Phase 1 dose-escalation study of pegylated arginine deiminase, cisplatin and pemetrexed in patients with argininosuccinate synthetase 1-deficient thoracic cancers

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PURPOSE: Pegylated arginine deiminase (ADI-PEG 20) depletes essential amino acid levels in argininosuccinate synthetase 1 (ASS1) negative tumors by converting arginine to citrulline and ammonia. The main aim was to determine the recommended dose, safety and tolerability of ADI-PEG 20, cisplatin and pemetrexed in patients with ASS1-deficient malignant pleural mesothelioma (MPM) or non-small cell lung cancer (NSCLC).

PATIENTS AND METHODS:

Using a 3+3+3 dose escalation study, nine chemonaïve patients (5 MPM, 4 NSCLC) received weekly ADI-PEG 20 doses of 18 mg/m^2, 27 mg/m^2, or 36 mg/m^2, alongside three-weekly pemetrexed 500 mg/m^2 and cisplatin 75 mg/m^2 (maximum of six cycles) (ADIPemCis). Patients achieving stable disease or better could continue ADI-PEG 20 monotherapy until disease progression or withdrawal. Adverse events (AE) were assessed by CTCAE version 4.03, pharmacodynamics and immunogenicity were also evaluated. Tumor response was assessed by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 for NSCLC and modified RECIST criteria for MPM.

RESULTS: No dose limiting toxicities (DLT) were reported; 9/38 reported AE (all Grade 1/2) were related to ADI-PEG 20. Circulating arginine concentrations declined rapidly and citrulline levels increased, both changes persisted at 18 weeks. Partial responses were observed in 7/9 (78%) patients, including three with either sarcomatoid or biphasic MPM.

CONCLUSION: Target engagement with depletion of arginine was maintained throughout treatment with no DLT. In this biomarker selected group of patients with ASS1-deficient cancers clinical activity was observed in patients with poor prognosis tumors. Taken together, we recommend a dose for future studies of weekly ADI-PEG 20 (36 mg/m^2) plus three-weekly cisplatin (75 mg/m^2) and pemetrexed (500 mg/m^2).

Introduction

Standard of care first-line cytotoxic chemotherapy for many patients with advanced malignant pleural mesothelioma (MPM) and non-squamous, non-small cell lung cancer (NSCLC) is cisplatin plus pemetrexed. The overall prognosis for these patients remains poor despite treatment and the majority of patients still only survive 12 months.[1,2] The development of novel treatment approaches is therefore of paramount importance for these patients.

Arginine is a semi-essential amino acid, which in normal cells, can be synthesized de-novo in the urea cycle from citrulline combined with aspartate in the presence of ATP, in addition to direct uptake of extracellular arginine. However, it has been found that a number of tumor types (e.g. melanoma, prostate and ovarian cancers) have abnormalities in arginine synthesis pathways such that tumor cells are not able to synthesize arginine de novo and are dependent on an exogenous supply for growth (termed arginine auxotrophy).[3] Arginine promotes tumor growth, and the observation that arginine-depleted feed is associated with reduced xenograft tumor growth was made in 1930.[4] More recently, arginine deprivation studies performed using selected in vitro cancer cell lines reported apoptosis of up to 80% of cells.[5]

A key enzyme in the biosynthesis of arginine is argininosuccinate synthetase 1 (ASS1), which combines citrulline with asparate to form argininosuccinate. Intratumoral deficiency of ASS1 has been detected in significant numbers of patients with cancers including mesothelioma and NSCLC.[6,7] Epigenetic modification via aberrant methylation of the ASS1 promoter is proposed to underlie this deficiency, especially in mesothelioma.[7,8] Importantly, low ASS1 expression is associated with a more aggressive clinical phenotype and worse clinical outcome in several different cancer types.[9-12]Moreover, it is known that patients resistant to the antifolate pemetrexed have high levels of thymidylate synthase (TS) and low levels of ASS1.[11] Indeed, recent work indicates that low ASS1 promotes diversion of aspartate for pyrimidine

synthesis and enhanced tumor cell proliferation.[13] Thus, it is noteworthy that patients selected on the basis of low ASS1 expression are likely to be a cohort with worse prognosis and poor response to currently used chemotherapy regimens.

