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Arginine Deprivation With Pegylated Arginine Deiminase in Patients With Argininosuccinate Synthetase 1–Deficient Malignant Pleural Mesothelioma
A Randomized Clinical Trial

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IMPORTANCE Preclinical studies show that arginine deprivation is synthetically lethal in argininosuccinate synthetase 1 (ASS1)-negative cancers, including mesothelioma. The role of the arginine-lowering agent pegylated arginine deiminase (ADI-PEG20) has not been evaluated in a randomized and biomarker-driven study among patients with cancer.

OBJECTIVE To assess the clinical impact of arginine depletion in patients with ASS1-deficient malignant pleural mesothelioma.

DESIGN, SETTING, AND PARTICIPANTS A multicenter phase 2 randomized clinical trial, the Arginine Deiminase and Mesothelioma (ADAM) study, was conducted between March 2, 2011, and May 21, 2013, at 8 academic cancer centers. Immunohistochemical screening of 201 patients (2011-2013) identified 68 with advanced ASS1-deficient malignant pleural mesothelioma.

INTERVENTIONS Randomization 2:1 to arginine deprivation (ADI-PEG20, 36.8 mg/m^2, weekly intramuscular) plus best supportive care (BSC) or BSC alone.

MAIN OUTCOMES AND MEASURES The primary end point was progression-free survival (PFS) assessed by modified Response Evaluation Criteria in Solid Tumors (RECIST) (target hazard ratio, 0.60). Secondary end points were overall survival (OS), tumor response rate, safety, and quality of life, analyzed by intention to treat. We measured plasma arginine and citrulline levels, anti–ADI-PEG20 antibody titer, *ASS1* methylation status, and metabolic response by ^{18}F-fluorodeoxyglucose positron-emission tomography.

RESULTS Median (range) follow-up in 68 adults (median [range] age, 66 [48-83] years; 19% female) was 38 (2.5-39) months. The PFS hazard ratio was 0.56 (95% CI, 0.33-0.96), with a median of 3.2 months in the ADI-PEG20 group vs 2.0 months in the BSC group (P = .03) (absolute risk, 18% vs 0% at 6 months). Best response at 4 months (modified RECIST) was stable disease: 12 of 23 (52%) in the ADI-PEG20 group vs 2 of 9 (22%) in the BSC group (P = .23). The OS curves crossed, so life expectancy was used: 15.7 months in the ADI-PEG20 group vs 12.1 months in the BSC group (difference of 3.6 [95% CI, −1.0 to 8.1] months; P = .13). The incidence of symptomatic adverse events of grade at least 3 was 11 of 44 (25%) in the ADI-PEG20 group vs 4 of 24 (17%) in the BSC group (P = .43), the most common being immune related, nonfebrile neutropenia, gastrointestinal events, and fatigue. Differential *ASS1* gene-body methylation correlated with ASS1 immunohistochemistry, and longer arginine deprivation correlated with improved PFS.

CONCLUSIONS AND RELEVANCE In this trial, arginine deprivation with ADI-PEG20 improved PFS in patients with ASS1-deficient mesothelioma. Targeting arginine is safe and warrants further clinical investigation in arginine-dependent cancers.

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The incidence of malignant pleural mesothelioma is increasing in many parts of the world, with a median survival from diagnosis of less than 12 months.[1] The US and European mesothelioma incidence of 3000 and 5000 cases per year, respectively, reflects a continuing population at risk. Developing countries will be affected similarly as a result of widespread use of asbestos.[2] Systemic treatment is by means of platinum and antifolate chemotherapy.[3,4] Therapeutic advances have stalled for more than a decade.[5]

To our knowledge, we were the first to show that an exogenous supply of the amino acid arginine is critical for the survival of mesothelioma cell lines displaying loss of the urea cycle and arginine biosynthetic enzyme argininosuccinate synthetase 1 (ASS1).[6] Arginine is essential for biosynthesis of proteins, nitric oxide, and polyamines and contributes to proline and glutamate production.[7] A wide therapeutic window exists because exogenous arginine is dispensable for normal cells due to ASS1 expression, whereas its supply is essential for ASS1-negative cancers.[8] Tumors deficient in ASS1 display increased tumorigenesis due to diversion of the precursor aspartate for enhanced pyrimidine synthesis.[9,10] Loss of the tumor suppressor *ASS1* in mesothelioma cell lines, due partly to epigenetic silencing, was observed in 63% of archival mesotheliomas by immunohistochemical analysis, warranting therapeutic stratification of an arginine-depleting agent.[6]

Various ASS1-negative tumors have been shown to be sensitive to the arginine depleters, mycoplasmal-derived pegylated arginine deiminase (ADI-PEG20) and recombinant human arginases, in preclinical studies.[11,12] This led to several arginine deprivation studies in patients with hepatocellular carcinoma and melanoma with single-agent ADI-PEG20, showing low toxicity and evidence of efficacy.[13-16] A phase 3 registration trial in patients with hepatocellular cancer, a tumor with frequent ASS1 deficiency, is ongoing.[17]

We report the first prospectively biomarker-driven, randomized trial of ADI-PEG20 in patients with cancer (mesothelioma), the Arginine Deiminase and Mesothelioma (ADAM) study. We hypothesized that exogenous arginine is a critical amino acid for ASS1-deficient mesothelioma and that arginine deprivation would improve progression-free survival (PFS).

