



Mini-review

Tackling pancreatic cancer with metronomic chemotherapy



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ABSTRACT

Pancreatic tumours, the majority of which arise from the exocrine pancreas, have recently shown an increasing incidence in western countries. Over the past few years more and more new selective molecules directed against specific cellular targets have become available for cancer therapy, leading to significant improvements. However, despite such advances in therapy, prognosis of pancreatic cancer remains disappointing. Metronomic chemotherapy (MCT), which consists in the administration of continuous, low-dose anticancer drugs, has demonstrated the ability to suppress tumour growth. Thus, it may provide an additional therapeutic opportunity for counteracting the progression of the tumour. Here we discuss evidence arising from preclinical and clinical studies regarding the use of MCT in pancreatic cancer. Good results have generally been achieved in preclinical studies, particularly when MCT was combined with standard dose chemotherapy or antiinflammatory, antiangiogenic and immunostimulatory agents. The few available clinical experiences, which mainly refer to retrospective data, have reported good tolerability though mild activity of metronomic schedules. Further studies are therefore awaited to confirm both preclinical findings and the preliminary clinical data.

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Introduction

Pancreatic cancer (PC), with an increasing incidence in western countries around 20:100,000 new cases per year, ranks as 12th among cancer types worldwide. The majority of pancreatic malignancies pertain to the exocrine pancreas and of these pancreatic ductal adenocarcinoma (PDAC) is by far the most common type of PC.

At a metastatic stage, therapy mainly consists of chemotherapy, while gemcitabine for Westerners and S-1 for East Asian populations are regarded as backbone drugs [1,2]. Combination regimens such as FOLFIRINOX (5-fluorouracil, leucovorin, irinotecan, oxaliplatin) [3], gemcitabine plus nab-paclitaxel [4] and erlotinib plus gemcitabine [5] represent new standards in the first-line therapy of patients that show a good performance status. There is also evidence for the benefits of second-line chemotherapy as compared to best supportive care alone even though in randomized studies only limited results were reported [6–15].

Pancreatic cancer characterized by both a 5-year survival rate below 10% [16] and a mortality rate that nearly overlaps the

incidence [17] represents a great challenge for cancer therapy. Undoubtedly, the strong commitment of recent research has provided a deeper knowledge concerning molecular pathways underlying tumour development and yielded positive clinical achievements [18]. High levels of secreted protein acidic and rich in cysteine (SPARC), which are observed in about 50% of PC, have been recognised to correlate with better survival following nab-paclitaxel [19–21].

Epidermal growth factor receptor (EGFR) amplification and KRAS mutations, which lead to a permanent activation of the signalling pathway below the EGFR molecule, are frequently detected in PC cells. However, only the combination of gemcitabine plus erlotinib, which is an EGFR tyrosin-kinase inhibitor, was associated with a modest but statistically significant increase in survival when compared to gemcitabine alone [5]. Early studies testing other molecular targeted therapies showed marginal outcomes [22–24]. Even, strategies aimed at overcoming drug resistance are currently being investigated [21]. Particularly, the hedgehog signalling activation through stroma proliferation [25], hypoperfusion and impaired drug delivery, determined resistance to gemcitabine in animal models while the use of hedgehog inhibitor increased intratumoral concentration of the drug [26]. Moreover, the inhibition of the epithelial to mesenchymal transition in a genetically engineered mouse model enhanced sensitivity to gemcitabine [27].

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Future perspectives of PC therapy certainly involve a more accurate recognition of genetic and histopathological features [28] as well as the support of immunotherapy [15].

Furthermore, metronomic chemotherapy (MCT), by demonstrating that repetitive low-doses of antiproliferative agents can suppress tumour growth with acceptable toxicity, provides an additional therapeutic chance of tackling PC [29]. Here we consider reports from preclinical and clinical studies about MCT in PC. The search strategy and selection criteria are reported in Table 1 (supplementary material).

