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MONOGRAPH

GEMCITABINE ELAIDATE

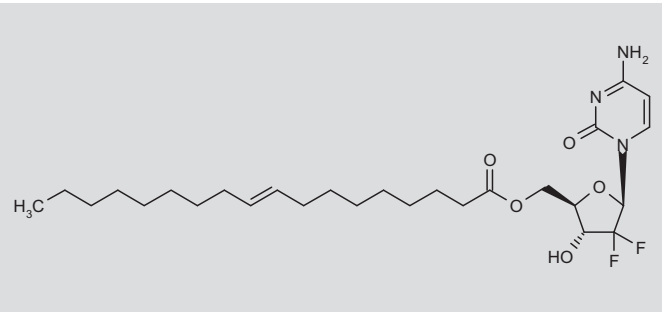
Cytotoxic Agent
Oncolytic

CO-1.01
CO-101
CP-4126

9(*E*)-Octadecenoic acid 2'-deoxy-2',2'-difluorocytidin-5'-yl ester

2'-Deoxy-2',2'-difluoro-5'-*O*-[9(*E*)-octadecenoyl]cytidine

InChI: 1S/C27H43F2N3O5/c1-2-3-4-5-6-7-8-9-10-11-12-13-14-15-16-17-23(33)36-20-21-24(34)27(28,29)25(37-21)32-19-18-22(30)31-26(32)35/h9-10,18-19,21,24-25,34H,2-8,11-17,20H2,1H3,(H2,30,31,35)/b10-9+/t21-,24-,25-/m1/s1



$C_{27}H_{43}F_2N_3O_5$
Mol wt: 527.6442
CAS: 210829-30-4
EN: 395985

SUMMARY

Gemcitabine is an established chemotherapeutic agent for the treatment of a variety of malignancies, including pancreatic, bladder, breast and non-small cell lung cancers. Gemcitabine requires specialized nucleoside transporters, which facilitate its entry into cells, and there is a growing body of evidence suggesting that these nucleoside transporters play a key role in gemcitabine sensitivity. Gemcitabine elaidate (CP- 4126, gemcitabine-5'-elaidic ester), is a novel cytotoxic agent that permeates the cell membrane independent of nucleoside transporters. A phase I trial of CP-4126 in patients with solid malignancies has shown promising antitumor activity. In this review, we discuss the origin, mechanism of action, preclinical and clinical evidence of antitumor activity of this novel drug.

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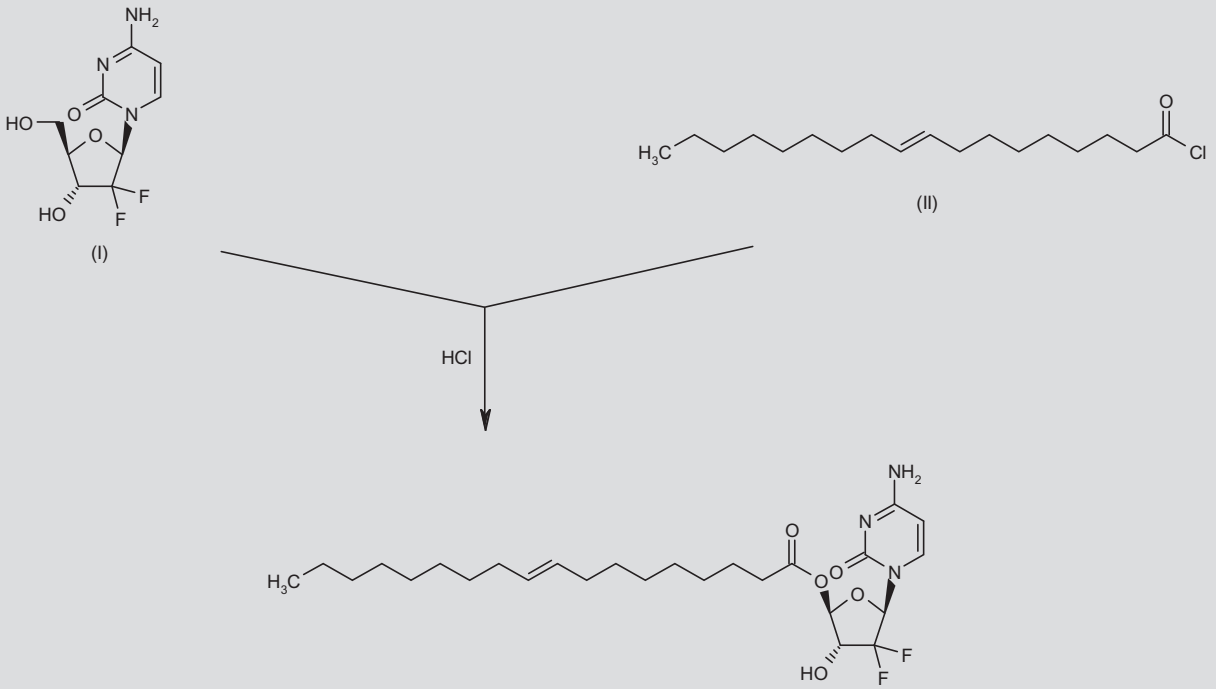
SYNTHESIS

