thymidylate synthase expression in prognosis and outcome of adjuvant chemotherapy in patients with rectal cancer. J Clin Oncol. 1994; 12:2640–2647. [PubMed: 7989939]
22. Hu YC, Komorowski RA, Graewin S, Hostetter G, Kallioniemi OP, et al. Thymidylate synthase expression predicts the response to 5-fluorouracil-based adjuvant therapy in pancreatic cancer. Clin Cancer Res. 2003; 9:4165–4171. [PubMed: 14519641]
23. Tsujie M, Nakamori S, Nakahira S, Takahashi Y, Hayashi N, et al. Human equilibrative nucleoside transporter 1, as a predictor of 5-fluorouracil resistance in human pancreatic cancer. Anticancer Res. 2007; 27:2241–2249. [PubMed: 17695509]
24. Greenhalf W, Ghaneh P, Neoptolemos JP, Palmer DH, Cox TF, et al. Pancreatic cancer hENT1 expression and survival from gemcitabine in patients from the ESPAC-3 trial. J Natl Cancer Inst. 2014; 106:1347–1349. [PubMed: 24931456]
25. Mini E, Nobili S, Caciagli B, Landini I, Mazzei T. Cellular pharmacology of gemcitabine. Ann Oncol. 2006; 17(Suppl 5):v7–12. [PubMed: 16807468]
26. Giovannetti E, Mey V, Danesi R, Mosca I, Del Tacca M. Synergistic cytotoxicity and pharmacogenetics of gemcitabine and pemetrexed combination in pancreatic cancer cell lines. Clin Cancer Res. 2004; 10:2936–2943. [PubMed: 15131028]
27. Ueno H, Kiyosawa K, Kaniwa N. Pharmacogenomics of gemcitabine: can genetic studies lead to tailor-made therapy? Br J Cancer. 2007; 97:145–151. [PubMed: 17595663]
28. Bachet JB, Marechal R, Van Laethem JL. Treatment of pancreatic cancer: what can we really predict today? Cancers (Basel). 2011; 3:675–699. [PubMed: 24212636]
29. Heinemann V, Hertel LW, Grindey GB, Plunkett W. Comparison of the cellular pharmacokinetics and toxicity of 2′,2′-difluorodeoxycytidine and 1-beta-D-arabinofuranosylcytosine. Cancer Res. 1988; 48:4024–4031. [PubMed: 3383195]
30. Heinemann V, Xu YZ, Chubb S, Sen A, Hertel LW, et al. Inhibition of ribonucleotide reduction in CCRF-CEM cells by 2′,2′-difluorodeoxycytidine. Mol Pharmacol. 1990; 38:567–572. [PubMed: 2233693]
31. Gandhi V, Plunkett W. Modulatory activity of 2′,2′-difluorodeoxycytidine on the phosphorylation and cytotoxicity of arabinosyl nucleosides. Cancer Res. 1990; 50:3675–3680. [PubMed: 2340517]
32. Huang P, Chubb S, Hertel LW, Grindey GB, Plunkett W. Action of 2′,2′-difluorodeoxycytidine on DNA synthesis. Cancer Res. 1991; 51:6110–6117. [PubMed: 1718594]
33. Ross DD, Cuddy DP. Molecular effects of 2′,2′-difluorodeoxycytidine (Gemcitabine) on DNA replication in intact HL-60 cells. Biochem Pharmacol. 1994; 48:1619–1630. [PubMed: 7980627]
34. Heinemann V, Xu YZ, Chubb S, Sen A, Hertel LW, et al. Cellular elimination of 2′,2′-difluorodeoxycytidine 5′-triphosphate: a mechanism of self-potentiation. Cancer Res. 1992; 52:533–539. [PubMed: 1732039]
35. Mackey JR, Mani RS, Selner M, Mowles D, Young JD, et al. Functional nucleoside transporters are required for gemcitabine influx and manifestation of toxicity in cancer cell lines. Cancer Res. 1998; 58:4349–4357. [PubMed: 9766663]
36. Michalski CW, Erkan M, Sauluiute D, Giese T, Strahtmann R, et al. Ex vivo chemosensitivity testing and gene expression profiling predict response towards adjuvant gemcitabine treatment in pancreatic cancer. Br J Cancer. 2008; 99:760–767. [PubMed: 18728667]
37. Bergman AM, Pinedo HM, Peters GJ. Determinants of resistance to 2′,2′-difluorodeoxycytidine (gemcitabine). Drug Resist Updat. 2002; 5:19–33. [PubMed: 12127861]
38. Andersson R, Aho U, Nilsson BI, Peters GJ, Pastor-Anglada M, et al. Gemcitabine chemoresistance in pancreatic cancer: molecular mechanisms and potential solutions. Scand J Gastroenterol. 2009; 44:782–786. [PubMed: 19214867]
39. Florea AM, Busselberg D. Cisplatin as an anti-tumor drug: cellular mechanisms of activity, drug resistance and induced side effects. Cancers (Basel). 2011; 3:1351–1371. [PubMed: 24212665]
40. Costello BA, Borad MJ, Qi Y, Kim GP, Northfelt DW, et al. Phase I trial of everolimus, gemcitabine and cisplatin in patients with solid tumors. Invest New Drugs. 2014; 32:710–716. [PubMed: 24740268]
41. Alberts SR, Townley PM, Goldberg RM, Cha SS, Sargent DJ, et al. Gemcitabine and oxaliplatin for metastatic pancreatic adenocarcinoma: a North Central Cancer Treatment Group phase II study. Ann Oncol. 2003; 14:580–585. [PubMed: 12649105]
42. Alshmanna K, Kim R. Second-line therapy for gemcitabine-refractory pancreatic cancer: is there a standard? Oncology (Williston Park). 2008; 22:1176–1183. [PubMed: 18935928]
43. Boudvillain M, Dalbiès R, Aussourd C, Leng M. Intrastrand cross-links are not formed in the reaction between transplatin and native DNA: relation with the clinical inefficiency of transplatin. Nucleic Acids Res. 1995; 23:2381–2388. [PubMed: 7630715]