Metronomic chemotherapy: preclinical studies

Effects of metronomic chemotherapy on cancer cells and the tumour microenvironment

The choice of experimental model is essential in the evaluation of drugs, particularly those targeting the tumour microenvironment [30]. Unlike in the case of pancreatic neuroendocrine tumours (PNET), which are dependent on angiogenic factors, tumours arising from the exocrine pancreas show apparently limited dependence on an excess of angiogenesis. Indeed, PDAC is essentially characterized by an extensive stromal reaction resulting in a hypovascular and hypoxic microenvironment, which supports tumour growth. Moreover, the rigidity of the extracellular matrix of PDAC by disrupting the normal architectural vasculature could influence therapeutic response by preventing the delivery of drugs to neoplastic cells [30]. Notwithstanding, targeting tumour vasculature remains an attractive strategy also in PC, aimed at further impairing perfusion or, alternatively, at improving intra-tumour drug delivery. For this reason, MCT, which is endowed with multiple anti-tumour functions including antiangiogenic properties [29,31], is proposed for the role of targeting both PC cells and their microenvironment (Fig. 1).

An early set of preclinical studies has investigated the feasibility of delivering metronomic cyclophosphamide (CTX) in human PNET xenografts of mice [32–34] (Table 1). In the first study, the chronic administration of CTX at low doses on a daily basis through the

drinking water of mice proved safe, reasonably efficacious and potentially applicable to chronic treatment [32]. This protocol further demonstrated itself to be particularly well suited for integration with antiangiogenic drugs. The second study has provided the evidence for enhanced efficacy of metronomic CTX using imatinib [33]. This receptor tyrosine kinase inhibitor was used to disrupt platelet-derived growth factor receptor (PDGFR) mediated pericyte support of tumour endothelial cells. CTX at maximum tolerated dose (MTD) prompted transitory regression followed by rapid regrowth of tumours, in contrast to metronomic CTX plus imatinib, which produced stable disease along with increased endothelial cell apoptosis. Moreover, a “chemo-switch” (C-S) protocol, using sequential MTD and MCT by means of a multitargeted inhibition of PDGFR and vascular endothelial growth factor receptor (VEGFR) produced enduring responses and improved survival. The third study, evaluating the cysteine cathepsin inhibitor JPM-OEt, alone or in combination with CTX, supplied further evidence in favour of the C-S strategy [34]. Three dosing schedules for CTX, MTD regimen, a metronomic continuous low-dose regimen or a C-S regimen consisting of MTD followed by metronomic dosing, were compared. The cathepsin inhibitor in combination with two distinct regimens of chemotherapy administration (MTD or C-S) resulted in tumour regression, decreased tumour invasiveness and increased survival. The cathepsin inhibitor plus the C-S regimen of CTX led to the most pronounced reduction in tumour burden and the greatest increase in OS. These results indicate that the initial MTD chemotherapy is able to debulk the tumour, whereas the metronomic therapy evidently reduces regrowth/relapse and tumour progression. Notably, the addition of JPM-OEt to the C-S regimen led to an even further regression in tumour burden. Conversely, metronomic CTX administered either alone or in combination with JPM-OEt did not significantly affect total tumour volume.

Aimed at targeting various components of the tumour microenvironment to relieve vessel compression and aid drug delivery in a xenograft model of PC, lenalidomide, an immunomodulatory agent, sunitinib and low dose metronomic CTX, alone and in combination have been tested [35]. The combination strategy was shown to significantly inhibit the growth of primary tumours in