The condensation of gemcitabine (I) with elaidic acid chloride (II) by means of HCl in DMF gives the target gemcitabine elaidate (1). Scheme 1.

BACKGROUND

Gemcitabine (difluorodeoxycytidine, dFdC) has been in clinical practice since 1995 and has been approved either as monotherapy or in combination with other cytotoxic agents in the treatment of pancreatic, bladder and non-small cell lung cancers (2-5). Despite the advent of biological therapies and a better understanding of the biology of pancreatic cancer, the prognosis of patients with pancreatic cancer remains poor and gemcitabine continues to be the cytotoxic therapy with proven clinical benefit in advanced disease, although it has only a modest effect on overall survival (6). Entry of gemcitabine into the cells through the plasma membrane is facilitated by human nucleoside transporters (7). Upon entry into the cell, dFdC is intracellularly phosphorylated by deoxycytidine kinase to difluorodeoxycytidine monophosphate (dFdCMP), and this is the rate-limiting step in the metabolism of gemcitabine. dFdCMP is further phosphorylated by kinases to difluorodeoxycytidine diphosphate (dFdCDP), and finally to its active metabolite difluorodeoxycytidine triphosphate (dFdCTP), which competes with deoxycytidine triphosphate (dCTP) for incorporation into DNA (8). This eventually leads to inhibition of DNA synthesis in the S phase (9). dFdC is inactivated to form difluorodeoxyuridine (dFdU) by a deamination process that is catalyzed by cytidine deaminase and excreted in urine. It is now thought that dFdU could also contribute to the cytotoxic action of gemcitabine through its conversion to difluorodeoxyuridine monophosphate (dFdUMP) (10). Gemcitabine metabolites, notably dFdCDP, inhibit ribonucleotide reductases and deplete the pool of deoxy nucleotide triphosphates (dNTPs), in particular dCTP, which are required for DNA synthesis. The depletion of dCTP pools possibly self-potentiates the cytotoxicity of dFdC by increasing the activity of deoxycytidine kinase and decreasing the activity of cytidine deaminase (11).

