Phase I trial of M_2ES, a novel polyethylene glycosylated recombinant human endostatin, plus gemcitabine in advanced pancreatic cancer

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Abstract. Pancreatic cancer is one of the most lethal and resistant to treatment of solid tumors. Combination therapies with various types of drugs against pancreatic cancer have been extensively investigated. Endostatin is a potent endogenous inhibitor of angiogenesis, which may be administered in combination with various chemotherapeutic agents in the treatment of several types of cancer. To the best of our knowledge, this phase I trial was the first clinical study to determine the tolerance, safety and efficacy of M_2ES, a novel polyethylene glycosylated recombinant human endostatin, administered concurrently with full-dose gemcitabine in patients with inoperable, locally advanced or metastatic pancreatic adenocarcinoma. A total of 16 patients were treated with gemcitabine (1,000 mg/m^2 on days 1, 8 and 15) and M_2ES (5-45 mg/m^2 on days 1, 8, 15 and 21) of each 28-day cycle. In 15 evaluable patients, the stable disease rate (SDR) was 40% (95% CI: 11.9-68.1%). In particular, a 75% SDR was observed in 3 out of 4 patients with a M_2ES dose level of 7.5 mg/m^2. The most noticeable M_2ES-related adverse events observed during the trial were grade 2 liver function abnormalities (6.3%) and grade 1 skin rash (6.3%). No dose-limiting toxicity was observed in any patients from all the dose levels. Therefore, there was no increased toxicity associated with the addition of M_2ES to gemcitabine and this combination was well tolerated.

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Introduction

Pancreatic cancer is amongst the deadliest types of cancer, with a 5-year survival rate of 3-5% (1,2). The diversity of the abnormalities in multiple aspects during malignant transformation, resulting in tumor heterogeneity in the majority of cancers, is one of the major challenges in cancer therapeutics. Strategies that combine various anticancer agents with activity against multiple targets have emerged as mainstream applications in the era of drug development.

Endostatin, a 20-kDa C-terminal fragment of type XVIII collagen, is a potent endogenous angiogenesis inhibitor (3). Endostatin potently constrains the proliferation and migration of endothelial cells, therefore hindering tumor angiogenesis and tumor growth (3,4). Our previous studies demonstrated that endostatin exhibits therapeutic activities against multiple levels of angiogenesis, lymphangiogenesis and tumor progression (5-7). Although the clinical trials of wild type recombinant human endostatin were terminated at early phase II in the United States (8), an N-terminal modified recombinant human endostatin has been approved by the China Food and Drug Administration for the treatment of non-small-cell lung cancer (9). The N-terminal modified recombinant human endostatin has been one of the most widely-used anti-angiogenic drugs in the clinics for seven years in China. However, due to its relatively small molecular mass, endostatin has a short circulating half-life *in vivo*, requiring a frequent dosing regimen (daily intravenous infusion). In order to increase the molecular mass of endostatin and improve its pharmacokinetics, we utilized polyethylene glycol (PEG), a widely used polymer for the covalent modification of biological/pharmaceutical macromolecules (10), to produce an N-terminal mono-PEGylated recombinant human endostatin, M_2ES, with a significantly longer half-life.

Accumulating evidence indicate that antiangiogenic inhibitors may achieve optimal therapeutic efficacy upon combination with other antitumor agents, such as chemotherapeutic drugs (11). Gemcitabine (2',2',-difluoro-2',-deoxycytidine) is a

nucleoside analogue of deoxycytidine that is considered to be effective in the treatment of inoperable, locally advanced or metastatic pancreatic adenocarcinoma (12,13). Gemcitabine has been broadly utilized as a cytotoxic chemotherapeutic agent in several human solid tumors, including non-small-cell lung, breast, bladder, ovarian and pancreatic cancers (14-16).