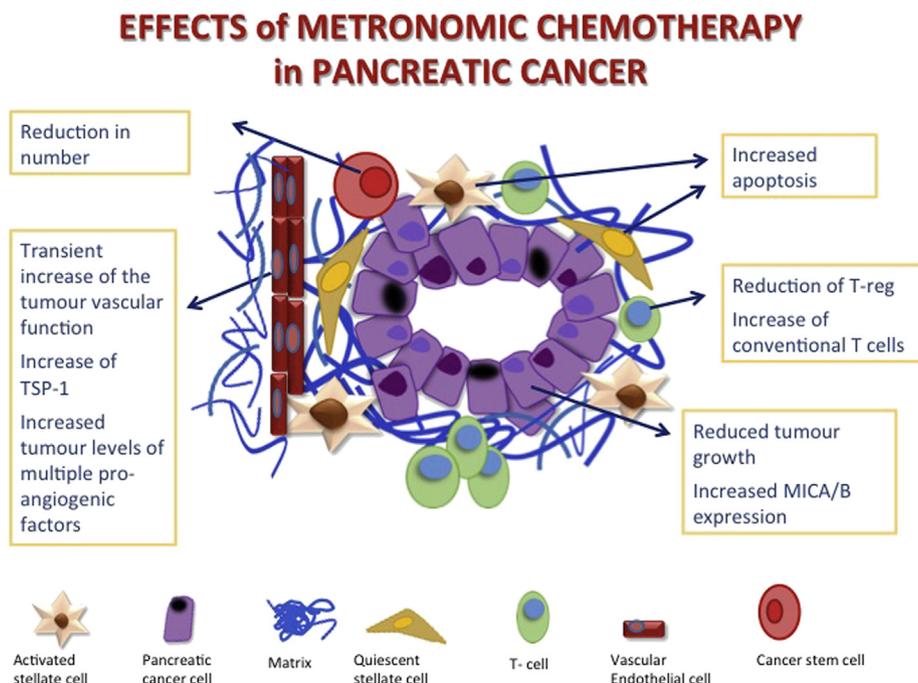


Fig. 1. Effects of metronomic chemotherapy on pancreatic cancer cells and tumour microenvironment.

Table 1
Preclinical studies evaluating metronomic chemotherapy regimens in rodent models of pancreatic cancer or pancreatic cancer cell lines.

Animal model/Cancer cell lines	Drug used	Reference
Rip-Tag transgenic of pancreatic islet cells carcinoma	CTX	Man et al. [32]
Rip1-Tag2 transgenic model of PNEC	CTX +/- imatinib (Chemo-switch)	Pietras et al. [33]
Rip1-Tag2 transgenic of pancreatic islet cells carcinoma	CTX (Chemo-switch)	Bell-McGuinn et al. [34]
MiaPaca-2 xenograft model	CTX +/- lenalidomide, sunitinib	Blansfield et al. [35]
AsPC-1 PDAC murine xenograft model	GEM + nab-paclitaxel or docetaxel	Awasthi et al. [36]
Orthotopic human NP-18 pancreatic cancer xenograft	GEM	Laquente et al. [37]
Orthotopic PaCa-8 and PaCa-13 human pancreatic cancer xenografts	GEM	Cham et al. [38]
Orthotopic MIA PaCa-2-RFP xenograft model	GEM +/- sunitinib	Tran Cao et al. [39]
Orthotopic human NP9 or TP11 xenografts	GEM (Chemo-switch)	Vives et al. [40]
Orthotopic human PaCa-8 and PaCa13 PC xenografts	GEM + DC101	Yapp et al. [41]
Orthotopic L3.6 pl pancreatic cancer model	EndoTAG TM -1 + GEM cisplatin	Eichhorn et al. [42]
Orthotopic AsPC-1, PANC-1, MiaPaca-2 xenografts	GEM (gold nanoparticles)	Kudgus et al. [43]
Genetically engineered model	GEM + DMAPT + sulindac	Yip-Schneider et al. [44]
Orthotopic model of human GER pancreatic tumour xenograft	Gimatecan +/- CpG-oligodeoxynucleotide	Petrangolini et al. [48]
PANC-1, BxPC-3, HPAF-II, MIA PaCa-2, Capan-1 and AsPC-1	GEM	Miyashita et al. [49]
Orthotopic Panc02 PC model	GEM	Schevchenko et al. [52]
Orthotopic human Panc-02 and murine PC model	GEM (+Anti-Bv8 Ab)	Hasnis et al. [53]

PC: pancreatic cancer; PNEC: pancreatic neuroendocrine cancer; GEM: gemcitabine; CTX: cyclophosphamide; DMAPT: bioavailable nuclear factor-kB inhibitor; EndoTAGTM-1: cationic lipid complexed paclitaxel.

mice more than any single agent alone, by reducing the number of both proliferating cancer cells and blood vessels.

Gemcitabine, which is a milestone drug in managing PC, has been evaluated in several, mostly orthotopic, xenografts of PC, as well as in a genetically engineered model [36–44] (Table 1). An early study has provided evidence of the antiangiogenic effect of gemcitabine, especially used at metronomic dosages [37]. This effect correlated with the induction of thrombospondin-1, which is a natural inhibitor of angiogenesis. By comparing low-dose metronomic gemcitabine with standard gemcitabine administration, the effect on established tumours was found equivalent in the two treatment groups.