Key Points

Question What is the effect of arginine deprivation in patients with argininosuccinate synthetase 1 (ASS1)-deficient malignant pleural mesothelioma?

Findings In this phase 2 randomized clinical trial of 68 patients with ASS1-deficient mesotheliomas, arginine deprivation with pegylated arginine deiminase led to improved progression-free survival compared with patients receiving best supportive care.

Meaning Arginine deprivation with pegylated arginine deiminase warrants further clinical investigation in patients with ASS1-deficient malignant mesothelioma.

Methods

Patients

From March 2, 2011, to May 21, 2013, we screened 201 patients. Eligible patients were at least 18 years old with histological evidence of advanced ASS1-deficient malignant pleural mesothelioma (defined by >50% low expressor cells; BD Biosciences ASS1 antibody, 1:500 dilution with the BioGenex Super Sensitive Polymer-IHC Detection System and human liver controls); measurable disease by modified Response Evaluation Criteria in Solid Tumors (RECIST) criteria[18]; Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and life expectancy of at least 3 months; adequate bone marrow, hematologic, hepatic, and renal function; and gave written, informed consent. Patients who had received prior platinum-based chemotherapy were eligible after progression. A rebiopsy was permitted for ASS1 reassessment if a prechemotherapy baseline biopsy was ASS1 positive (n = 13).

Study Design

Randomized phase 2 nonblinded trial conducted across 8 cancer centers in the UK Clinical Research Network, after multicenter ethics approval (see trial protocol in Supplement 1).

Randomization

Patients were enrolled by research nurses. Randomization (2:1) and allocation concealment was performed by telephoning the Trials Center, where a computer program (generated by a programmer without further involvement in the trial) allocated patients to a treatment arm using minimization, stratified according to sex, sarcomatoid or nonsarcomatoid subtype, chemonaive or prior chemotherapy, and hospital.

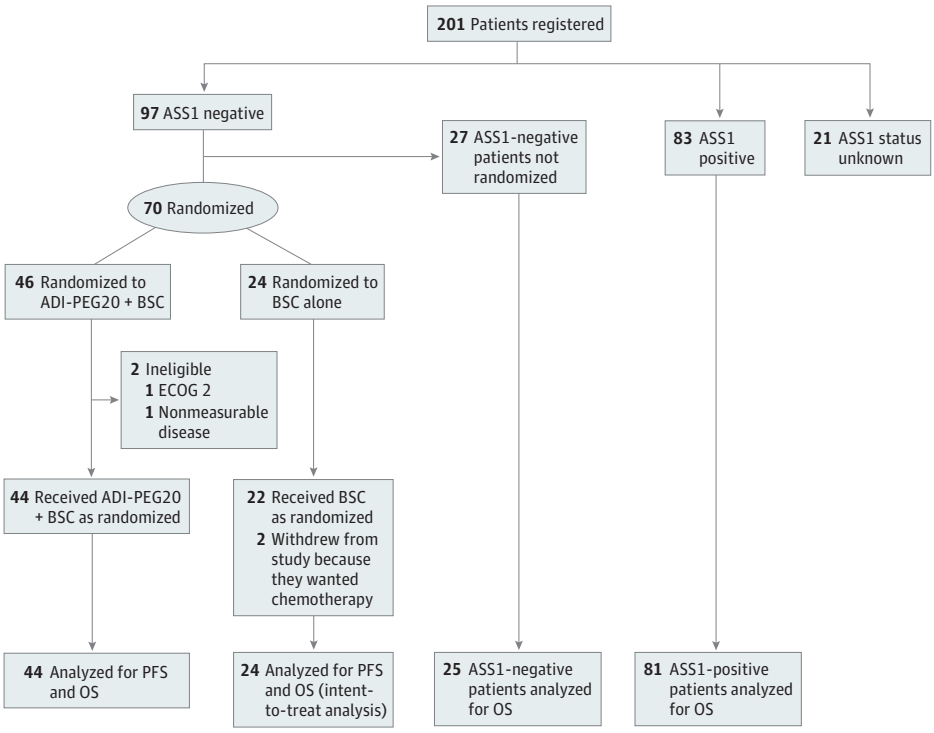
Treatment and Procedures

Sixty-eight patients were randomized to receive a weekly intramuscular injection of ADI-PEG20 (36.8 mg/m^2) for up to 6 months (cycles) into the buttock plus best supportive care (BSC), or BSC alone. Patients continued to receive study treatment, with regular blood sampling, until disease progression, withdrawal of consent, or unacceptable toxic effects. ADI-PEG20–treated patients with disease control were allowed to exceed 6 cycles. Chemotherapy-naive patients were offered chemotherapy on progression. Patients receiving BSC alone were not allowed to cross over to ADI-PEG20. Computed tomographic scans were scheduled at the end of month 2, 4, 6, end of treatment, and 6 months after finishing treatment. Quality-of-life questionnaires were scheduled at baseline, then at the end of 2 and 4 months, and end of treatment. We also collected survival data on patients with low ASS1 expression who were not randomized, and from ASS1-positive patients ("high expressor"; ≤50% low expressor cells) who were not eligible for randomization.