To the best of our knowledge, this study is the first clinical trial of a PEGylated recombinant human endostatin in patients with advanced cancer. In this phase I trial, a M_2ES dose escalation was conducted in combination with gemcitabine, which was administered at the current recommended dose (1,000 mg/m^2 three times weekly for every 28-day cycle). The primary objective was to determine the tolerance, safety and efficacy of M_2ES concurrently administered with full-dose gemcitabine in patients with inoperable advanced pancreatic adenocarcinoma. The overall hypothesis was that this combination regimen with M_2ES and chemotherapy, which targets endothelial cells (angiogenesis) and tumor cells, respectively, may improve tumor control in multiple aspects, whereas data regarding tolerance and efficacy may support further investigation of this combination regimen.

Patients and methods

Eligibility. Patients with inoperable, locally advanced or metastatic pancreatic adenocarcinoma (TNM stage III or IV) were considered to be eligible for this trial. Histological or cytological confirmation of pancreatic adenocarcinoma was required. Patients who had received no prior chemotherapy were considered eligible, whereas patients who had received prior radiotherapy were eligible when the irradiated lesion was not the only measurable lesion.

Additional eligibility criteria included the following: i) age 18-60 years; ii) Karnofsky performance status score ≥60; iii) evaluable disease; iv) adequate hematopoietic (white blood cell count ≥4x10^9/l; absolute neutrophil count ≥1.5x10^9/l; platelet count ≥100x10^9/l; and hemoglobin concentration ≥9 g/dl), hepatic (bilirubin ≤2 x the upper limit of normal (ULN); aspartate aminotransferase and alanine aminotransferase ≤1.5 x ULN or ≤3.0 x ULN for subjects with hepatic metastases) and renal function (creatinine clearance ≥50 ml/min; serum creatinine ≤1.5 x ULN); and v) no clinically evident severe cardiovascular disorders (e.g., congestive heart failure, severe cardiac arrhythmias, active coronary artery disease or ischemia). The patients provided written informed consent in accordance with the local and institutional guidelines prior to enrollment.

Study design. This open-label, non-placebo-controlled phase I trial was conducted to determine the tolerance, safety, efficacy, and recommended phase II dose (RPTD) of M_2ES administered concurrently with full-dose gemcitabine in patients with advanced pancreatic cancer. Gemcitabine was administered weekly at a fixed dose of 1,000 mg/m^2 in a 30-min intravenous infusion for 3 consecutive weeks (on days 1, 8 and 15), followed by a 1-week break. M_2ES was administered at escalating doses intravenously 1 h after gemcitabine administration on days 1, 8, 15 and 21. Each cycle was defined as 28 days, with dose escalation decisions made based on the safety data of each cohort from the first cycle of concomitant administration of M_2ES plus gemcitabine. Gemcitabine was purchased from Eli Lilly (Indianapolis, IN, USA) and M_2ES was provided by Protgen Ltd. (Beijing, China).

Table I. Dose escalation schema.

Dose levels	1	2	3	4	5
M_2ES (mg/m^2)	5	7.5	15	30	45
Gemcitabine (mg/m^2)	1,000	1,000	1,000	1,000	1,000
No. of patients	3	4	3	3	3

Table II. Patients' characteristics (n=16).

Characteristics	No. (%)
Total number of patients	16
Age (years)	
Median	46
Range	28-50
Gender, n (%)	
Male	12 (75%)
Female	4 (25%)
Ethnicity	
Asian	16 (100%)
Stage, n (%)	
III	8 (50%)
IV	8 (50%)
Metastasis	
Yes	8
No	8

In this dose-escalation phase I study, 5 dose levels were designed. The gemcitabine dose was fixed at 1,000 mg/m^2 with escalating doses of M_2ES as follows: dose level 1, 5 mg/m^2; dose level 2, 7.5 mg/m^2; dose level 3, 15 mg/m^2; dose level 4, 30 mg/m^2; and dose level 5, 45 mg/m^2. Three patients were initially enrolled in the first dose level. In the absence of a dose-limiting toxicity (DLT) at the end of the first 4-week treatment cycle, 3 additional patients were enrolled into the next dose level. If in any dose level, ≥2 patients developed a DLT, the maximum tolerated dose (MTD) was considered exceeded and the dose level immediately preceding was considered as the RPTD. If the frequency of DLT encountered at the highest dose level did not fulfill the MTD definition, 45 mg/m^2 M_2ES with 1,000 mg/m^2 gemcitabine was considered as the RPTD. DLT was defined as follows: ≥grade 2 neurotoxicity; ≥grade 3 haematological toxicity; ≥grade 3 non-haematological toxicity (except alopecia and unpremedicated nausea/vomiting) and elevations in alkaline phosphatase levels. The toxicities and adverse events of this protocol were graded according to the National Cancer Institute Common Toxicity Criteria, version 3.0 (17).