Arginine deiminase (ADI) catalyzes conversion of arginine to citrulline, thereby depleting the former in ASS1-deficient tumor cells. The addition of polyethylene glycol (PEG) to arginine deiminase increases bioavailability and decreases immunogenicity such that ADI-PEG 20 was developed for clinical use.[14] ADI-PEG 20 was well-tolerated in early clinical trials as a single agent with promising biological activity including patients with hepatocellular carcinoma, metastatic melanoma and mesothelioma.[15-20] More recently, we showed for the first time that single agent ADI-PEG 20 significantly extended progression-free survival for patients with ASS1-deficient mesothelioma compared with best supportive care alone (ADAM study).[8]

In preclinical studies, it has been shown that the combination of arginine deprivation, (using ADI-PEG 20) alongside pemetrexed leads to a potentiation of cytotoxicity in ASS1-negative tumor cells. This was accompanied by suppression of de novo thymidine synthesis with decreased levels of thymidylate synthase (TS) and the salvage pathway via reduced thymidine kinase 1 (TK1).[11] Indeed, several pharmacologic and metabolic tracing studies support the enhanced sensitivity to antifolate agents in arginine auxotrophs exposed to arginine depleting agents.[13,21] Furthermore, the addition of cisplatin to ADI-PEG 20 exerted at least additive anti-cancer effects in both tissue culture and xenograft models of melanoma and this mechanism is thought to be due, at least in part, to inhibition of DNA repair enzymes.[22]

We undertook a phase 1 dose escalation study of ADI-PEG 20 combined with pemetrexed and cisplatin (ADIPemCis) in the first line treatment of patients with non-squamous NSCLC or mesothelioma tumors that were ASS1-deficient. The main aims of the study were to define the toxicity profile of the combination, to recommend phase 2 doses for ADIPemCis, and to investigate pharmacodynamic alterations in arginine metabolism.

Patients and Methods

Study Design and Treatment

This was a multi-center, open label, phase 1 dose-escalation trial designed to evaluate the safety and tolerability of ADI-PEG 20 combined with cisplatin and pemetrexed in patients with histologically proven advanced MPM and stage IIIB/IV non-squamous NSCLC. A 3+3+3 phase 1 dose-escalation design was utilized to accommodate the predicted toxicity of pemetrexed and cisplatin.[1,23]

Pre-planned dose levels of weekly intramuscular ADI-PEG 20 were at 18 mg/m^2, 27 mg/m^2, and 36 mg/m^2 alongside intravenous treatment with intravenous cisplatin (75 mg/m^2) and pemetrexed (500 mg/m^2) every three weeks. The initial dose of ADI-PEG 20 was administered at least 48 hours before the first dose of cytotoxic drugs. To ameliorate toxicity from pemetrexed patients received daily folic acid supplementation (400 mcg) and intramuscular hydroxycobalamin (1000 mcg, every 9 weeks) both started at least 7 days prior to the first dose.

At least three patients were investigated at each dose level for a minimum of 21 days before escalation to the next cohort dose level. There was no intra-patient dose escalation. Patients continued ADIPemCis combination therapy for a maximum of 6 cycles (18 weeks). Patients with clinical benefit (stable disease or better) were eligible to receive continued single agent ADI-PEG 20 treatment until disease progression.

The primary objectives of the trial were to evaluate the safety and tolerability of the combination (ADIPemCis) treatment, and to establish a recommended phase 2 dose (RP2D). Secondary objectives were to determine the progression free survival, overall survival at one year, and the pharmacodynamics of ADI-PEG 20 in combination with pemetrexed and cisplatin.

The study was performed in accordance with good clinical practice (GCP) and the EU Clinical Trials Directive with approval from Leeds East (Yorkshire and The Humber) ethical review board. All patients provided written, informed consent. The study was registered on the National Institutes of Health clinical trials database, NCT02029690.