The anti-tumour efficacy and effects on the tumour microenvironment of metronomic vs MTD gemcitabine were investigated in two other human PC xenografts [38]. Both metronomic and MTD gemcitabine significantly reduced tumour volume in both xenografts. However, by using dynamic contrast-enhanced magnetic resonance imaging, a better tissue perfusion was shown in metronomic gemcitabine-treated tumours than in their MTD-treated counterparts. Moreover, metronomic gemcitabine significantly increased apoptosis in cancer-associated fibroblasts and markedly reduced tumour levels of multiple pro-angiogenic factors. The frequent dosing of gemcitabine at relatively low doses compared with a single MTD of gemcitabine would increase the probability of eliminating or inducing differentiation in both stromal cells and PC. Indeed, these assorted populations are unlikely to all be in a susceptible growth phase under once a week drug administration [45].

Gemcitabine either at metronomic or MTD schedule, with or without sunitinib, has also been trialled [39]. Although the MTD schedule showed greater efficacy on primary tumour growth, metronomic gemcitabine had comparable anti-metastatic effects but lower toxicity. Moreover, the combination of sunitinib with metronomic gemcitabine significantly prolonged OS compared with MTD alone or in combination with sunitinib. Therefore, the schedule was suggested as a possible adjuvant or maintenance treatment for PC.

The efficacy of a C-S has also been explored in a PC model, using gemcitabine as a reference drug [40]. Once the tumour volume was palpable, mice were randomly distributed to four cohorts: control and three different treatment groups, receiving the MTD gemcitabine, the metronomic gemcitabine schedule or the C-S schedule (MTD followed by metronomic gemcitabine until sacrifice). The

authors showed that the C-S schedule produced the best anti-tumour response, reducing tumour growth by up to 80%, with lower toxicity than MTD regimen. Moreover, this effect on tumour volume correlated with a significantly lower expression of the proliferation marker Ki67. The remarkable anti-tumour and anti-dissemination effects of C-S could be due to the combined advantages of different therapeutic strategies: that by MTD directly attacks tumour cells, while metronomic administration inhibits angiogenesis (inducing thrombospondin-1) and reduces cancer stem cells in number.

The benefits of low-dose combination treatment of gemcitabine with docetaxel or nab-paclitaxel on local tumour growth and animal survival were observed in AsPC-1 PDAC murine xenograft models [36]. These low-dose combinations compared with controls or gemcitabine alone increased both inhibition in tumour growth and animal survival but were not superior to regular dose nab-paclitaxel alone.

Different effects of metronomic gemcitabine and the anti-angiogenic agent DC101 on patient-derived xenografts of PC have recently been described [41]. Metronomic gemcitabine improved the tumour's vascular function transiently and retarded cell proliferation. The overall effect was to significantly slow tumour growth. Metronomic gemcitabine was not only cytotoxic but also affected tumour vasculature and reduced the levels of proangiogenic factors such as VEGF. It was suggested that both the increase in tumour perfusion and reduced hypoxia following metronomic gemcitabine might be used as a therapeutic window for the administration of a second drug or radiation therapy.

Nanotechnology, having the potential to improve patients' quality of life through overcoming the adverse effects of chemotherapy, has attracted increasing attention in recent years, especially for cancer treatment. Vascular targeting therapy has been realized by means of liposomal compounds such as paclitaxel encapsulated in cationic lipid complexes (EndoTAGTM-1). EndoTAGTM-1 was investigated in combination with conventional gemcitabine or cisplatin therapy in an orthotopic PC model [42]. Interestingly, the best therapeutic results were achieved using a metronomic rather than a MTD schedule. Moreover, the combination of EndoTAGTM-1 and cytotoxic chemotherapy (cisplatin or gemcitabine) significantly decreased tumour growth, while only EndoTAGTM-1 in combination with gemcitabine was able to inhibit the incidence of metastasis in PC. Moreover, the approach with anti-EGFR monoclonal antibodies conjugated to gold nanoparticles

has proven to be effective in targeting cancer cells with an over-expression of EGFR [43]. Low dose gemcitabine delivered in the form of gold nanoparticles was demonstrated to inhibit tumour growth in an advanced stage of an orthotopic model of PC.

