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Pegylated arginine deiminase: a novel anticancer enzyme agent

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Abstract

Pegylated arginine deiminase (ADI-PEG20) is a novel anticancer enzyme that produces depletion of arginine, which is a nonessential amino acid in humans. Certain tumours, such as malignant melanoma and hepatocellular carcinoma, are auxotrophic for arginine. These tumours that are sensitive to arginine depletion do not express argininosuccinate synthetase, a key enzyme in the synthesis of arginine from citrulline. ADI-PEG20 inhibits human melanomas and hepatocellular carcinomas *in vitro* and *in vivo*. Phase I – II trials in patients with melanoma and hepatocellular carcinomas have shown the drug to have antitumour activity and tolerable side effects. Large Phase II trials and randomised, controlled Phase III trials are needed to determine its overall efficacy in the treatment of these malignancies and others.

Keywords

anticancer enzyme; arginine; argininosuccinate synthetase expression; hepatocellular carcinoma; melanoma

1. Introduction

Amino acid depletion is one method of treating certain cancers. The best known of these anticancer enzymes is asparaginase, which lowers blood levels of asparagine, a nonessential amino acid in humans. This agent is particularly effective in acute lymphoblastic leukaemia, a common type of leukaemia in children and young adults [1,2]. The drug is active in this disease and generally well tolerated, because most human body cells do not require aspargine, whereas the leukaemic cells require this amino acid for their growth and survival. Thus there is a precedence for the elimination of a nonessential amino acid as cancer therapy in which the malignant cells are auxotrophic for a particular amino acid.

Another nonessential amino acid in humans is arginine, which is synthesised from citrulline using two enzymes, argininosuccinate synthetase (ASS) and argininosuccinate lyase (ASL). ASS converts citrulline to argininosuccinate, which (in turn) is converted to arginine by ASL. Cells that do not express ASS can be sensitive to arginine-depleting enzymes such as arginine deiminase (ADI). Human melanoma and hepatocellular cell lines have been found to lack ASS expression and hence are auxotrophic for arginine. *In vitro* studies have suggested that arginine depletion can be effective in treating these tumours and possibly other tumours [3-15].

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Malignant melanoma of the skin is rapidly increasing in incidence throughout the world, and accounts for 4% of all cancers in USA. The disease is curable with surgery when detected early; however, melanoma can be highly lethal when the disease is detected in the advanced stages. Treatment for advanced melanoma remains unsatisfactory, despite advances in the therapy for other cancers [16]. Hepatocellular carcinoma is also increasing in incidence worldwide, most likely due to the effects of hepatitis B and C infections. It is the eighth biggest cause of cancer mortality in men and the twelfth in women in USA. Total surgical resection or liver transplantation remains the only known cure for this disease although long-term, disease-free survival has been observed for certain patients after thermal ablation or percutaneous ethanol injection for early-stage disease. For patients with unresectable disease, treatment options may include radiofrequency ablation or chemoembolisation of the liver; however, the prognosis is dismal for most patients because drug therapy has not been demonstrated to improve survival [17,18]. Thus there is a great need for more effective therapy for cancers such as melanoma and hepatocellular carcinoma. ADI-PEG20 is a novel targeted therapy that appears to have antitumour activity in melanoma and hepatocellular carcinoma, tumours that are auxotrophic for arginine. This review discusses the available literature regarding the pharmacokinetics, pharmacodynamics, and preclinical and clinical studies with ADI-PEG20.

2. Background

ADI catalyses the conversion of L-arginine into L-citrulline and ammonia. Arginine is not considered to be an essential amino acid in human adults and an arginine-deficient diet does not produce hyperammonaemia or orotic aciduria in humans [19-23]. Although mice do not appear to require arginine in their diet, certain other animal species (including rats, dogs and cats) require dietary arginine [19].

Similar to L-asparaginase, ADI is derived from microorganisms. It is not produced by mammals such as humans. Consequently, the enzyme has a very short circulating half-life and is immunogenic. Due to the fact that ADI is a foreign protein, multiple injections are needed to produce the desired effect of arginine deprivation.