44. Goodsell DS. The molecular perspective: Cisplatin. Stem Cells. 2006; 24:514–515. [PubMed: 16582013]

45. Alcindor T, Beauger N. Oxaliplatin: a review in the era of molecularly targeted therapy. Curr Oncol. 2011; 18:18–25. [PubMed: 21331278]

46. Chao Y, Wu CY, Wang JP, Lee RC, Lee WP, et al. A randomized controlled trial of gemcitabine plus cisplatin versus gemcitabine alone in the treatment of metastatic pancreatic cancer. Cancer Chemother Pharmacol. 2013; 72:637–642. [PubMed: 23912692]

47. Stordal B, Pavlakis N, Davey R. Oxaliplatin for the treatment of cisplatin-resistant cancer: a systematic review. Cancer Treat Rev. 2007; 33:347–357. [PubMed: 17383100]

48. Siddik ZH. Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene. 2003; 22:7265–7279. [PubMed: 14576837]

49. Wang X, Guo Z. Towards the rational design of platinum(II) and gold(III) complexes as antitumour agents. Dalton Trans. 2008; 1521–1532. [PubMed: 18335133]

50. Heinemann V, Quietzsch D, Gieseler F, Gonnermann M, Schönekäs H, et al. Randomized phase III trial of gemcitabine plus cisplatin compared with gemcitabine alone in advanced pancreatic cancer. J Clin Oncol. 2006; 24:3946–3952. [PubMed: 16921047]

51. Chen Y, Wang XL, Wang JH, Yan ZP, Cheng JM, et al. Transarterial infusion with gemcitabine and oxaliplatin for the treatment of unresectable pancreatic cancer. Anticancer Drugs. 2014; 25:958–963. [PubMed: 24800885]

52. Azmy A, Abdelwahab S, Yassen M. Oxaliplatin and Bolus-Modulated 5-Fluorouracil as a Second-Line Treatment for Advanced Pancreatic Cancer: Can Bolus Regimens Replace FOLFOX When Considered for Second Line? ISRN Oncol. 2013; 2013:358538. [PubMed: 23533808]

53. Omura N, Goggins M. Epigenetics and epigenetic alterations in pancreatic cancer. See comment in PubMed Commons below. Int J Clin Exp Pathol. 2009; 2:310–326. [PubMed: 19158989]

54. Dhayat S, Mardin WA, Mees ST, Haier J. Epigenetic markers for chemosensitivity and chemoresistance in pancreatic cancer—a review. Int J Cancer. 2011; 129:1031–1041. [PubMed: 21413017]