Outcomes

The primary end point was PFS, measured from the randomization date to first progression or death from any cause. Progression was assessed by means of imaging (modified RECIST) and examined by blinded central review (which matched the local review in 65 patients; in the other 3, the progression date was judged to be earlier than the local review). Secondary end points were overall survival (OS), response rate, toxicity, and

Figure 1. Consolidated Standards of Reporting Trials Diagram



The trial protocol indicated that patients who miss a dose of pegylated arginine deiminase (ADI-PEG20) could be withdrawn from the study, unless the chief investigator gave authorization to continue based on clinical information; this occurred for 2 patients. Follow-up information (outcomes) was available on all patients (ie, no trial withdrawals or dropouts). ASS1 indicates argininosuccinate synthetase 1; BSC, best supportive care; ECOG, Eastern Cooperative Oncology Group; OS, overall survival; and PFS, progression-free survival.

quality of life using the Lung Cancer Symptom Scale.[19] Exploratory additional end points included plasma concentrations of arginine (and duration of arginine deprivation), citrulline, and anti-ADI-PEG20 antibodies, the methylation status of the *ASS1* gene using the Illumina Infinium HumanMethylation450 BeadChip array, and metabolic response as assessed by ^{18}F-fluorodeoxyglucose positron-emission tomography (FDG-PET) in patients receiving ADI-PEG20.[20-23] Plasma samples were planned weekly during treatment for ADI-PEG20 patients and at weeks 9, 17, 25 for BSC-alone patients.

Statistical Analysis

The target sample size was 66 patients (2:1 allocation), based on detecting a hazard ratio (HR) of 0.60, assuming a median PFS of 4.5 months with BSC alone, 80% power, and 15% 1-sided statistical significance (phase 2 studies typically use 10%-20%). Time-to-event end points were analyzed using Kaplan-Meier curves, the log-rank test, and Cox regression, all measured from the date of randomization, and by intention to treat (SAS, version 9.3). *P* values for OS and PFS were either 1 sided (consistent with the design; significance level, .15), or 2 sided (to be conservative) and are indicated throughout; all other *P* values were 2 sided. For PFS, an event was modified RECIST progression (using the central review) or death from any cause, and those without an event were censored when last seen alive (ie, seen in clinic). Overall survival, but not PFS, violated the proportional hazards assumption, so we also estimated the restricted mean survival time, a measure of life expectancy or mean survival (calculated as the area under each Kaplan-Meier curve).[24] Overall survival was also compared (Kaplan-Meier curves, log-rank test, and restricted mean survival times) between all registered patients who had BSC only and either low or high ASS1 expression, and the control group in the randomized trial, where OS was measured from the date of study registration. The purpose here was to examine the association between ASS1 expression as a prognostic marker for survival in patients receiving the same care. Toxic effects were based on the maximum National Cancer Institute Common Terminology Criteria for Adverse Events toxicity grade for each patient and event. Quality of life was examined as the difference in scores between baseline and each of 2 and 3 months after randomization (Wilcoxon test). To examine how within-patient arginine levels change over time and how this correlates with PFS, a time-varying Cox regression was used (model containing only PFS and the individual plasma levels for each patient where available). The Spearman correlation was used to examine the relationship between the duration of arginine depletion and PFS.

Results

A total of 201 patients were registered, with 97 (48%) identified as being ASS1 deficient; 70 were randomized, but 2 were found to be ineligible (ECOG 2 and nonevaluable disease by modified RECIST) (**Figure 1**). The main analyses were on 24 patients who received BSC alone and 44 who received ADI-PEG20 + BSC; median follow-up was 38 months (range, 2.5-39 months). Overall, 4 of 9 (44%) patients with prior exposure to platinum-antifolate chemotherapy were rescreened and displayed ASS1 deficiency on tumor rebiopsy compared with the baseline tumor. Baseline patient characteristics were balanced (**Table 1**).

Adherence to ADI-PEG20 Treatment

Nineteen of 44 (43%) patients completed 2 4-week cycles of ADI-PEG20, and 10 (23%) had at least 6 cycles (eFigure 1 in Supplement 2). Twenty-two (50%) patients had at least 9 injections in total. Only 2 patients missed 1 week's dose during the treatment period; and 2 patients had a lower than target dose based on their body surface area (1 patient had 51% of the full dose for 1 injection out of 7 received in total; 1 patient had 49% of the full dose for 4 injections out of 8 in total. Eight patients stopped ADI-PEG20 treatment early: 4 due to toxic effects, 3 because of a clinical decision, and 1, a patient decision (unrelated to toxic effects).

Efficacy

No partial or complete radiological responses were observed. Among patients who had evaluable disease at 4 months (using modified RECIST), the best response was stable disease assessed by central review: 12 of 23 (52%) in the ADI-PEG20 + BSC group vs 2 of 9 (22%) in the BSC group (Fisher exact 2-tailed $P = .23$). Twenty-one of 44 patients (48%) receiving ADI-PEG20 experienced disease progression by the first 8-week scan. Also, using baseline ^{18}F-FDG-PET imaging and during the first cycle of treatment in the ADI-PEG20 + BSC group only, 18 of 39 patients exhibited partial metabolic responses (46%), with stable maximum standardized uptake value in 12 (31%), mixed (ie, a decrease and an increase in maximum standardized uptake value in the same patient) in 3 (8%), and progression in 6 (15%) patients.