Scheme 1. Synthesis of Gemcitabine Elaidate



Despite displaying cytotoxic activity against a wide range of solid malignancies, gemcitabine is limited by both inherent and acquired drug resistance. Resistance to gemcitabine can be multifactorial, with various factors, including cellular uptake, intracellular phosphorylation of gemcitabine, incorporation of dFdCTP into DNA, inhibition of ribonucleotide reductases by dFdCDP and deamination by deoxycytidine deaminase, being implicated as key players (12). In addition, decreased drug accumulation plays a role in both acquired and intrinsic drug resistance. Gemcitabine, and most other nucleoside analogues, are hydrophilic compounds that require specialized nucleoside transporters present in tissues and human cells, which facilitate their uptake into the cells. This nucleoside transporter-mediated uptake is a prerequisite step for gemcitabine to exert its cytotoxic activity. In humans, these nucleoside transporters are broadly classified into human equilibrative nucleoside transporters (hENTs) and human concentrative nucleoside transporters (hCNTs). Among the four subtypes of hENT proteins, hENT1 and hENT2 play a key role in the concentration gradient-dependent, bidirectional transport of nucleoside analogues, including gemcitabine, across the cell membrane. In addition, it is now known that some hCNTs also play a role in gemcitabine transport (7, 13, 14).

Spratlin et al. retrospectively analyzed tissue samples of patients with pancreatic cancer who were treated with gemcitabine. Patients with high expression of hENT1 were noted to have superior overall survival (13.0 months) compared to patients with low expression of hENT1 (4.0 months), supporting the notion that hENT1 plays a role in gemcitabine transport and could be a predictive biomarker of response to gemcitabine (15). Similarly, gene expression analysis of pancreatic cancer specimens has demonstrated that higher expression of hENT1 significantly correlated with a favorable clinical outcome (16). The role of hENT1 in gemcitabine transport has also been studied in a large phase III trial, in which patients were randomly assigned to adjuvant treatment using gemcitabine or 5-fluorouracil following resection of their pancreatic cancer. The expression of hENT1 was associated with increased overall survival and prolonged disease-free interval in patients receiving gemcitabine. Such an effect was not noticeable in the cohort treated with 5-fluorouracil (17). All of these studies underpin the dependence of gemcitabine on the expression of hENT1 for its cytotoxic activity, and the need for novel drugs to treat pancreatic cancer patients with tumors that have low expression of hENT1.

CP-4126 is a fatty acid derivative of gemcitabine, wherein an elaidic ester (*trans*-9-octadecenoic fatty acid) is attached to the 5′ position of the sugar moiety. The molecular weight of CP-4126 is 527.7 g/mole, which is roughly twice that of gemcitabine (18, 19). CP-4126 was developed by Clavis Pharma based on lipid vector technology, which involves chemical linking of lipids to selected pharmaceutical agents, thereby producing a novel chemical entity (20). The tagging of fatty acids to the parent compounds alters the physicochemical property of the novel chemical entity and allows enhanced permeability across the cell membranes. A similar novel chemical entity, CP-4055, or elacytarabine (Elacyt™), which is a cytarabine-5′-elaidic ester, has already been tested in a phase I trial (21), and promising antitumor activity has been demonstrated in hematolog-