A method of increasing a drug's circulating half-life and decreasing its immunogenicity is to link the drug with PEG [24-27]. A number of other drugs have been formulated with PEG. These include (for example) PEG-interferon and asparaginase. The latter drug is also derived from microbes and is an enzyme that has been used successfully in leukaemia. By formulating asparaginase with PEG, the frequency of injections has been reduced and the half-life of the drug prolonged. Its immunogenecity has been greatly reduced, which (in turn) reduces the amount of enzyme needed and thus side effects (including serious anaphylactic reactions) have been subsequently diminished.

The chemical linker used to formulate PEG with ADI was the succinimidyl succinate linker. This linker is also used in the formulation of PEG asparaginase (Oncaspar®; pegaspargase) and PEG adenosine deaminase (Adagen®; pegademase bovine). Formulation of PEG to ADI resulted in ~ 50% of its specific enzyme activity.

The effectiveness of ADI-PEG is determined by its ability to deplete circulating arginine levels *in vivo*. Mice do not require arginine in their diet and were used for these studies; they have measurable levels of arginine and citrulline, which are very similar to that of humans. A single dose of native or non-PEG ADI at a dose of 800 U/m^2 injected into the tail vein resulted in lowering of the plasma arginine levels by 50% compared with baseline concentration. This reduction persisted for 12 –24 h after injection. On the other hand, a single injection of PEG-ADI produced undetectable (< 6 μM) blood levels of arginine for several days.

Pharmacodynamic studies in mice were performed using HPLC to measure blood arginine and citrulline levels. Various formulations of PEG of different molecular weight (MW) were used. The ADI-PEG with a MW of 20,000 (ADI-PEG20) produced a similar reduction of blood arginine, but for a more prolonged duration compared with PEG of a different molecular weight.

In terms of immunogenecity, native or non-PEG ADI was highly immunogenic using measurements of titre of IgG anti-ADI antibody. These experiments were performed in both mice and rabbits. On the other hand, ADI-PEG20 had significantly reduced immunogenicity. ELISA studies with a secondary antibody that recognised all subclasses of antibody, or with a secondary antibody that recognised only IgG, confirm the reduced immunogenecity of ADI-PEG20 compared with native ADI.

Human melanoma and hepatocellular cell lines have been shown to be auxotrophic for arginine [3,7-12]. Sugimura *et al.* [11,12] demonstrated that all five human melanoma cell lines were auxotrophic for arginine. Ensor *et al.* [28] confirmed these results in human melanoma and human hepatocellular carcinoma cells lines (16 each) tested *in vitro* and showed that this was due to absence of ASS in these cells. Furthermore, their data showed that native ADI reduced arginine levels in mice to 40 – 50% at 24 h after injection, whereas ADI-PEG20 reduced circulating arginine levels to nondetectable levels for ~ 7 days.

The incidence and distribution of ASS deficiency has been evaluated in a number of human tumour types from biopsy specimens. Using a monoclonal antibody, Dillon *et al.* [29] found that the incidence of ASS deficiency varied greatly depending on the tumour type. For melanoma, all 119 human tumour biopsy specimens showed lack of ASS expression. For hepatocellular carcinoma, 51 of 51 biopsy specimens showed ASS deficiency. Prostate carcinoma showed ASS deficiency in 13 of 13 specimens. Generally, other tumours (such as breast and colon cancer) did not show ASS deficiency, but some tumour types (such as renal cell carcinoma and sarcoma) sometimes showed ASS deficiency. In addition, the *ASS* gene has been found to be downregulated in renal cell carcinoma [30]. Thus ADI-PEG20 may likely be useful to treat human melanoma, hepatocellular carcinoma and prostate carcinoma and, perhaps, certain renal cell and sarcoma cancers.

Other types of cancers may be sensitive to arginine depletion. ADI has been shown to enhance dexamethasone-induced cytotoxicity in human T-lymphoblastic leukaemia CEM cells [31]. Both ADI and glucocorticoids arrest tumour cells in G1 phase. It was theorised that the combination of a G1-specific anticancer drug would increase the cytotoxic effect of dexamethasone on leukaemic cells.

3.1 Pharmacology

ADI-PEG20 is a recombinant protein cloned from *Mycoplasma hominus* and subsequently produced in *Escherichia coli*.