Eligibility

Patients were aged 18 years or over with histologically proven advanced MPM, or stage IIIB/IV non-squamous NSCLC. In addition, tumors were ASS1-deficient by immunohistochemistry (IHC), which was defined as loss of ASS1 expression (0 or 1+ IHC staining)[7] in >50% tumor cells. The 50% threshold was selected on the assumption that this would enrich the fraction of tumor cells likely to respond to arginine deprivation based on multiple prior cell line studies showing an inverse correlation between ASS1 expression and sensitivity to arginine depletion.[3,24] Specifically, a statistically significant improvement in progression-free survival for patients with mesothelioma was observed in the ADAM study using the 50% cutoff, with the hazard ratio decreasing further for tumors with >75% ASS1 loss.[8] Patients had evaluable disease by modified RECIST criteria for MPM and by RECIST 1.1 criteria for non-squamous NSCLC. All patients were chemotherapy naïve. Additional criteria included ECOG performance status 0 or 1, adequate hematological, hepatic and renal function, and minimum expected survival of 3 months. Exclusion criteria included: anti-cancer therapy within 4 weeks of entering study; ongoing toxic manifestations of previous treatments; symptomatic brain or spinal cord metastases; significant concomitant or uncontrolled intercurrent illness; recent major surgery; therapeutic anticoagulation; participation in another interventional clinical study; history of another primary cancer (unless curatively treated or unlikely to affect patient outcome); allergy to platinum salts, pegylated or *E. coli* products; pregnancy; history of seizure disorder and prior therapy with ADI-PEG 20.

Safety Evaluations

Baseline characteristics of age, sex, performance status and histology were collected for all patients. Physical examination was performed on Day 1 of every cycle and at other times as clinically indicated. All treated patients were evaluated for safety by laboratory tests, physical examination, and adverse event assessment at screening, and at every 3 weeks during therapy. All adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 4.03. Adverse event (AE) monitoring continued up until 30 days after the final treatment, and AEs related to ADI-PEG 20 were continued until stabilization or resolution. Dose limiting toxicities (DLT) were assessed during cycle one (21 days) as AEs that were possibly, probably or definitely related to study treatment including: Grade 4 neutropenia (> 7 days duration); febrile neutropenia; Grade 4 anemia (requires transfusion therapy); Grade 4 thrombocytopenia; or Grade 3/4, non-hematologic toxicity (except Grade 3 nausea, vomiting or diarrhea that resolves to a lower grade with supportive treatment within 7 days, Grade 3 AST/ALT elevation without accompanying increase in bilirubin, alopecia, electrolyte abnormalities/ other Grade 3-4 asymptomatic laboratory evaluations only be deemed as DLT if assessed by the Investigator as clinically significant); delay in cycle 2 more than 3 weeks. Patients were evaluable for DLT if they had received at least 1 dose of ADI-PEG 20. Non-evaluable patients (those not receiving at least 1 dose of ADI-PEG 20) were replaced.

Pharmacodynamic Evaluations

Blood samples were taken prior to each dose of ADI-PEG 20 to analyze arginine and citrulline levels and immunogenicity analyses, as described previously.[8]

Efficacy Evaluations

Computed tomography (CT) scans were performed every 6 weeks during ADIPemCis combination dosing and after every 8 weeks during ADI-PEG 20 only treatment. Tumor measurements were recorded and assessed according to RECIST 1.1 criteria or modified RECIST criteria.

Results

Nine eligible (4 NSCLC and 5 MPM) patients were treated. Three patients were treated in each of the planned 18mg/m^2, 27mg/m^2, and 36mg/m^2 ADI-PEG 20 cohorts. A majority of patients managed to complete 6 cycles of combination treatment, median weeks on treatment was 23.5 (range 13 – 47) for patients with NSCLC and 31.0 (range 30 - 47) for patients with MPM. Patients' characteristics and disposition are summarized in Table 1 and Figure 1.

Safety

The majority of side effects were Grade 1 (83% of total) or Grade 2. All adverse events are summarized in Table 2. The most common toxicities were fatigue, nausea, vomiting, oropharyngeal toxicity (stomatitis, mucositis and oral candidiasis) and rash. Hypersensitivity was observed for 1 patient and was attributed to cisplatin; this patient was successfully re-challenged with ADI-PEG 20. Febrile neutropenia was not observed; Grade 3 neutropenia was reported in 1/9 (11%) patients. There were no DLTs or treatment-related deaths. Most adverse events were attributed to (and expected as a result of) pemetrexed and/ or cisplatin therapy, only 9/38 (Grade 1/2) reported adverse events were related to ADI-PEG 20. In this small number of patients there was no clear dose-response relationship between ADI-PEG 20 dose level and toxicity.

Pharmacodynamics

Despite variability due to the small sample size, circulating plasma arginine concentrations were rapidly depleted in all patients and remained suppressed at approximately 30% of baseline levels, throughout the 18 weeks of triplet therapy. Correspondingly plasma levels of the ADI-PEG20 product citrulline increased rapidly and remained elevated during the same dosing period (See Figure 2). Anti-ADI-PEG 20 antibody titers are shown in Figure 3. In

summary, titers of anti-drug antibodies gradually increased, appeared to plateau at week 8-10, and remained below 10^{-4} by week 18.