Patient evaluations. The stable disease rate (SDR) evaluated in the present trial was defined as the percentage of patients

Table III. Response types and stable disease rate (SDR=CR+PR+SD).

Dose levels	1	2	3	4	5	Total
No. of patients	3	4	3	3	3	16
Response (n)						
SD	1	3	-	1	1	6
PD	2	1	2	2	2	9
Not evaluable	-	-	1	-	-	1
SDR, % (95% CI)	33.3	75	0	33.3	33.3	40 (11.9-68.1)

SDR, stable disease rate; CR, complete response; PR, partial response; SD, stable disease; CI, confidence interval.

Table IV. Toxicities experienced during the observation period of the study.

	Grade			
Toxicity, n (%)	1	2	3	4
Myelosuppression	1 (6.3%)	2 (12.5%)	-	-
Thrombocytopenia	2 (12.5%)	-	-	-
Leukopenia	1 (6.3%)	-	-	-
Hyponatremia	1 (6.3%)	-	-	-
Electrolyte disturbance	1 (6.3%)	-	-	-
Haemorrhage	-	-	-	1 (6.3%)
Arrhythmia	1 (6.3%)	-	-	-
Dyspnea	1 (6.3%)	-	-	1 (6.3%)
Abnormal liver function	1 (6.3%)	2 (12.5%)	-	-
Jaundice	-	1 (6.3%)	1 (6.3%)	-
Cutaneous	2 (12.5%)	-	-	-
Fever	2 (12.5%)	1 (6.3%)	-	-
Pain	-	3 (18.8%)	-	-
Fatigue	1 (6.3%)	-	-	-
Diarrhoea	1 (6.3%)	-	-	-
Urinary abnormalities	1 (6.3%)	-	-	-

who exhibited complete response (CR), partial response (PR) and stable disease (SD). CR was defined as complete resolution of all evidence of measurable tumor during the time of evaluation. PR was defined as ≥50% reduction in the tumor volume, without the appearance of new lesions. SD was defined as a <50% reduction to a <25% increase in tumor volume. Progressive disease (PD) was defined as an increase of the tumor lesions by >25% or the occurrence of new lesions. Chemotherapy-related toxicities were assessed according to the common toxicity criteria for the grading of acute and subacute side effects. Radiographic assessments were conducted to determine tumor response according to the Response Evaluation Criteria in Solid Tumor (18,19).

Results

Patient characteristics. Between July 8, 2010 and January 13, 2011, a total of 16 patients with inoperable, locally advanced or metastatic pancreatic adenocarcinoma (stage III or IV) were enrolled and assigned to 5 dose levels (Table I). The patient characteristics are summarized in Table II. The enrolled patients had received no prior gemcitabine or gemcitabine-based chemotherapy combination. Eight patients presented with locally advanced (stage III) and 8 patients had metastatic (stage IV) disease. The median age of the patients was 46 years (range, 28-50 years).

Response. Fifteen out of the 16 patients were evaluable for response. The response to combination therapy is summarized in Table III. Of the 15 evaluable patients, 6 patients achieved SD with therapy. The SDR was 40% (95% CI): 11.9-68.1]. In particular, 3 of the 4 patients in dose level 2 (7.5 mg/m^2 M_2ES) had SD, exhibiting a significantly higher SDR (75%) compared to that in other dose levels.

Toxicity, adverse events and mortality. The toxicities occurring during this study are presented in Table IV. There were 2 deaths during the study, not treatment-related but rather due

to PD. The first patient death (in dose level 3) on day 43 was attributed to bleeding from a duodenal metastasis (grade 4 haemorrhage). The second patient death (in dose level 4) on day 56 was attributed to grade 4 respiratory failure. No severe adverse events attributable to treatment were observed.