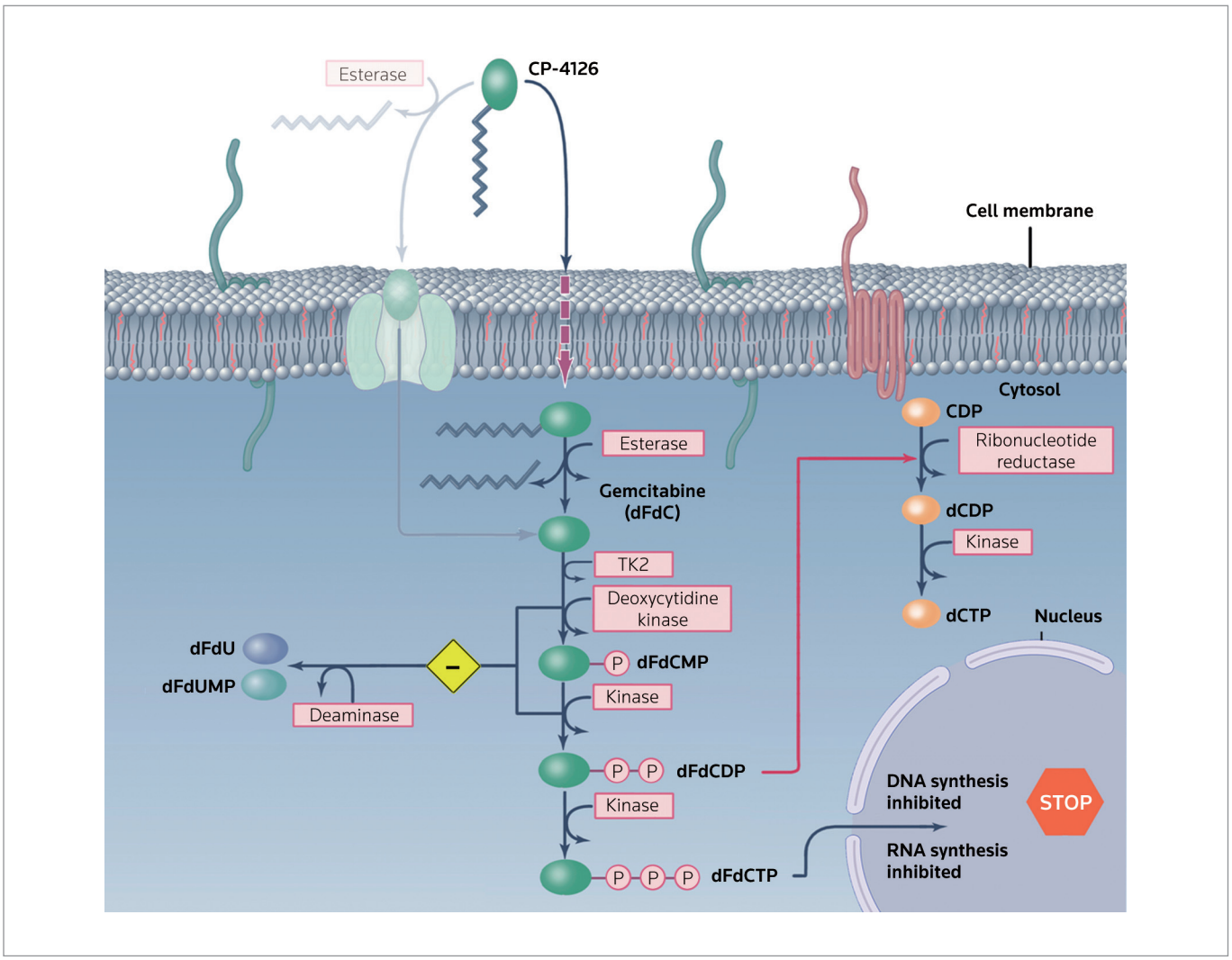


Figure 1. Cellular uptake and metabolism of CP-4126 (reproduced with permission from Ref. 19).

ical malignancies (22). Clavis Pharma has entered an agreement with Clovis Oncology for the further development of CP-4126 under a new name, CO-1.01, but for the purpose of this review, CO-1.01 will not be used further.

CP-4126, due to its lipophilic properties, enters cells by passive diffusion, independent of nucleoside transporters. Upon entry into cells, the fatty ester undergoes hydrolytic cleavage by esterases in blood or extracellular tissue, thereby releasing free gemcitabine. Subsequent to this conversion, gemcitabine undergoes intracellular anabolism and exerts its antiproliferative effects (Fig. 1). In addition, CP-4126 is not a substrate for cytidine deaminase and might also autopotentiate its cytotoxic activity by inhibiting cytidine deaminase (as discussed below).