Response

All patients experienced a best response of stable disease or better. Furthermore, 7/9 (78%) patients achieved a partial response, which was seen at all dose levels investigated. Median overall survival (OS) was 55.7 weeks and median progression free survival (PFS) was 30.1 weeks. The median OS was 56.4 (range 30.7 – 105.1+) weeks and median PFS was 30.7 (range 27.9 – 38.0) weeks for patients with MPM, whereas for patients with NSCLC median OS was 55.5 (range 25.7 – 56.7) weeks and median PFS was 23.0 (range 12.7 – 41.0) weeks. Individual patient data are summarized in Figure 4.

Discussion

In this biomarker-directed study we have presented data on the first combination of the arginine-depleting agent (ADI-PEG 20) with cisplatin and pemetrexed (ADIPemCis). Patients with ASS1-deficient thoracic tumors were treated for up to six cycles and treatment was well-tolerated overall. The most common side effects were nausea and vomiting at a frequency comparable to that seen in previous studies of cisplatin and pemetrexed. The addition of ADI-PEG 20 prompted an increase in rash as the most common side effect. One hypersensitivity reaction occurred and this was during the administration of cisplatin. This patient was subsequently re-challenged with ADI-PEG 20 successfully. All of the toxicities reported as being possibly or probably related to ADI-PEG 20 were Grade 1 or 2 in nature. No dose limiting toxicities were reported.

Arginine suppression with the ADIPemCis triplet was more prolonged than observed previously with ADI-PEG 20 monotherapy. In the ADAM monotherapy study, arginine was depleted to below 30% of baseline for 7 weeks, but by 9 weeks arginine concentrations returned to pre-treatment levels with a corresponding fall in citrulline concentrations.[8] Arginine suppression was also detected for the first 8 weeks only before reaching baseline line levels by week 12 in a recent phase 1 combination study of ADI-PEG20 combined with docetaxel in solid tumors.[25] In contrast, with the ADIPemCis regimen, arginine concentrations remained depleted compared to baseline levels, until the end of treatment, and citrulline concentrations were persistently elevated. Anti-ADI-PEG 20 antibodies increased gradually then appeared to reach a plateau around 10^{-4} by 13 weeks. Interestingly, this was in contrast to samples taken from patients treated within the monotherapy and docetaxel combination study where anti-ADI-PEG 20 antibodies increased rapidly and reached 10^{-4} by 9 weeks followed by continued increase in titers. Prolonged arginine depletion may be due to suppression of neutralizing anti-ADI-PEG 20

antibodies by pemetrexed and cisplatin, especially cisplatin and the use of concomitant steroids as chemotherapy premedication.[26]

We report an interesting clinical activity signal in this small patient cohort, with an overall RECIST response rate of 78%. Previous studies reported response rates for cisplatin and pemetrexed given to patients with NSCLC and MPM of 31% and 41% respectively.[1,2] Importantly, two patients with biphasic and one with sarcomatoid MPM treated with ADIPemCis in this study have achieved partial response. Sarcomatoid histology is considered resistant to chemotherapy, and with the combination of pemetrexed, cisplatin and bevacizumab there were no responses reported amongst the 5 patients with sarcomatoid MPM.[27] A recent epidemiological study reported a median OS of 13.3 versus 6.2 months for patients with epithelioid and non-epithelioid MPM respectively.[28] A recently reported genomic study of MPM confirmed that the prognosis of patients with sarcomatoid subtype was worse than biphasic (predominantly sarcomatoid), compared to biphasic (predominantly epithelioid) and compared to epithelioid. Interestingly, this outcome correlated with significant downregulation of the ASS1 gene in sarcomatoid compared to epithelioid MPM.[29]One of our patients with biphasic MPM remains alive 26 months following presentation with a debilitating steroid-refractory anti-cyclic citrullinated peptide (CCP) antibody positive paraneoplastic arthritis of the hands, described previously with several cancers but not MPM.[30]The arthritis resolved within the first cycle of ADIPemCis, and then fluctuated at a lower intensity while on ADI-PEG20 monotherapy.