ADI-PEG20 is formulated as a sterile solution containing: arginine deiminase (12 – 17 mg); PEG (40 – 45 mg); mono-basic sodium phosphate (USP, 1.25 mg plus 5%); dibasic sodium phosphate (USP, 3 mg + 5%); NaCl (USP, 7.6 mg plus 5%); pH (6.8 – 7); and water for injection (query size to 1 ml).

3.2 Structural formula

ADI is a recombinant protein of ~ 46,000 MW, with the following sequence:
MSVFDSKFNGIHVYSEIGELETVLVHEPGREIDYITPARLDELLFSAILESHDARKEH
QSFVKIMKDRGINVVELTDLVAETYDLASKAAKEEFIETFLEETVPVLTEANKEAVR
AFLLSKPTHEMVEFMMSGITKYELGVESENELIVDPMPNLYFTRDPFASVGNGVTIH

FMRYIVRRRETLFARFVFRNHPKLVKTPWYYDPAMKMSIEGGDVFIYNNETLVVGV
SERTDLDTITLLAKNIKANKEVEFKRIVAINVPKWTNLMHLDTWLTMLDKNKFLYS
PIANDVFKFWDYDLVNGGAEPQPQLNGLPLDKLLASIINKEPVLIPIGGAGATEMEIA
RETNFDGTNYLAIKPGLVIGYDRNEKTNAALKAAGITVLPFHGNQLSLGMGNARC
MYMPLSRKDVKW.

ADI is formulated by covalent attachment to PEG of 20,000 MW. Each molecule of ADI is connected to ~ 13 –16 PEG molecules.

3.3 Preclinical and clinical studies

Takaku [3,8,9] demonstrated that adding ADI to culture media could produce cell death in a variety of tumours, including melanoma and hepatocellular carcinoma. Sugimura [10-12] showed that melanoma cells could be inhibited by the depletion of arginine and the addition of citrulline to the media did not provide protection from this inhibition. It was hypothesised that melanoma cells may lack ASS expression, which accounted for these findings.

Further studies with ADI have confirmed this inhibitory effect in melanomas and hepatocellular carcinomas [27,28]. The human melanoma cell lines (SK-mel2 and SK-mel28) were inhibited in a dose-dependent manner by adding ADI to the culture media. Northern blot analysis showed that these cells do not express ASS, but they express ASL mRNA. These cell lines were subsequently transfected with an expression plasmid containing the human ASS cDNA. These transfected cells were made very resistant to the growth-inhibitory effect of ADI.

ADI inhibits the growth of human melanomas and hepatocellular carcinomas implanted in mice and also extends the survival of these tumour-bearing animals [3]. ADI-PEG20 was also shown to inhibit the growth of SK-mel2 and SK-mel28 implanted in athymic mice. In addition, ADI-PEG20 also inhibited the growth of human hepatocellular carcinomas implanted in severe combined immune deficient (SCID) mice and prolonged survival.

In a comparative study, ADI-PEG20 was more efficacious than native ADI in inhibiting human melanomas and hepatocellular carcinomas implanted in mice. This sensitivity to ADI-PEG20 appears to be due to lack of expression of ASS mRNA.

Both pharmacokinetic and pharmacodynamic studies have been performed in mice. These studies demonstrate that ADI-PEG20 has a plasma half-life in mice of ~ 6 days. The pharmacokinetic data with ADI-PEG20 correlated well with the pharmacodynamic data. Immunogenicity testing of ADI-PEG20 in CD-1 mice and New Zealand rabbits showed that ADI-PEG20 was much less immunogenic than native ADI. Animals were assayed weekly for the production of anti-ADI antibodies determined by ELISA after weekly injections of ADI-PEG20.

3.4 Dog studies

Oral melanoma in dogs is similar to malignant melanoma in humans in that both are very aggressive in their biology and behaviour, with the propensity for distant metastases. Surgery, if feasible, remains the only cure for oral melanoma in dogs, as chemotherapy is usually ineffective. Unlike mice and humans, dogs have a requirement for arginine and arginine depletion can lead to rapid death in these animals. Therefore, drugs such as ADI-PEG20 have to used with caution in the treatment of oral melanoma in dogs, and only a partial depletion of arginine can be tolerated. A study conducted by the University of Pennsylvania treated 10 dogs with inoperable oral melanoma (Clark, personal communication). After 8 weeks of treatment, the dogs were evaluated for response. Arginine levels were measured periodically. The results of the study showed that partial arginine

depletion was well tolerated in these animals. Furthermore, three dogs had a partial response, one had stable disease and two had a complete response.