PRECLINICAL PHARMACOLOGY

The preclinical activity of CP-4126 was evaluated by Bergman et al. (18). Chemosensitivity tests were conducted in vitro in both gemcitabine-sensitive and -resistant human ovarian cancer and murine colon cancer cells, and also in leukemia cells, by determining the IC_{50} (concentration that results in 50% growth inhibition when compared with untreated cells after 72 hours of drug exposure). The growth-inhibitory effects of CP-4126 were comparable to gemcitabine in solid tumor cell lines and were less pronounced in leukemia cells. The nucleoside transporter-independent transport of CP-4126 was demonstrated by chemosensitivity tests conducted in the presence and absence of the nucleoside transporter inhibitors dipyridamole and nitrobenzyl mercaptopurine ribonucleoside. Whereas the IC_{50} of gemcitabine was increased (indicating decreased sensitivity) in the presence of the nucleoside transporter inhibitors, there was no difference in the IC_{50} of CP-4126. This nucleoside transporter-independent transport of CP-4126 across the cell membrane was seen in a panel of human cancer cell lines, including melanoma and leukemia cells.

In addition, assays performed to test the effect of CP-4126 on the activity of cytidine deaminase (the key enzyme responsible for the

Table I. *Clinical trials with CP-4126.*

Clinical trial	Number of patients	Dose range explored	MTD	DLT	Clinical response
Phase I trial of intravenous CP-4126	43	30-1600 mg	1600 mg	Neutropenia, thrombocytopenia, death, grade 3 fatigue	Stable disease lasting more than 3 months (range: 3-11 months) in seven patients
Phase I trial of oral CP-4126	26	100-3000 mg	Not reached	Grade 3 AST elevation	Stable disease in nine patients

MTD, maximum tolerated dose; DLT, dose-limiting toxicity; AST, aspartate aminotransferase.

deamination of gemcitabine) suggest that CP-4126 partially inhibited the deamination of gemcitabine. Although inhibition of deoxycytidine deaminase could lead to increased levels of gemcitabine, and in theory to higher cytotoxicity and side effects, the evidence available hitherto suggests that CP-4126 is generally well tolerated in humans (19).

The ability of CP-4126 to cross the cell membrane independent of nucleoside transporters was also tested in matched lymphoma cell lines with functional nucleoside transporter (CEM) and deficient nucleoside transporter (5CEM/araC/8C). Whereas the 5CEM/araC/8C cells were resistant to gemcitabine, with an IC_{50} in the millimolar range, the same cells were sensitive to CP-4126, with an IC_{50} in the nanomolar range. Similarly, whereas the apoptotic activity was similar for gemcitabine and CP-4126 in CEM cells, there was no apoptotic activity in 5CEM/araC/8C cells treated with gemcitabine. In contrast, 5CEM/araC/8C cells treated with CP-4126 showed an enhanced induction of apoptosis (23). These observations suggest that CP-4126, by virtue of nucleoside trasnporter-independent transport into the cell and inhibition of its catabolism by cytidine deaminase, has increased accumulation and retention in the cells.

In addition to in vitro cytotoxicity assays, the antitumor activity of CP-4126 was evaluated in vivo in nude mice bearing subcutaneous solid tumor xenografts by Bergman et al. (18). In selected pancreatic tumor xenografts, CP-4126 exhibited high antitumor activity with limited toxicity. The antitumor activity of orally administered CP-4126 at varying doses and schedules was also evaluated in nude mice bearing human colon cancer xenografts. Clinically relevant antitumor activity was seen when CP-4126 was given orally at a dose of 10 mg/kg on days 1-5 in three weekly cycles.

PHARMACOKINETICS AND METABOLISM

Upon administration of the recommended dose of 1250 mg/m^2, CP-4126 was detected for up to 24 hours after dosing at concentrations (0.01-0.03 μmol) that have shown antitumor activity. dFdU, the inactive metabolite of gemcitabine was excreted in urine mostly within the first 24 hours of CP-4126 infusion (19). Concordant with the cellular pharmacokinetic data indicating increased accumulation and retention of CP-4126, exposure of the tissue to the drug, as measured by the $AUC_{0-\infty}$ value for dFdC after 1250 mg/m^2 of CP-4126, was significantly higher than after a 30-minute infusion of gemcitabine at 640 mg/m^2 ($P = 0.006$). This is also consistent with the longer half-life of dFdC after CP-4126 than after gemcitabine ($P < 0.001$).