The median PFS and OS outcomes for the study overall are within the range expected for platinum and pemetrexed doublets but with less aggressive cancers. The clinical activity signal described, in particular in patients with MPM, is being studied further in an expansion cohort including 18F-fluorothymidine positron emission tomography (FLT-PET) scanning and will be reported separately.[31]

Several other triplet combination phase I trials containing ADI-PEG 20 are ongoing including the combination of ADI-PEG 20 with gemcitabine and nab-paclitaxel in pancreatic cancer (NCT02101580) and ADI-PEG 20 with FOLFOX in the treatment of advanced gastrointestinal malignancies, especially hepatocellular carcinoma (NCT02102022). The key rationale is similar in that significant proportions of these tumors are auxotrophic for arginine and that disruption of arginine supply with ADI-PEG 20 suppresses in particular nucleotide metabolism.[13,32,33] These trials seek to emulate the success seen with asparaginase combination therapy in the treatment of acute lymphoblastic leukemia many years earlier where a signal was observed with monotherapy but a significant number of cures were not seen until it was combined with multiple other agents.[34] Further work will be needed to optimize anti-metabolite combinations based on careful biomarker analyses to identify the role of arginine deprivation in the clinic.[24]

In summary, our results are consistent with pre-clinical data that support a synergistic interaction of platinum-based and anti-folate chemotherapy with concomitant arginine depletion for patients selected for tumors deficient in ASS1. The triplet regimen has achieved very high response rates in this small trial, whilst being well-tolerated in patients with MPM and NSCLC. With limited treatments available for these patients this triplet combination warrants further study. The RP2D was weekly, intramuscular, ADI-PEG 20 (36 mg/m^2) plus three-weekly intravenous cisplatin (75 mg/m^2) and pemetrexed (500 mg/m^2). A randomized phase 2/3 trial for ASS1-deficient patients with MPM has opened (NCT02709512).

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TABLES

Table 1. Patient Characteristics

Age	62-77 years
Sex	6 Male : 3 Female
Diagnosis	4 NSCLC (adenocarcinoma)
	5 MPM (1 epithelioid, 2 biphasic, 2 sarcomatoid)
Performance status	PS0 = 1; PS1 = 8
ASS1 screening	38 patients screened
	17 (45%) negative for ASS1 expression
	Of those patients with ASS1 negative tumours 9/17 (53%) patients enrolled 8/17 (47%) were not eligible (rapid disease progression) 9 patients evaluable for primary endpoint.

Table 2. Adverse events reported for escalating doses of ADI-PEG 20 in combination with cisplatin and pemetrexed.

Toxicity	**Cohort 1 18 mg/m^2**		**Cohort 2 27 mg/m^2**		**Cohort 3 36 mg/m^2**		**Toxicity related to ADI-PEG 20** (all doses)**
CTCAE Grade (vs. 4.03)	**1/2**	**3**	**1/2**	**3**	**1/2**	**3**	**1/2**
Fatigue	3		2		1		
Nausea/Vomiting	2	1	2	1	2	1	**2**
Oropharyngeal toxicity (sore mouth, oral candidiasis)			1		3		
Dehydration	1		1		1		
Tinnitus	1				2		
Rash					2		**2**
Diarrhea					1		
Pruritus					1		
Dizziness					1		**1**
Peripheral Neuropathy			1				
Anorexia					1		
Expressive dysphasia			1				**1**
Thrombophlebitis			1				**1**
Syncope					1		**1**
Hypersensitivity*				1			
Neutropenia				1			
Lymphopenia			1				**1**

Toxicities listed are those possibly or probably related to treatment and are the number of events per dose and Grade.

***** Related to cisplatin, re-challenged with ADI-PEG 20 successfully. ****** Toxicity listed as possibly or probably related to ADI-PEG 20. These were all grade 1 or 2.

No Grade 4 toxicities were reported.

FIGURE LEGENDS:

Figure 1: Patient screening and enrollment

Commented [PS1]: New figure included of patients' disposition from screening to enrollment

Figure 2: Pharmacodynamics of arginine and citrulline in patients treated with ADIPemCis. Median serum concentrations of both arginine and citrulline are shown by week of treatment. Error bars shown are ± standard error of the mean.

Figure 3: Mean serum levels of anti-ADI-PEG 20 antibodies in all patients by week of treatment with ADIPemCis. Error bars shown are ± standard error of the mean.

Figure 4: (A) Progression-free survival shown for ADIPemCis alongside corresponding best tumor response (percentage change compared to baseline) for all patients. Dose cohort of each patient is indicated (1-3). **(B)** Sarcomatoid MPM displaying partial (PR) and stable (SD; resolution of the pleural effusion) responses (cohort 3)

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