3.5 Human clinical studies

ADI-PEG20 was tested in a patient with hepatocellular carcinoma treated as a single patient exemption [32]. The patient was treated with escalating doses of ADI-PEG20. There were reductions in tumour size and serum α-fetoprotein levels. No toxicity attributed to the drug was noted. This single case report led to a Phase I or II trial of ADI-PEG20 in patients with nonresectable hepatocellular carcinoma [33]. Patients were accrued from the Pascale Cancer Institute in Naples, Italy; 19 patients were entered into the study in 1 of 4 cohorts. The first 3 cohorts were composed of 3 patients, each treated with an initial dose of ADI-PEG20 of 20, 40 or 80 U/m^2. Subsequent patients were enrolled into cohort 4 and treated initially at a dose level of ADI-PEG20 160 U/m^2. The latter dose was determined to be the optimum biological dose (OBD) that lowered the plasma arginine level to undetectable amounts (< 2 μM/l) for > 7 days. ADI-PEG20 was administered intramuscularly.

As unresectable hepatocellular carcinoma is uniformly fatal, it was determined that all of the patients should receive 3 cycles of ADI-PEG20 at the OBD, providing toxicity was tolerable. The initial cycle of therapy consisted of three treatments on days 1, 15 and 22. Subsequent cycles consisted of 4 weekly treatments on days 1, 8, 15 and 22. After 1 week's rest, subsequent cycles of treatment were initiated.

For the pharmacodynamic study (Clark, personal communication), amino acid analysis was performed on plasma samples at various times after drug administration. The pharmacokinetics of ADI-PEG20 were measured using two different assays; one assay measured the amount of ADI enzyme activity and the other assay measured the amount of ADI protein in plasma. Antibodies to ADI-PEG20 were also periodically measured using two assays: one ELISA, to measure antibody titre to ADI-PEG20, and the other assay was to measure the neutralising antibody in plasma.

Of the 19 patients, 15 completed all of the cycles of treatment and 2 patients each stopped treatment due to progressive disease and complication of cirrhosis. Side effects were tolerable and consisted of pain at the injection site, elevation of serum uric acid and fibrinogen, and occasional elevation of lipase and amylase levels. No patient had clinical pancreatitis. The elevated serum uric acid level appeared to correlate with tumour necrosis. Patients who developed hyperuricaemia were treated intravenously with urate oxidase, and none developed evidence of tumour-lysis syndrome. No patient developed allergic reactions to ADI-PEG20. There was no evidence of neutralising antibody production. Pharmacodynamic studies confirmed that ADI-PEG20 160 U/m^2 was able to reduce plasma arginine levels to undetectable amounts for ≥ 7 days. In terms of response, 2 patients (10.5%) had complete response and 7 (36.8%) had a partial response for a total response rate of 47%. A total of 7 patients (36.8%) had stable disease and 3 (15.9%) had progressive disease. Duration of response was defined as the time from start of therapy until progression. The mean duration of response was > 400 days (range: 37 – > 680 days). In addition, the overall Karnofsky performance status (KPS) of the patients was evaluated and the mean KPS improved from 66% at start of therapy to 91% at end of therapy.

A second Phase I/II clinical trial of weekly intramuscular ADI-PEG20 was performed at the MD Anderson Cancer Center [34], the results of which were published in abstract form. The Phase I part consisted of escalating doses of the drug up to the OBD of 160 U/m^2. The second part of the study was a Phase II trial consisting of a starting dose of 160 U/m^2 with dose escalation allowed of ≤ 240 U/m^2. Of the 35 patients enrolled on the study, the response data include: 1 patient who became resectable (partial response?), 16 who had

stable disease, 4 who did not complete the study (due to either allergic reaction or intercurrent disease) and 28 patients who progressed; the mean time before progression was 3.4 months (range: 1 – 13 months). All of the patients achieved an undetectable plasma arginine level (< 2 μM). In the patient who became resectable, death occurred postoperatively due to portal vein thrombosis leading to liver failure. Pathology evaluation showed minimal viable tumour. In terms of toxicity, 12 patients had grade 3 toxicity (mostly due to liver function or electrolyte abnormalities) and 3 had grade 4 toxicity (2 liver function abnormalities and 1 elevation of serum lipase).