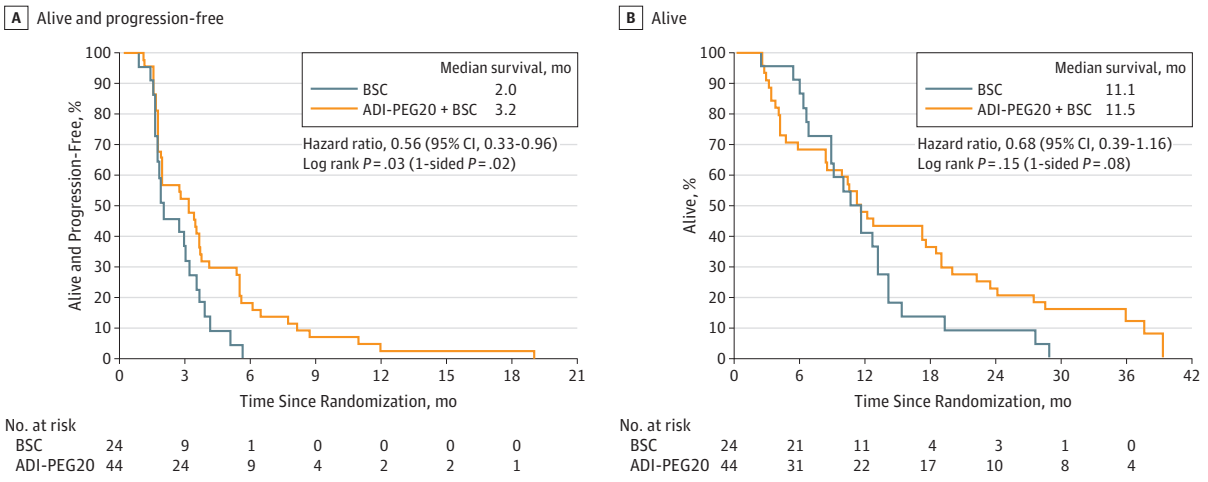
Sixty-six of 68 (97%) patients had a PFS event. Two patients allocated to BSC alone withdrew soon after randomization because they wanted chemotherapy and so were censored at the date of withdrawal. The median PFS in the ADI-PEG20 group was 3.2 (interquartile range, 1.8-5.5) months vs 2.0 (interquartile range, 1.8-3.6) months in the BSC-alone group, with HR of 0.56 (95% CI, 0.33-0.96; $P = .03$ [1-sided $P = .02$]), which was close to our target of 0.60 (**Figure 2**). The 6-month PFS rate was 18% vs 0%, acknowledging the small number of patients (10 patients at risk at this time point).

Sixty-four of 68 (94%) patients had died at the time of data-lock (June 26, 2015). Three BSC-alone patients lived beyond 2 years, before dying between 27 and 29 months, compared with 10 ADI-PEG20 patients, of whom 4 were still alive as of August 2015 (survived 32-38 months). The median OS in the ADI-PEG20 group was 11.5 (IQR, 4.2-22.9) months vs 11.1 (IQR,

Table 1. Baseline Characteristics of Patients Receiving Pegylated Arginine Deiminase (ADI-PEG20) Plus Best Supportive Care (BSC) vs BSC Alone