55. Chen M, Shabashvili D, Nawab A, Yang SX, Dyer LM, et al. DNA methyltransferase inhibitor, zebularine, delays tumor growth and induces apoptosis in a genetically engineered mouse model of breast cancer. Mol Cancer Ther. 2012; 11:370–382. [PubMed: 22203734]

56. Yoo CB, Cheng JC, Jones PA. Zebularine: a new drug for epigenetic therapy. Biochem Soc Trans. 2004; 32:910–912. [PubMed: 15506921]

57. Cheng JC, Matsen CB, Gonzales FA, Ye W, Greer S, et al. Inhibition of DNA methylation and reactivation of silenced genes by zebularine. J Natl Cancer Inst. 2003; 95:399–409. [PubMed: 12618505]

58. Cheng JC, Yoo CB, Weisenberger DJ, Chuang J, Wozniak C, et al. Preferential response of cancer cells to zebularine. Cancer Cell. 2004; 6:151–158. [PubMed: 15324698]

59. Billam M, Sobolewski MD, Davidson NE. Effects of a novel DNA methyltransferase inhibitor zebularine on human breast cancer cells. Breast Cancer Res Treat. 2010; 120:581–592. [PubMed: 19459041]

60. Neureiter D, Zopf S, Leu T, Dietze O, Hauser-Kronberger C, et al. Apoptosis, proliferation and differentiation patterns are influenced by Zebularine and SAHA in pancreatic cancer models. Scand J Gastroenterol. 2007; 42:103–116. [PubMed: 17190770]
Figure 1.
Structure of 5FU with the fluor group in carbon 5-position. 5FU is a pyrimidine analog drug whose mechanism of action is through irreversible inhibition of thymidylate synthase (TS). Clinically it has been used in the treatment of anal, breast, colorectal, esophageal, stomach, pancreatic and skin cancers.
Figure 2.
Mechanism of 5FU leading to RNA and DNA damage. Thymidylate synthase inhibition is the main mechanism of action of 5FU through its active metabolite FdUMP. Synthesis of the pyrimidine thymidine, which is required for DNA synthesis, is the result of blocking thymidylate synthase. Thymidylate synthase methylates deoxyuridine monophosphate (dUMP) to for thymidine monophosphate (dTMP). The use of 5FU in cancer causes there to be a reduction leading to a scarcity of dTMP so that rapidly dividing cancer cells die from a lack of thymine.
Figure 3.
Gemcitabine is a nucleoside analog in which the hydrogen atoms on the 2′ Carbon of deoxycytidine are replaced by fluorine atoms. Like other analogues of pyrimidines, the triphosphate analogue of gemcitabine replaces the important cytidine building block of nucleic acids during DNA replication arresting tumor growth and resulting in apoptosis. Gemcitabine has been used to treat various carcinomas including lung, pancreatic, bladder and breast cancers. It is being investigated for the possible use against esophageal cancers and lymphomas.
Figure 4.
The broad spectrum mechanism of action of Gem, depending on its phosphorylation state, can inhibit Ribonucleotide Reductase, Polymerase and Deaminase activities. Once these enzymes are irreversibly inhibited, the cell cannot produce the deoxyribonucleotides required for DNA replication and repair and the cell dies via apoptosis.
Figure 5.
Cisplatin has two chloride ions and two amine groups attached to the center platinum ion. Cisplatin has been used to treat various cancers which include sarcomas carcinomas of the lung and ovary, lymphomas and germ cell tumors and is especially effective in treatment of testicular cancer.
Figure 6.
Similar to Cisplatin, Oxaliplatin contains a doubly charged platinum ion in the center. It, however, contains diamnocylohexane and carboxylate compounds. These platinum complexes bind to and crosslink DNA *in vivo* which triggers apoptosis.
Figure 7.
Zebularine’s structure includes a $2(1H)$-pyrimidinone ring. It is a nucleoside analog of cytidine and works by inhibiting cytidine deaminase by binding to the active site as a covalent hydrates. It has also been shown to inhibit DNA methylation and tumor growth in vivo and in vitro. Though entirely experimental at this time, it has been suggested that it could be used as a chemoprevention agent or even in epigenetic therapy.