There is a discrepancy in terms of objective radiographic responses in these two clinical trials that basically used the same dose of ADI-PEG20. The survival in the abstract of the study by Delman *et al.* [34] was not reported, but it is similar to that in the study of Izzo *et al.* [33] (~ 12 months). The Italian study [33] reported a high response rate of 47% with both partial and complete responders. The MD Anderson trial [34] did not report major responses, although one patient apparently became resectable after treatment with ADI-PEG20. The differences may be explained by different selection factors and patient demographics; for example, it is not clear how many patients in either study had prior chemotherapy, which may have influenced the response rate. In addition, the major responders in the Italian study may have had minimal disease, whereas the patients in the second study may have had more bulky disease. The average amount of tumour burden in the patients is not presented in either paper. It is well known that bulky disease is less likely to respond to drug therapy. Finally, it is not clear how many patients had tumours that were ASS positive and whether there is a correlation between ASS expression and response to arginine depletion. A recent study using arginase to deplete plasma arginine suggests that mechanisms other than ASS expression may be important in terms of antitumour response [34].

A Phase I/II trial of ADI-PEG20 was performed in patients with metastatic melanoma [35]. A Phase I trial was initiated in USA, which enrolled 15 patients into 4 cohorts. Treatment consisted of weekly intramuscular injections on days 1, 15 and 22. The first 3 cohorts were each composed of 3 patients and each cohort was treated with ADI-PEG20 20, 40 or 80 U/m^2; 6 subsequent patients were enrolled at 160 U/m^2, which is the OBD.

In the Italian Phase I trial, patients were enrolled onto one of six cohorts. The first 4 cohorts were composed of 3 patients each, and each cohort was treated with 1 cycle consisting of ADI-PEG20 40, 80, 160 or 320 U/m^2. A total of 6 patients received 1 cycle ADI-PEG20 of 640 U/m^2. The first cycle consisted of treatment on days 1, 15 and 22. In addition, six patients were treated onto a cohort of three cycles consisting of four weekly injections at the OBD. In terms of response, none of the patients from USA on the Phase I part of the study had an objective response. In the Italian Phase I/II trial, there was one complete response and 5 partial responses for a total response rate of 25%. A total of 14 patients had stable disease for ≥ 1 cycle of treatment and 6 had stable disease for 3 months of the study. All stable or responding patients had received the OBD or higher dose. The most common side effect was mild pain at the injection site. A total of 2 patients had hypotension within 20 – 40 min of therapy; 1 of these had a history of hypertension and was on antihypertensive medications. The second patient had hypotension shortly after receiving the fourth injection and was subsequently treated with a further six treatments, without recurrence of the hypotension. Laboratory abnormalities that were noted included elevation of serum uric acid levels, mild increases in blood fibrinogen levels, and occasional elevation of serum lipase and amylase levels.

No patient developed clinical evidence of pancreatitis or coagulopathy. Elevation of serum uric acid levels was treated with allopurinol or urate oxidase and no clinical evidence of

tumour-lysis syndrome was noted. Interestingly, ADI-PEG20 treatment resulted in a decrease in nitric oxide synthesis, but no measurable effect of this treatment on blood pressure or heart rate was found.

Immunogenicity studies were performed as part of the trial. None of the plasma samples from any of the 39 patients showed measurable enzyme-neutralising activity. These results correlate with the lack of allergic reactions observed clinically. As this was a Phase I trial, one objective was to determine the maximum-tolerated dose. A total of 6 patients received the highest dose of 640 IU/m^2/week. Except for pain at the injection site, no major toxicities were observed. As this was considered to be the highest dose that could be easily administered by the intramuscular route, the maximum-tolerated dose for AD-PEG20 was considered to be > 640 IU/m^2 weekly. Pharmacodynamic studies showed that a dose of 160 IU/m^2 was sufficient to deplete serum arginine level to undetectable (< 2 μM) levels for ≥ 7 days.