There is a further study that explored the efficacy of intervention with a bioavailable nuclear factor- κ B inhibitor (DMAPT), the cyclooxygenase inhibitor sulindac, and low-dose gemcitabine in a genetically engineered mouse model of PC [44]. Here, the percentage of normal pancreatic ducts was significantly increased by the combination compared with placebo.

In conclusion, MCT, especially when used in combination with immunomodulating and target agents [35,39] or in C-S protocols [33,34,40], has shown to significantly inhibit the growth of pancreatic tumours in mice and improve survival. Antiangiogenic effects usually ensue from the metronomic drug delivery [33,37,40,41] but a reduction in cancer stem cells [40] and increased apoptosis in cancer-associated fibroblasts have also been reported [38] confirming the multiple anti-tumour function of MCT.

Effects of metronomic chemotherapy on immune response

Low concentrations of selected antineoplastic agents can also modulate the immune response improving efficacy of immunotherapy [46,47]. Indeed, an increased immunogenicity of cancer cells, enhanced antigen presentation through modulation of dendritic cells and potentiated cytotoxic activity of immune effectors have all been observed in several animal tumour models [46].

The efficacy of MCT plus immunostimulatory agents was investigated in the preclinical study by Petrangolini et al. [48]. Low-dose protracted treatment of gimatecan, an orally active lipophilic camptothecin, significantly increased mice survival over control mice, in contrast to gimatecan high/intermittent dose or intravenous gemcitabine. Orally metronomic gimatecan was also experimented in combination with CpG-oligodeoxynucleotide (CpG-ODN), which is an immunomodulating agent capable of stimulating the expression of TRAIL (a member of TNF family) which can in turn induce the apoptosis of cancer cells by engaging TRAIL-receptors expressed on the cancer cell surface. TRAIL was released in the peritoneal washings of CpG-ODN-treated mice. This study showed that mice treated with the combination of metronomic gimatecan and CpG-ODN had a better survival rate than control mice, with no increased toxicity. This is possibly related to the apoptotic synergistic effect of metronomic therapy and the immunomodulating agent on cancer cells.

The effect of gemcitabine on the expression of cell surface major histocompatibility complex class I chain-related gene A/B (MICA/B) in PC cell lines has recently been examined [49]. Because MICA/B can be recognized by gamma delta T cells and natural killer cells through natural killer group 2, member D (NKG2D) activating receptors [50,51], cytotoxicity of gamma delta T cells was also evaluated. Gemcitabine increased MICA/B expression on cell surface in MICA/B positive PC cell lines [49]. This effect was higher at a low concentration of gemcitabine that did not affect cell growth but the enhancement of NKG2D-dependent cytotoxicity was not observed.

Moreover, preferential depletion and functional impairment of T regulatory cells (Treg), have also been reported using metronomic schedules [46,52]. The PC microenvironment with a focus on conventional and regulatory T cells has been investigated in a murine model of PC [52]. An accumulation of Treg with the effector/memory phenotype, suggesting their enhanced suppressive activity and higher proliferation capacity, was observed in these tumours. Low-dose gemcitabine (10 times lower than the dose needed to completely eradicate the tumour) led to a decrease in the Treg percentage and a concomitant increase in the percentage of

conventional T cells, resulting in a modestly increased survival of PC mice.

Furthermore, it has been hypothesized that Bv8 factor, secreted by myeloid-derived suppressor cells (MDSC), would play a critical role in the resistance of PC to MTD gemcitabine [53]. The presence of MDSC in the tumour microenvironment is recognized to be one of the major contributors to PC growth. Moreover, it has been suggested that the colonization of MDSC in the pancreatic tumours inhibits immune cells such as T-cells, which are responsible for the suppression of tumour growth by the secretion of interferon- γ [54]. Tumour-bearing mice treated with metronomic gemcitabine exhibit controlled tumour growth as compared to those receiving weekly gemcitabine [53]. Weekly gemcitabine, but not continuous metronomic gemcitabine or the combination of the two drug regimens, was demonstrated to prompt rebound MDSC mobilization and increase angiogenesis in this tumour model. Moreover, the use of anti-Bv8 antibodies improved PC treatment outcome following weekly gemcitabine therapy. Thus, both Bv8 inhibition and MCT have been proposed as “add-on” treatments for preventing post-chemotherapy PC recurrence, progression and metastasis. Finally, possible apoptotic synergistic effects on cancer cells with immunostimulating agents [48], an increased expression of cell surface major histocompatibility complex class I in PC cell lines [49], decreased immunosuppressive cells (T-reg and MDSC) and concomitant increase in the percentage of conventional T cells [52,54] have all been reported following metronomic schedules, disclosing that MCT can modulate the immune response.