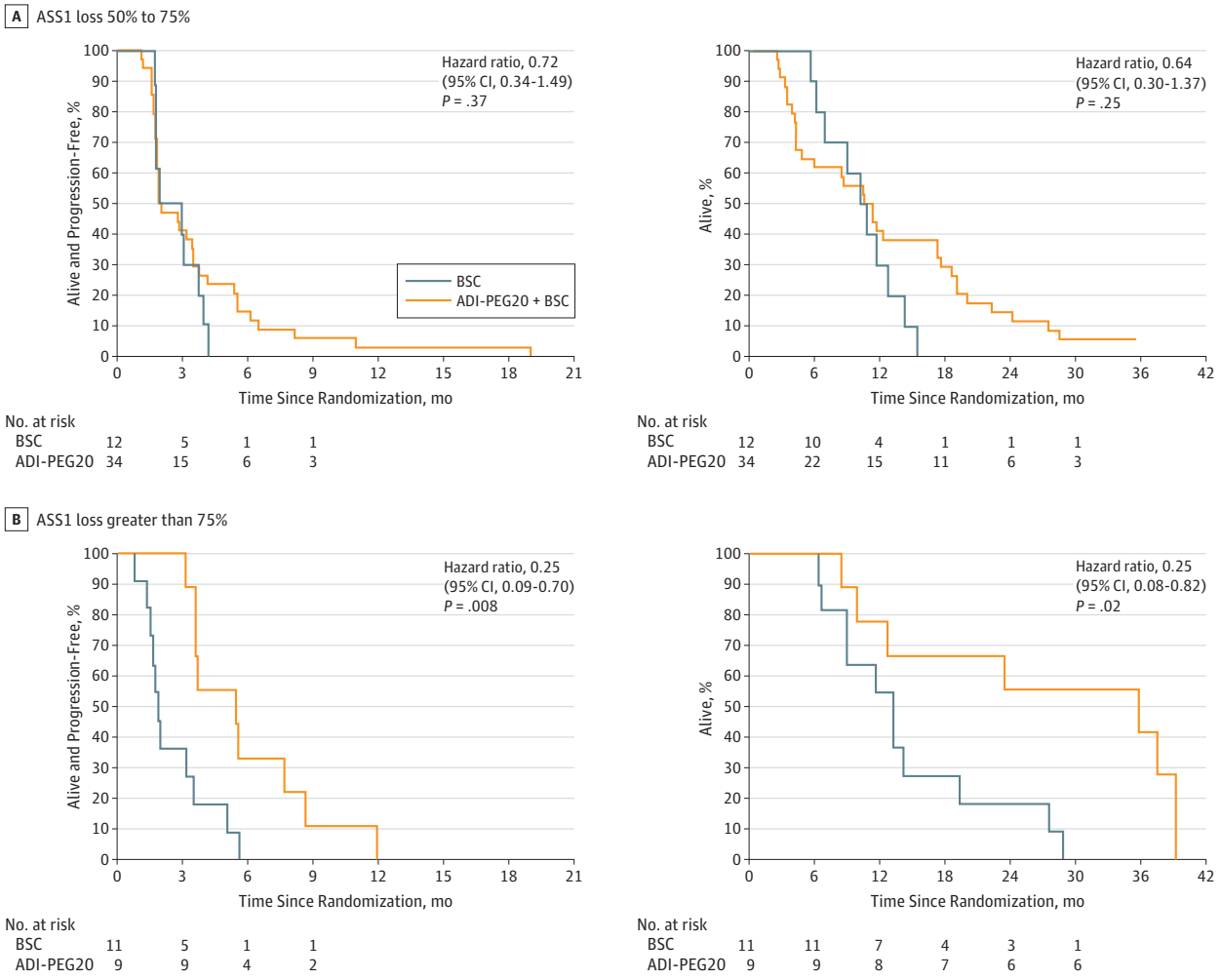
Characteristic	No. (%)	
	BSC Alone (n = 24)	ADI-PEG20 + BSC (n = 44)
Age, median (range), y	64 (48-83)	67 (54-79)
Sex		
Male	19 (79)	36 (82)
Female	5 (21)	8 (18)
Eastern Cooperative Oncology Group performance status		
0	7 (29)	9 (20)
1	17 (71)	35 (80)
Smoking history		
Never smoker	7 (29)	18 (41)
Current smoker	1 (4)	1 (2)
Ex-smoker	16 (67)	25 (57)
Time since stopping, median (range), y	20 (0.5-71)	25 (0.5-50)
Histological subtype		
Sarcomatoid	1 (4)	1 (2)
Nonsarcomatoid	23 (96)	43 (98)
Prior chemotherapy		
None	11 (46)	17 (39)
Platinum doublet	13 (54)	27 (61)

Figure 2. Progression-Free Survival (PFS) and Overall Survival (OS) According to Trial Group



The PFS hazard ratio adjusted for the randomization stratification factors (sex, hospital, and prior chemotherapy; histologic subtype was excluded because only 2 patients had sarcomatoid) was 0.47 (95% CI, 0.25-0.86). A test for proportional hazards produced $P = .30$ for PFS and $P = .02$ for OS. The restricted mean survival times (life expectancy) for PFS were 4.1 months for the pegylated arginine deiminase (ADI-PEG20) group vs 2.7 months for the best supportive care (BSC) group, for a difference of 1.4 months (95% CI, 0.2 to 2.6 months; $P = .02$ [1-sided $P = .01$]). For OS, they were 15.7 months for the ADI-PEG20 group vs 12.1 months for the BSC group, for a difference of 3.6 months (95% CI, −1.0 to 8.1 months; $P = .13$ [1-sided $P = .06$]).

Figure 3. Progression-Free Survival (PFS) and Overall Survival (OS) by Degree of Argininosuccinate Synthetase 1 (ASS1) Loss



There were 46 patients with 50% to 75% loss and 20 with 76% to 100% loss; data were unavailable for 2 patients. Interaction test between ASS1 loss group and treatment group resulted in $P = .21$ for PFS and 0.16 for OS. Restricted mean survival times (OS) for ASS1 loss of 75% or less were 12.8 months in the pegylated arginine deiminase (ADI-PEG20) group and 10.5 months in the best supportive care (BSC) group. The *P* values in the figure are all 2 sided (1-sided *P* values are half of these).

6.9-14.2) months in the BSC-alone group, with HR of 0.68 (95% CI, 0.39-1.16; $P = .15$ [1-sided $P = .08$]) (Figure 2). However, the proportional hazards assumption failed ($P = .02$), and an analysis of restricted mean survival times produced a measure of life expectancy of 15.7 months in the ADI-PEG20 group and 12.1 months in the BSC group, that is, an increase of 3.6 months (95% CI, −1.0 to 8.1 months; $P = .13$ [1-sided $P = .06$]). We could not explain why the curves crossed; it could be a spurious feature within a phase 2 trial of limited size.

Prespecified subgroup analyses for sex and prior chemotherapy did not show a differential treatment effect for either PFS or OS (eFigures 2 and 3 in Supplement 2). Among patients who had prior chemotherapy, the PFS HR for ADI-PEG20 treatment was 0.54 (95% CI, 0.26-1.14), compared with 0.60 (95% CI, 0.27-1.37) for chemotherapy-naive patients, with corresponding OS HRs of 0.68 (95% CI, 0.33-1.43) vs 0.60 (95% CI, 0.26-1.40) (interaction $P = .95$ for PFS and .56 for OS).