4. Expert opinion and conclusion

ADI-PEG20 represents a novel targeted therapy for both hepatocellular carcinoma and malignant melanoma. Both of these diseases are difficult to treat with drug therapy, and response rates and survival after standard treatment for unresectable patients remains poor. Thus there is a great need for drugs that have antitumour activity in these malignancies without major side effects.

Preclinical studies with ADI-PEG20 demonstrated the rationale and feasibility of prolonged arginine depletion. There are strong data from experimental and animal studies to support the use of ADI-PEG20 for patients with melanoma and for patients with hepatoma. Studies with human tumour cell lines showed that hepatocellular carcinoma and melanoma are most likely to respond to this treatment, as these cell lines most commonly stain negative for ASS [29].

With regards to the mechanism(s) of antitumour activity of ADI, a recent study used pegylated recombinant human arginase to deplete arginine with the addition of 5-fluorouracil to test *in vitro* and *in vivo* antiproliferative potential and apoptotic activity [35]. In this study, all four human hepatocellular cell lines tested had ASS expression by real-time PCR. The authors conclude that mechanism(s) other than tumour ASS deficiency may be important in arginine depletion. Thus the exact mechanism of cell death by arginine depletion using drugs such as ADI-PEG20 is not clear and requires further investigation. ADI can inhibit the proliferation of cultured neuroblastoma cells and vascular endothelial cells, by arresting the cell cycle and inducing apoptosis, as well as potentiate the effects of radiation therapy on neuroblastoma cells [14]. Interestingly, serum vascular endothelial growth factor levels in animals treated with ADI were reported to be significantly lower compared with control animals, consistent with the antiangiogenetic effect of ADI [14]. ADI can inhibit cell proliferation by arresting the cell cycle in G1 and/or S phase and inducing apoptosis [37]. Other investigators have looked at the modulation of arginine metabolic pathways as a potential mechanism of ADI's anti-tumour effect [38]. ADI was found to inhibit *de novo* protein synthesis in cells with low ASS activity, but not in cells with high ASS activity, whereas polyamine synthesis was not significantly affected. Thus the inhibitory effect of ADI on ADI-sensitive tumour cells may be due to inhibition of *de novo* protein synthesis [38]. The authors' laboratory studies suggest that apoptosis by ADI-PEG20 appears to involve the TNF-related apoptosis-inducing ligand (TRAIL) pathway [39].

ADI-PEG20 appears to be active in patients with either hepatocellular carcinoma or malignant melanoma. Anti-tumour activity has been observed in these cancers in Phase I/II

clinical trials. In the Phase I/II trials performed in hepatocellular carcinoma at an Italian cancer centre and MD Anderson Hospital, the response rate was encouraging (47%), with 2 complete and 7 partial responses. However, an apparently separate Phase I/II trial performed at the MD Anderson Cancer Center alone reported that (of 35 patients enrolled) 1 patient became resectable and 16 had stable disease. Clearly, survival is more important than radiographic responses. On the other hand, the response rate was much lower in the second trial. It is unclear why patients in the Italian–American study should have a higher response rate than the single institutional American trial. In both trials, serum arginine levels were measured periodically and were generally not detectable after dosing. It is also conceivable that serum arginine levels do not reflect actual tumour tissue levels of arginine and higher doses of the drug may need to be given. Selection factors (e.g., racial factors and selection bias) may have also played a role. The lower response rate is particularly puzzling when it is considered that, when human hepatocellular tumour biopsy specimens were tested for ASS expression, 100% (51 of 51 samples) were ASS-negative [29]. Thus it should be expected that the response rate should be much higher in clinical trials. Clearly, although ASS expression is important [40], other factors may also be critical. These may include tumour heterogeneity or rapid development, and/or induction of drug resistance and other factors could be important in determining antitumour responses.

Data from the Phase I/II trial in melanoma also showed that ADI-PEG20 has activity. No patient in the Phase I study in USA showed a response (including 6 patients treated with the OBD of 160 IU/m^2 but only 3 doses over 4 weeks were given; this was a Phase I study and response was not an end point. On the other hand, 6 of the 24 Italian patients with melanoma had a response (for example, did racial factor or dose intensity play a role?). The authors are currently performing a Phase II trial of ADI-PEG20 in patients with advanced melanoma. Antitumour responses have been observed in some patients lasting > 12 months [41]. Thus the drug appears to be active in melanoma.