The most frequently reported treatment-related adverse events in the study were grade 1/2 myelosuppression (3 patients; 18.8%), grade 1 thrombocytopenia (2 patients; 12.5%) and grade 1 leukopenia (1 patients; 6.3%), all of which were commonly associated with gemcitabine administration. The most noticeable M_2ES-related adverse events observed during the trial were grade 2 liver function abnormalities (1 patient; 6.3%) and grade 1 skin rash (1 patient; 6.3%). As no DLT of M_2ES in combination with gemcitabine was observed in any of the dose levels, the MTD was not reached in this study.

Discussion

Pancreatic cancer is among the most lethal and resistant to treatment solid tumors and is associated with a high mortality. Combination therapies with gemcitabine and other agents (such as 5-fluorouracil, cisplatin, oxaliplatin and irinotecan) have been extensively investigated (20-23). Recent studies on erlotinib, an epidermal growth factor receptor inhibitor, plus gemcitabine, provided the foundation for approval of such regimens for the treatment of advanced pancreatic adenocarcinoma (24) and reported a SDR of 53-59.3% (25,26). The most frequently reported treatment-related toxicities of gemcitabine, alone or in combination with other drugs, are leukopenia, thrombocytopenia and neutropenia (26,27).

This phase I trial demonstrates that M_2ES and gemcitabine may be safely administered in combination at full dose, with no consistent pharmacokinetic interaction between these two drugs. There were no reported treatment-related grade 3/4 haematological or non-haematological toxicities in this study, with the most common treatment-related adverse events being myelosuppression (18.8%), thrombocytopenia (12.5%), leukopenia (6.3%), liver function abnormalities (6.3%) and cutaneous reactions (6.3%). These adverse events were compatible with those expected with the administration of these two agents. Specifically, myelosuppression, thrombocytopenia and leukopenia were attributed to the administration of gemcitabine, whereas liver function abnormalities and cutaneous reactions are presumably associated with M_2ES treatment. In general, M_2ES exhibited good safety and tolerability when combined with gemcitabine, with only few, if any, adverse effects. Furthermore, this PEGylated endostatin did not exhibit increased toxicity when compared to the original recombinant endostatin (28,29).

Since the frequency of DLTs encountered at the highest dose level did not fulfill the MTD definition, 45 mg/m^2 M_2ES (1,000 mg/m^2 with gemcitabine) may be considered as the RPTD. Notably, we observed a 75% SDR in 3 out of the 4 patients in one of the moderate dose levels (7.5 mg/m^2 M_2ES). Thus, 7.5 mg/m^2 is a potential option for the RPTD of M_2ES. Further evaluations, such as in a small-scale phase II clinical trial of this combination therapy with 7.5 and 45 mg/m^2 M_2ES, are recommended, in order to compare the efficacy of these two doses of M_2ES when combined with gemcitabine in pancreatic cancer patients.

In this phase I study, the preliminary efficacy evaluations yielded relatively encouraging results. Although in this limited cohort of patients we did not observe a significantly improved objective response rate with the combination of M_2ES and gemcitabine when compared to single-agent gemcitabine, we observed a clinical benefit (SDR, 40%) in 6 of the 15 patients. Specifically, we observed a 75% SDR in one of the moderate dose levels (7.5 mg/m^2 M_2ES), which is considered a rather favorable result for a phase I trial in non-operable advanced pancreatic cancer patients and supports further development of this combination with M_2ES and gemcitabine in this type of cancer.

In conclusion, to the best of our knowledge, this study was the first clinical trial of a PEGylated recombinant human endostatin, M_2ES, in advanced pancreatic cancer patients. The combination of M_2ES and gemcitabine was generally well tolerated, with no pharmacokinetic interaction in patients with inoperable, locally advanced or metastatic pancreatic adenocarcinoma. Full therapeutic doses of M_2ES (5-45 mg/m^2) and gemcitabine (1,000 mg/m^2) may be administered without reaching an MTD. A phase II clinical study is required to further evaluate the safety and efficacy of the combination of M_2ES and gemcitabine for the treatment of patients with advanced pancreatic cancer.

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