Metronomic chemotherapy: clinical studies

Pancreatic neuroendocrine tumours

Anecdotal clinical experiences pertaining to the use of MCT in patients affected with PNET tumours have become available (Table 2). These tumours arising from cells of the diffuse endocrine system constitute a small proportion, around 10%, of PC. Chemotherapy has limited indications in the locally advanced and metastatic disease. The alkylating agents streptozocin and temozolomide have demonstrated the most pronounced anti-tumour activity in these neoplasms [55]. The molecularly targeted agents, everolimus and sunitinib, have been shown to have anti-tumour activity too, as well as improving PFS in patients with advanced PNET.

The Piemonte Region Oncology Network conducted a multicentre non randomized phase-II trial to assess the activity and safety profile of a combination regimen of continuous/metronomic 5-FU infusion and long-acting release (LAR) octreotide in patients with locally advanced or metastatic, well-differentiated neuroendocrine carcinomas [56]. Twenty-nine patients with radiological documentation of progressive disease were entered into the study. 5-FU treatment was administered for at least 6 months. LAR octreotide was continued until disease progression. This combination regimen was well tolerated while most toxicities were non haematological. Moreover, it was associated with an overall objective response rate (RR) of 24%, a biochemical RR of 48% and a symptomatic RR of 60%.

In addition, the activity and toxicity of another metronomic regimen in patients with metastatic well-to-moderately differentiated neuroendocrine neoplasms arising from various primary sites, not previously treated with chemotherapy, were recently analysed [57]. This was a single-arm multicentre Italian phase-2 study including 45 patients treated with LAR octreotide, metronomic capecitabine and intravenous bevacizumab without interruptions for nine months. Bevacizumab was continued until disease progression. The treatment was active and well tolerated.

Table 2
Clinical studies of metronomic chemotherapy in pancreatic cancer.

Regimen	Study design	Pts (N.)	RR (%)	PFS/TTP (months)	OS (months)	Reference
Pancreatic neuroendocrine tumours						
PVI 5-FU (200 mg/m ² daily), LAR octreotide (20 mg monthly)	II	29	24	22.6	Not reached	Brizzi et al. [56]
LAR octreotide (20 mg monthly), mCAP (2000 mg/daily), bevacizumab (5 mg/kg every 2 weeks)	II	45	26.3	14.3	Not attained	Berruti et al. [57]
Pancreatic adenocarcinoma						
PVI 5-FU (300 mg/mq, daily), cisplatin (20 mg/mq, weekly) 10 weeks on than 2 weeks off	II	56	16	2.5	5.8	Rothman et al. [58]
PVI 5-FU (225 mg/mq, daily) plus trimetrexate glucuronate (20–50 mg/mq, weekly) 6 weeks on every 8 weeks	II	22	9	2	6.9	Amado et al. [59]
S1 (80–120 mg/day, 28 days on, 14 days off), cisplatin (4 mg/mq, days 1–5, 8–12, 15–19, 22–26 of the first course)	II	30	17	4.6	9	Ina et al. [60]
Miscellaneous						
CTX (50 mg daily), rofecoxib (25 mg daily), vinblastine (3 mg/m ² weekly) + minocycline (100 mg twice daily)	II	47 (5 PC)	13	3.4	8.4	Young et al. [61]
CTX (500 mg/mq ² , iv bolus on day 1; 50 mg daily p.o. from day 2), UFT (100 mg/twice a day), CXB (200 mg/twice a day)	II	38 (2 PC)	0	2.7	7.1	Allegrini et al. [62]
CAP (1000 mg daily), CXB (800 mg daily)	II	37 (3 PC)	–	4	–	Steinbild et al. [63]
Maintenance CAP (1000 mg twice daily, 5 days on, 2 days off)	Retrospective	28 (10 PC)	–	–	–	Sun et al. [64]
CAP (500 mg twice a day)	Pilot Study	35	30.8	–	–	Miger et al. [65]
CAP (1500 mg daily)	Retrospective	47 (11 PC)	2	–	5	Roberto et al. [66]
GEM (1,000 mg/m ² days 1, 8, 15 q28) vs GEM (1,000 mg/m ² days 1, 8, 15 q28) plus induction regimen with: CTX (50 mg q.d.), CXB (400 mg twice daily), oral NAC (400 mg twice daily), GCSF plus autologous tumour antigens	I/II R	60	–	–	10.2 vs 18.0 (p = 0.036)	Lasalvia-Prisco et al. [67]
Chemoradiation						
GEM (40 mg/m ² twice weekly) + RT → GEM (1,000 mg/m ² g1, 8,15 q28)	II	43	–	6.1	8.2	Blackstock et al. [71]
GEM (40 mg/m ² twice weekly) + SFRT vs GEM (40 mg/m ² twice weekly) + HART	I	35	35	–	11.3	Maemura et al. [72]
GEM 200–400 mg weekly/biweekly + CTX 50 mg every other day + UFT 200–400 mg daily + RT	II	22	27	–	10.6	Nio et al. [73]