Interestingly, the authors have observed a patient who initially responded to a dose of 160 IU/m^2. The patient eventually developed disease progression; the dose of ADI-PEG20 was subsequently increased and the patient responded again to the treatment. This suggests the possibility of developing drug resistance or development of neutralising antibodies (for example) to explain the dose response. It is also interesting that two patients had melanoma tumour samples assayed for ASS expression prior to treatment and were ASS negative. Both patients responded to treatment, but subsequently progressed. Tumour samples obtained at the time of relapse stained ASS positive.

A patient whose tumour stained ASS negative prior to treatment did not respond to ADI-PEG20. On the other hand, 7 of 11 patients who were ASS-negative prior to treatment had some type of antitumour response (partial, minor or mixed response). Thus there may be some correlation between ASS staining and antitumour response. This requires further investigation.

Data from both studies in hepatoma and melanoma show that ADI-PEG20 is generally well tolerated. In the two hepatoma trials, with 54 patients combined, there were 14 grade 3 toxicities and 3 patients with grade 4 toxicities. The most common serious toxicity was related to liver function, electrolyte abnormality or elevation in serum lipase. In the melanoma trial, no grade 3 or 4 toxicities were noted. This suggests that impaired liver function, such as that which occurs in cirrhotic patients or patients with hepatitis, may lead to a greater risk of liver function toxicity with ADI-PEG20 compared with patients with no liver dysfunction. Furthermore, the long-term side effect of arginine depletion is not known. In Phase II trials, no long-term untoward side effects were noted, even in patients who received the drug for > 1 year. As this represents only a small number of patients, a larger

population will need to be followed to determine if chronic arginine depletion has delayed toxicities.

It is conceivable that ADI-PEG20 may be synergistic or have additive effects when combined with other agents. For melanoma, it seems reasonable to consider combining ADI-PEG20 with high-dose IL-2. First, the major serious side effect of high-dose IL-2 is hypotension. This hypotension has been related to the production of nitric oxide, which appears to play a key role in IL-2 therapy-induced capillary leak syndrome [42]. Arginine is the only endogenous source of nitric oxide. ADI-PEG20 has been shown to inhibit nitric oxide production and protect mice from the fatal effects of TNF-α and endotoxin [43,44]. NG-monomethyl-L-arginine, a nitric oxide synthase inhibitor, appears to alleviate the hypotensive effects of high-dose IL-2 in patients with metastatic renal cell cancer [45,46]. Thus pretreatment with ADI-PEG20 may also protect patients from hypotension induced by high-dose IL-2. Second, nitric oxide has been demonstrated to promote tumour growth and metastasis by stimulation of tumour cell migration, invasiveness and growth [47]. Lastly, metastatic melanoma cells may escape immunosurveillance through the mechanism of releasing nitric oxide to induce dysfunction of immunocytes [48]. Therefore, the combination of ADI-PEG20 with high-dose IL-2 seems to be a reasonable regimen for a Phase II trial in malignant melanoma.

Although ADI-PEG20 appears to be active in hepatoma and malignant melanoma, the true response rate for both diseases remains unknown. Larger Phase II trials will be needed to assess its overall activity. In addition, Phase III trials comparing ADI-PEG20 with standard drug therapy have not yet been initiated. The role of ADI-PEG20 in melanoma may be in early-stage disease (stage I, II or III) before the tumour has had a chance to develop ASS expression and drug resistance. It would be reasonable to design a clinical trial in which patients with high-risk melanoma (primary tumours > 4 mm or lymph node involvement) have their tumour assayed for ASS expression. A patient whose tumour is ASS negative could be randomised to receive either ADI-PEG20 or standard therapy (IFN-α for USA). In addition, further laboratory studies are needed to determine why only certain patients whose tumour stains negative for ASS may respond to ADI-PEG20 and others do not. In this way, the therapy could be targeted so that there is a greater chance of identifying patients who may benefit from treatment with ADI-PEG20.

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