The beneficial effect of ADI-PEG20 treatment seemed greatest for patients with an ASS1 loss of greater than 75%, vs 50% to 75% (PFS HRs of 0.25 [95% CI, 0.09-0.70] vs 0.72 [95% CI, 0.34-1.49], interaction $P = .21$; and OS HRs of 0.25 [95% CI, 0.08-0.82] vs 0.64 [95% CI, 0.30-1.37], interaction $P = .16$) (**Figure 3**); statistical significance of the interaction was not reached because of insufficient power for this particular analysis. Moreover, ASS1 loss of expression was associated with significant hypomethylation ($P = .02$; regularized *t* test) at a single CpG site of the *ASS1* gene in intron 1, whereas methylation changes were not detected at the *ASS1* promoter in the clinical samples (eFigure 4 in Supplement 2).

We compared OS from the BSC-alone patients (ASS1 negative/"low expressors") in the randomized trial with nonrandomized ASS1-positive ("high expressors") or ASS1-negative patients (eFigure 5 in Supplement 2). The nonrandomized ASS1-negative patients had worse OS, whereas the OS curves for the

Table 2. Reported Adverse Events Based on the Common Terminology Criteria for Adverse Events Grade for Each Patient and Each Event

	No. (%)					
	BSC Alone (n = 24)		ADI-PEG20 + BSC (n = 44)			
			All Reported Events		Considered to Be at Least Possibly Causally Associated With ADI-PEG2	
Adverse Event	Grade 1-2	Grade 3	Grade 1-2	Grade 3-4[a]	Grade 1-2	Grade 3-4[b]
Abnormal biochemical test result	1 (4)	0	6 (14)	1 (2)	2 (5)	1 (2)
Abnormal hematologic test result	2 (8)	0	12 (27)	2 (5)	7 (16)	2 (5)
Neutropenia	0	0	4 (9)	2 (5)	3 (7)	2 (5)
Allergic reaction and/or anaphylaxis	0	0	1 (2)	4 (9)	1 (2)	4 (9)
Injection site reactions	0	0	16 (36)	0	9 (20)	0
Serum sickness	0	0	0	1 (2)		2 (5)[c]
Chest pain and/or trouble breathing	8 (33)	2 (8)	24 (55)	1 (2)	6 (14)	1 (2)
Dizzy spell	0	1 (4)	6 (14)	0	3 (7)	0
Gastrointestinal events	6 (25)	2 (8)	23 (52)	2 (5)	6 (14)	0
Fatigue	6 (25)	0	19 (43)	3 (7)	5 (11)	2 (5)
Fever	1 (4)	0	1 (2)	1 (2)	0	1 (2)
Hypertension	0	0	2 (5)	1 (2)	0	1 (2)
Infection	3 (12)	0	12 (27)	1 (2)	0	1 (2)
Pain	4 (17)	1 (4)	22 (50)	1 (2)	7 (16)	1 (2)
Rash	1 (4)	0	17 (39)	2 (5)	6 (14)	1 (2)
Swelling in limbs	0	0	3 (7)	1 (2)	2 (5)	1 (2)
Other cancer	0	1 (4)		1 (2)	0	0
Other[d]	6 (25)	2 (8)	22 (50)	2 (4)	8 (18)	1 (2)

Abbreviations: ADI-PEG, pegylated arginine deiminase; BSC, best supportive care.

[a] All were grade 3 except 1 grade 4 event of allergic reaction and/or anaphylaxis and 1 grade 4 event of chest pain and/or trouble breathing.

[b] All were grade 3 except 1 grade 4 event of allergic reaction and/or anaphylaxis, 1 grade 4 event of chest pain and/or trouble breathing, and 1 grade 4 event of vomiting.

[c] For 1 patient, serum sickness was not reported as an adverse event on the case report forms but was apparent from the description of symptoms.

[d] Mostly less than 3 occurrences of each specific event, and none were grade 4.

BSC-alone group vs ASS1-positive patients separated after 12 months, in favor of the latter group. The restricted mean survival times were 8.8, 17.0, and 12.7 months for the ASS1-negative (nonrandomized), ASS1-positive, and ASS1-negative (randomized) groups, respectively. These data support the observation that ASS1 status is prognostic, that is, ASS1-positive patients tend to have better survival compared with ASS1-negative patients. The lower survival among nonrandomized ASS1-negative patients is likely due to having poor prognosis at baseline, which would have been why they were considered inappropriate for the trial.

Safety and Quality of Life

Forty of 44 (91%) in the ADI-PEG20 group vs 14 of 24 (58%) in the BSC-alone group had any reported grade 1 to 4 adverse event (P = .001), but mostly grade 1 or 2 (**Table 2**). There was no statistically significant difference in grade 3 or 4 events (13 of 44 [30%] vs 4 of 24 [17%]; P = .24); neither was there any difference in the incidence of physical and/or symptomatic grade 3 or 4 events (ie, excluding abnormal biochemical and hematological test results) (11 [25%] vs 4 [17%]; P = .43) for the ADI-PEG20 vs BSC alone group, respectively. Specific events more common in the ADI-PEG20 group were neutropenia, gastrointestinal problems (eTable in Supplement 2), fatigue/lethargy, injection site reactions, and grade 3 events for 4 patients with anaphylaxis, and 2 with serum sickness. These have been associated previously with ADI-PEG20 treatment, except serum sickness, which responded readily to steroid therapy. Fewer events were considered to be causally related to ADI-PEG20 by the treating clinician (Table 2), leading to a determination of 57% (25 of 44) in the ADI-PEG20 group vs 4% (1 of 24) in the BSC-alone group with any reported grade 1 to 4 event, and 16% (7 of 44) vs 0 with any physical grade 3 or 4 event. Quality of life (patient self-assessment and observer assessment) was generally similar between treatment groups at 2 and 3 months after randomization; importantly, ADI-PEG20–treated patients did not have noticeably worse quality of life for any domain (eFigures 6-7 in Supplement 2).