RR: Response Rate; PFS: Progression Free Survival; TTP: Time To Progression; OS: Overall survival; NR: Not Reported; CTX: cyclophosphamide; mCAP: metronomic capecitabine; LAR: long-acting release; PVI 5-FU: protracted venous infusion 5-fluorouracil; CXB: celecoxib; iv: intravenous; GCSF: granulocyte colony-stimulating factor; UFT: tegafur uracil; GEM: gemcitabine; NAC: N-acetylcysteine; RT: radiotherapy; SFRT: standard-fractionated radiotherapy; HART: hyperfractionated accelerated radiotherapy.

Partial response was obtained in 8 patients (17.8%) and stable disease in 29 patients (64.4%). Tumour response was more frequent in pancreatic than in non-pancreatic malignancies. Biochemical and symptomatic responses were observed in 52.9% and 82.3% of cases respectively. Most adverse events were mild to moderate and include nausea/vomiting, diarrhoea, hand-foot syndrome, hypotension, proteinuria and asthenia. Proteinuria was the best predictive factor of treatment efficacy.

Cancer of the exocrine pancreas

Cytotoxic drugs delivered at continuous, low-dose but with short treatment breaks had already been used to treat PC before the development of the metronomic concept [58,59]. Protracted infusion of 5-fluorouracil (5-FU) was for instance combined with either low-dose weekly cisplatin continuously delivered [58] or a weekly dose of the synthetic antifolate trimetrexate [59]. Overall, positive results were achieved with a tangible effect on survival in the first study and clinical benefit response experienced by 27.2% of patients in the second one.

The oral fluoropyrimidine S-1 combined with cisplatin in a phase-2 trial, which included 30 patients with unresectable advanced PC [60], was evaluated. S-1 was administered for 28 days followed by a rest of 14 days with cisplatin on days 1–5, 8–12, 15–19 and 22–26 of the first course. After the second course, S-1 was administered as maintenance chemotherapy. The overall RR was 17% and no grade-4 toxicity was detected.

Few other studies have dealt with the use of metronomic protocols in patients with PC. Young and colleagues in a phase-II trial tested a combination of low dose CTX, weekly vinblastine and

rofecoxib [61]. Among the 47 cancer patients enrolled, five cases had PC. Overall, 30% of patients obtained clinical benefit with moderate toxicity. Comparable results in patients with advanced refractory gastrointestinal cancer were achieved by a combination of metronomic Tegafur-uracil and CTX plus celecoxib [62]. Again, only five out of 38 recruited cases had cancer of pancreato-biliary origin. Steinbild and colleagues conducted a phase-II trial enrolling 37 patients with several tumour types, of whom seven had a pancreato-biliary cancer [63]. All patients received metronomic capecitabine plus celecoxib. The regimen was well tolerated and showed both antitumour and antiangiogenetic activity. Another study evaluated the safety profile of metronomic capecitabine as maintenance chemotherapy in patients with gastrointestinal cancer [64]. Toxicity was mild and mainly consisted of grade 1–2 fatigue and hand-foot-syndrome. Moreover, a significant increase in red blood cell mean corpuscular volume, which may have potential use as a biomarker to monitor therapeutic response, was observed.