Preliminary pharmacokinetic data indicate that CP-4126 at a dose of 1250 mg/m^2 results in plasma levels of dFdC comparable to or higher than those obtained with a gemcitabine dose of 640 mg/m^2. It should be noted that a CP-4126 dose of 1250 mg/m^2 is equivalent to a gemcitabine dose of 625 mg/m^2, since dFdC constitutes 50% of the CP-4126 moiety (24).

CLINICAL STUDIES

A phase I trial of CP-4126 was conducted in humans with the primary objective of determining the maximum tolerated dose (MTD) and establishing the recommended dose for phase II trials (19) (Table I). Secondary objectives were to evaluate the safety profile, pharmacokinetics and antitumor activity of CP-4126. Forty-three patients with histologically or cytologically confirmed solid malignancies who were refractory to standard therapies were treated with CP-4126, which was administered i.v. on days 1, 8 and 15 every 4 weeks (d1, d8, d15 q4w schedule) as a 30-minute infusion. The median age was 60 years (range: 19-78 years) and the patients had a wide range of solid tumors, e.g., colon cancer (13 patients), pancreatic cancer (9 patients) and ovarian cancer (5 patients). The MTD was established as 1600 mg/m^2/day and the recommended dose for further phase II trials was 1250 mg/m^2/day when given on a d1, d8, d15 q4w schedule. The median number of cycles administered was two (range: 1-9 cycles). The dose-limiting toxicity was CTCAE grade III lethargy. CP-4126 was generally well tolerated, with the most frequent treatment-related toxicities being nausea, vomiting, fatigue and anorexia. Seven patients had clinical benefit, with stabilization of disease lasting more than 3 months, including patients with pancreatic, colon and ovarian cancer. The antitumor activity of CP-4126 in this group of heavily pretreated patients is promising.

In view of in vivo data suggesting antitumor activity for orally administered CP-4126, a phase I trial of oral CP-4126 was conducted in patients with advanced solid tumors (25). This trial was terminated before the MTD could be reached as the bioavailability of oral CP-4126 was too low and patients were required to take up to 12 capsules per day. This is in keeping with our knowledge that orally administered gemcitabine has poor bioavailability, probably due to extensive first-pass metabolism (26).

Given the promising results from the phase I trial, to prospectively test the hypothesis of nucleoside transporter-independent transport of CP-4126, a randomized phase II trial comparing CP-4126 with gemcitabine as a first-line therapy in patients with metastatic pan-

creatic adenocarcinoma is currently recruiting patients (27). The primary objective of this study is to compare the overall survival in patients with low expression of hENT1 undergoing treatment with CP-4126 or gemcitabine. This study should provide more information about the efficacy of CP-4126 and prospectively validate the suitability of hENT1 as a predictive marker of response to gemcitabine.

CONCLUSION

CP-4126 is novel cytotoxic agent that has the potential to make a meaningful difference in the outcome of patients with pancreatic cancer who have low levels of expression of hENT1. In addition to pancreatic cancer, further studies need to be conducted in other solid tumors, such as lung and bladder cancers (where gemcitabine has been effective), to exploit the nucleoside transporter-independent transport of CP-4126 either as a single agent or in combination with other agents, such as cisplatin. CP-4126 may increase therapeutic options and translate into better clinical outcome for patients with pancreatic cancer.

SOURCES

Clavis Pharma ASA (NO); licensed to Clovis Oncology, Inc. (US).

DISCLOSURES

T.R.J. Evans has received research funding from Clavis Pharma to support the phase I study of CP-4126 and from Clovis Oncology to support a phase II study of CO-1.01 (CP-4126) in pancreatic cancer. Dr. Venugopal states no conflicts of interest.

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