Pharmacodynamics

To validate the pharmacodynamic effects of ADI-PEG20, we compared plasma arginine and citrulline levels in the 2 arms of the study. As expected, ADI-PEG20 treatment (42 of 44 with samples) led to a rapid decrease in arginine level following the first dose (levels were <0.12 mg/dL by week 2 in almost all patients [to convert to micromoles per liter, multiply by 57.05]), with a reciprocal increase in plasma citrulline level, whereas little change was seen in BSC-alone patients (21 of 24 with samples) (eFigure 8 in Supplement 2). In 27 ADI-PEG20 patients, arginine levels increased by the third cycle due to the emergence of neutralizing anti-ADI-PEG20 antibodies (eFigure 8 in Supplement 2), while levels remained low in 15 patients. From a time-varying Cox regression analysis, for every increase in arginine level of 0.35 mg/dL, the risk of progressing or dying also increased: PFS HR in the BSC-alone group was 1.66 (95% CI, 1.06-2.60; P = .02), in the ADI-PEG20 group was

1.11 (95% CI, 0.97-1.27; P = .13), and for all patients (adjusted for treatment group) was 1.13 (95% CI, 0.99-1.28; P = .06). The effect was strongest in the BSC-alone group, whose arginine levels were not depleted.

We found a positive moderate correlation between duration of arginine deprivation and PFS among 27 patients in whom the arginine concentration became low after treatment with ADI-PEG20 but later increased (to ≥40% of the patient's baseline value; Spearman correlation of 0.38; P = .05) (eFigure 9 in Supplement 2). The association was strongest among 15 patients in whom arginine levels remained low (<0.11 mg/dL; correlation, 0.93; P < .001). Among 21 BSC-alone patients, there was an expected negative correlation between baseline arginine level and PFS (−0.27), although not statistically significant (P = .24) (eFigure 9 in Supplement 2). On disease progression with ADI-PEG20 treatment at 8 months, 1 patient underwent a repeated biopsy, which revealed a continuing absence of ASS1 expression (eFigure 10 in Supplement 2).

Poststudy Treatments

Fourteen (32%) ADI-PEG20 patients received further treatments other than ADI-PEG20: 8 received platinum/pemetrexed disodium; 2, vinorelbine tartrate; 1, gemcitabine hydrochloride/platinum; 1, irinotecan hydrochloride/cisplatin/mitomycin; and 2, unknown treatment. These were known to be after progression in all 12 patients for whom treatment dates were recorded. Four patients who had stable disease continued ADI-PEG20 treatment beyond the 6-month study treatment period.

Eleven (46%) BSC-alone patients received systemic therapy: 3 received platinum/pemetrexed; 3, carboplatin plus either gemcitabine or vinorelbine; and 5, unknown treatment. These were known to be after progression in 8 patients and before progression in 1 patient with treatment dates available.

Discussion

Our phase 2 trial shows that depletion of the nonessential amino acid arginine in ASS1-deficient mesothelioma reduced progression times in patients with advanced disease, warranting further investigation. The PFS HR (0.56) represents a 44% reduction in the risk of progressing and/or dying, a clinically important effect for patients with advanced cancers with a poor prognosis. Also, it was close to that expected (HR, 0.60), with a 1-sided P = .02), which was well within that specified in the design (P = .15). The improvement in median PFS from 2.0 to 3.2 months scores 33 (out of a maximum of 55) using the American Society of Clinical Oncology assessment framework.[25]

Early-phase clinical studies of ADI-PEG20 treatment in melanoma and liver cancer have completed, but without biomarker selection on the premise that these tumors display a high degree of ASS1 loss. In contrast, our analysis showed that ASS1 loss is lower than the 63% frequency derived from tissue microarray studies and, at 48% loss in ADAM, reflects sampling and heterogeneity of expression.[6] The PFS HR of 0.56 in favor of ADI-PEG20 supports the preselected 50% threshold for ASS1 loss, although determining the treatment benefit from ADI-PEG20 with different levels of enzyme expression will require larger studies. We observed a greater advantage among patients with tumors with at least 75% ASS1 deficiency, with an HR of 0.25, indicating that biomarkers may enable targeting arginine deprivation for cancer therapy. Moreover, hypomethylation within the *ASS1* gene body, rather than methylation at the *ASS1* transcription start site, was linked to the loss of ASS1 protein in mesothelioma (P = .02). Whereas several cell line studies show good correlation between methylation at the *ASS1* promoter and inactivation of ASS1 expression, our findings indicate that gene-body hypomethylation appears to be a more robust biomarker in clinical mesothelioma samples, consistent with other work.[26]

Intriguingly, in 4 of 9 (44%) rebiopsied patients, we noted a subsequent decrease in ASS1 expression, illustrating a potential role for arginine deprivation with disease progression in those treated previously with chemotherapy. As mesothelioma enters a more accelerated phase, ASS1 loss may increase tumor cell proliferation and invasion as seen in several translational studies of ASS1-deficient tumors.[9,10,27,28] Further prospective studies could validate this hypothesis.