Continuous low-dose capecitabine has recently been investigated in gastrointestinal cancer in two other studies [65,66]. The first one included 35 patients with either upper or lower gastrointestinal tract tumours [65]. There was a considerable disease control rate (76%) in patients affected with upper gastrointestinal tract cancer. Moreover, six out of 35 patients experienced symptoms palliation lasting more than two years. The second one has presented clinical outcome of metronomic capecitabine in 22 pre-treated patients with pancreato-biliary cancer [66]. Among 20 patients evaluable for response, seven (33%) achieved a disease stabilization lasting at least three months. A good performance status was associated with a longer survival.

The potential benefits of adding an induction regimen of anti-angiogenesis and antitumour immunity to chemotherapy in poor outcome disease has been explored in a prospective, randomized trial [67]. Patients affected with several advanced, unresectable cancers, including 60 cases of PDAC, were randomized to standard chemotherapy or chemotherapy plus an induction regimen of antiangiogenesis and antitumour immunity (metronomic CTX, high dose cyclooxygenase-2 inhibitor, granulocyte colony-stimulating factor, sulfhydryl donor, and a hemoderivative that contained autologous tumour antigens released from patient tumours into the blood). An increase in survival and higher blood levels of anti-angiogenesis and antitumour immunity mediators were found in patients treated with the induction regimen added to standard chemotherapy.

Radiotherapy combined with chemotherapy is usually considered as a means to control locally advanced PC without systemic metastases, although in a randomized study it was not observed to be superior to continuing chemotherapy in patients with controlled locally advanced PC after four months of induction chemotherapy [68].

Gemcitabine, which has demonstrated a potent radiosensitizer effect in both *in vivo* and *in vitro* experiments [69], is generally used at a weekly single dose *i.e.* 250 mg/m² when combined with radiotherapy.

Following preclinical data showing gemcitabine maximum radiation sensitization at a low-dose of 40 mg/m², twice weekly [70], clinical studies reported an improved local control of advanced PC [71,72] by using such schedule. The combination with either hyperfractionated accelerated or standard fractionated radiotherapy demonstrated both good tolerability and tumour regression rate up to 36% [72]. Disappointingly, no survival advantage was observed [71].

The antitumour effects of intravenous or intra-arterial low-dose gemcitabine plus oral tegafur-uracil and CTX in combination with RT were tested in 22 patients with recurrent and advanced PC [73]. This regimen was considered well-tolerated and worthy of further investigations on account of the clinical benefit response in 22.7% of the patients, classified by measuring the pain intensity, analgesic consumption, Karnofsky performance status and body weight.

Conclusions

The interesting theoretical premises of MCT account for a high number of studies carried out in several tumour types [29]. Pre-clinical data in the field of PC have shown effects of MCT on cancer cells, the tumour microenvironment and the immune response. Although different tumour models have been used, MCT and particularly the C-S strategy emerge as promising therapeutic options to counteract the growth of PC. However, because only a few, and not definitive, clinical studies have up to now reported results of MCT in patients affected with PC, conclusions applicable to clinical practice cannot be drawn. Notwithstanding, metronomic schedules have been described as moderately active and well tolerated with only mild toxicity. Thus, results from those phase II studies (NCT02368860, NCT02620800) evaluating the most effective drug combinations for PC as metronomic protocols are expected. In fact, if successful, they allow for further proceeding to confirmatory trials. Metronomic maintenance chemotherapy has already shown some advantages versus observation in both advanced colorectal cancer and early stage breast cancer [74]. Such achievements make maintenance therapy one of most worthwhile developments for MCT even in PC. Several strategies are under investigation with standard chemotherapy [NCT02368860, NCT02620800], target agents [NCT02620800, NCT01203306] and immunotherapy [NCT00727441, NCT01088789] but further efforts

are needed to fill the gaps of knowledge in this area. Furthermore, as recently emphasized, much research is awaited to define optimal metronomic schedules on the basis of their pharmacokinetic and pharmacodynamic properties [75]. Moreover, although recent reports have suggested some gene polymorphisms as putative biomarkers of response to metronomic capecitabine [76], the recognition and validation of predictive biomarkers remain to be addressed more fully.

Conflict of interest

None.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.canlet.2017.02.017>.

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