Our study also highlights that despite ASS1 biomarker selection, 48% of ADI-PEG20–treated patients experienced disease progression by the first 8-week scan, indicating early resistance. This group included 8 patients who had an initial metabolic partial response within the first month of treatment. Moreover, the overall partial metabolic response rate of 46% is almost double the rate recorded in a recent melanoma trial, indicating that mesothelioma is particularly sensitive to ADI-PEG20.[29] We found that PFS increased with the duration of arginine deprivation, as reported in a study of liver cancer patients.[16] Apart from neutralizing antibodies mediating resistance to ADI-PEG20, alternative explanations include reexpression of ASS1, which has been observed in cell line studies and in patients with melanoma treated with ADI-PEG20.[30,31] However, on rebiopsy of a progressing patient after 8 months of ADI-PEG20 treatment, there was no evidence of ASS1 reexpression (eFigure 10 in Supplement 2). Alternative resistance mechanisms may be operational, including autophagy and activation of alternate metabolic pathways, which are under investigation.[32,33]

Notably, ADI-PEG20 potentiates antifolate cytotoxicity specifically in ASS1-negative tumor cell lines.[28] Following ADAM, we have initiated the first triplet phase 1 study combining ADI-PEG20 with pemetrexed/cisplatin in patients with mesothelioma and nonsquamous non–small-cell lung cancer deficient for ASS1 (NCT02029690). Preliminary data from this antimetabolite combination are encouraging.[34]

We did not use placebo for the controls (approved by the ethics committee) because it was unfeasible for phase 2, requiring patients to attend clinic every week for sham (invasive) injections. Also, our results (including the subgroup analyses) require confirmation in larger studies.

Conclusions

ADAM is the first biomarker-driven trial showing that arginine deprivation using ADI-PEG20 significantly improves PFS, and possibly OS, in patients with mesothelioma who are deficient in the enzyme ASS1. Further cancer studies using tissue, fluid, and imaging biomarkers are warranted in tumors auxotrophic for arginine to optimize arginine deprivation as a novel antimetabolic strategy.

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Invited Commentary

The ADAM Trial
Visiting the Road Less Traveled

Surein Arulananda, MBBS; Thomas John, MBBS, PhD, FRACP

Until recently, cisplatin and pemetrexed disodium doublet chemotherapy was the only anticancer treatment with a median overall survival benefit in malignant mesothelioma.[1] The MAPS study published in 2016 confirmed a modest improvement in median survival with the addition of bevacizumab to this regimen.[2] Recent sequencing studies point to most mesotheliomas having a low mutational burden and few "druggable" oncogenes.[3] However, with the rapidly expanding field of immuno-oncology, most of the recent excitement in mesothelioma research involves immune checkpoint inhibitors, with some encouraging results but also some disappointment. Despite the lack of oncogenic driver mutations, other mechanisms of "personalized medicine" are under investigation including antimesothelin antibody drug conjugates,[4] vaccines, and small-molecule inhibition of growth factor receptors.[5]

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Arginine has long been postulated to be implicated in tumorigenesis; hence, exploiting this as a potential target has been a subject of interest.[6] Argininosuccinate synthase 1 (ASS1), which represents the rate-limiting enzyme in the production of arginine, is deficient in multiple tumor cell lines including mesothelioma.[6,7] Deficiency of ASS1 through either downregulation or epigenetic silencing enables cells to increase proliferation by facilitating pyrimidine biosynthesis and activating phosphorylation of S6K1 through the mammalian target of rapamycin (mTOR) pathway.[7] Depriving ASS1-deficient cells of arginine by using the enzyme arginine deiminase (ADI) results in cell apoptosis in vitro.[6] Early phase 1/2 trials using ADI-PEG20, a human recombinant arginine depleter, in melanoma and hepatocellular carcinomas showed minimal responses, with the highest response rate of 25% (6 of 24) in an Italian melanoma cohort.[8] In the hepatocellular carcinoma cohorts, the majority of responses were that of disease stability.[9] Larger studies in melanoma were not undertaken due to the emergence of immune checkpoint inhibitors with more impressive efficacy data.

In this issue of *JAMA Oncology*, Szlosarek and colleagues[10] present their findings from the ADAM study, a phase 2 multicenter study of ADI-PEG20 and best supportive care (BSC) vs BSC alone in patients with ASS1-deficient malignant pleural mesothelioma. Of 201 patients, 97 (48%) were found to be ASS1 deficient, in keeping with previous studies. It is noteworthy that the investigators screened a large group of patients with a relatively uncommon tumor. Of 97 ASS1-negative patients, 68 with ASS1 deficiency were randomized 2:1 to ADI-PEG20 vs BSC.

Disappointingly, there were no objective responses with ADI-PEG20 treatment in a carefully selected biomarker-based subgroup of patients, although this was a secondary end